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August 14, 2017

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Project: Optimizing Establishment of Corn in Cover Crops and Living Mulches to Maintain Yield while Reducing Nitrate Losses

Dear Chris:

Here is complete copy of the final report submitted to the Minnesota Department of Agriculture Pesticide and Management Division. The electronic copy was emailed to you on August 14, 2017.

I am submitting only one print copy. This report was prepared by the contractor and according to the project manager is not mandated by law.

Please contact me at (651) 201-6196 if you have questions.

Sincerely,

Kam Carlson

Kam Carlson
Contracts & Grants Coordinator
Pesticide & Fertilizer Management Division
Minnesota Department of Agriculture
625 Robert Street N.
St. Paul, MN 55155-2538

Enclosures: One copy of final report for project listed above

OPTIMIZING ESTABLISHMENT OF CORN IN COVER CROPS AND LIVING MULCHES TO MAINTAIN YIELD WHILE REDUCING NITRATE LOSSES

Minnesota Department of Agriculture Clean Water Fund Research and Evaluation Program Project Number 76922

JULIE GROSSMAN, JOHN BAKER, PEYTON GINAKES, MICHELLE DOBBRATZ, SCOTT WELLS

UNIVERSITY OF MINNESOTA

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EXECUTIVE SUMMARY

It is a formidable challenge for row crop production to continue without negatively affecting water quality based ecosystem services. Currently, nutrients exported from row crop production contribute to the eutrophication of local surface water, pollution of subsurface drinking water, and global environmental challenges such as the hypoxic dead zone in the Gulf of Mexico (Rabalais et al., 2002; Wolfe and Patz, 2002; Minnesota Polution Control Agency, 2015). As states in the Upper Midwest focus on reducing negative water quality impacts of grain corn production, there is a need for innovative production strategies that maintain profitability while minimizing the export of nutrients, agrochemicals, and soil. Maintenance of surface vegetative cover is probably the most effective means to protect soil, but it is challenging to do so in annual row crops (Carlson, 2017).

Zone tillage is a reduced-tillage compromise between no-till and full-width tillage that attempts to capture both the environmental advantages of year-round ground cover and the agronomic benefits of in-row tillage. This management tool is especially well-suited to the practice of cover cropping, where plants are grown between cash crops in space, time, or both. Localizing the use of tillage in cover cropped systems may preserve soil quality between crop rows where soil is not tilled, as measured by soil microbial activity and varying indicators of soil organic matter, while also making N available only in crop rows. In turn, increased soil organic matter increases paired with N availability synchronized with plant uptake may ultimately lead to enhanced water quality. This project determined the effect of differing levels of zone tillage intensity on soil carbon and nitrogen cycling in a corn-kura clover cropping system (*Zea maize-Trifolium ambiguum*) in an effort to determine impacts on soil N and C

Graduate student team: Michelle Dobbratz (far left), Peyton Ginakes (PhD far right)

(Chapter 1). Additionally, our research determined the impacts of these different zone tillage approaches on corn production (Chapter 2). Research took place in Rosemount, MN in 2015 and 2016 in an established kura clover stand. Soils and kura clover biomass were each sampled three times in crop rows per year in four treatments: NT (spray-down no-till), ST (shank-till, traditional strip till unit), ZT (zone-till, PTO-driven rotary zone tiller), and DT (double-till, ST+DT). Samples were analyzed for microbial biomass (MB), soil inorganic nitrogen, and permanganate oxidizable carbon (POXC). Additionally, potentially mineralizable nitrogen (PMN) was measured on 2016 post-till soils. Greater spring kura clover biomass in 2016 (2,449 kg ha^{-1}) relative to 2015 (187 kg ha^{-1}) appears to have influenced overall differences in soil quality between years. The double-till (DT) treatment had greater post-till soil inorganic N than the no-till (NT) treatment in 2016, and by harvest sampling time, both zone-till (ZT) and double-till (DT) had higher soil inorganic N than NT, indicating that the addition of kura clover biomass contributed to in-row, plant-available nitrogen. Double-till was also more effective in reducing kura clover encroachment into crop rows than NT. No effect of tillage treatment on PMN, MB, or POXC was observed at any sampling time, although decreasing POXC data trends paired with increasing MB trends over the 2016 growing season suggest that the amount of incorporated kura clover biomass governed belowground nutrient cycling and soil fertility.

Despite potential for environmental benefits, yields of zone tillage systems are often reduced relative to conventional, fully-tilled systems, likely due to early season competition between corn seedlings and the clover. We monitored kura clover health, soil moisture & temperature, corn emergence, corn development, and corn yield in three treatments (ZT, ST and NT). Our primary objective was to compare the novel rotary ZT with the traditional shank-based strip tillage unit. In 2015, corn grown in ZT plots emerged and developed faster than corn grown in ST plots, but this did not lead to a difference in grain or stover yield. However, in 2016, corn grown in ZT plots not only emerged and developed faster, but also produced 4.0 Mg ha^{-1} more grain and 3.5 Mg ha^{-1} more stover biomass than corn grown in ST plots. Kura clover biomass was not affected by treatment in either year. We conclude that rotary zone tillage is a promising row preparation strategy in kura clover living mulch for corn production with minimal herbicide use.

In total, this project supported the training of two graduate students, and five undergraduates. We are grateful to the MDA for the support of our project and the valuable training of these students.

Summary conclusions:

- Rotary zone tillage is a promising strategy for managing kura clover living mulch in corn systems in the Upper Midwest.
- Combining the shallower rotary zone tillage approach with the deeper shank tillage approach provided more soil inorganic nitrogen available for corn uptake than the traditional shank till approach alone.
- The quantity of aboveground kura clover biomass plays a critical role in governing C and N cycling in the living mulch systems, providing more labile soil C and higher available N when more kura clover biomass is present.
- Kura clover zone till management requires careful attention, particularly during the early season. During this critical period, corn grown in seedbeds prepared with rotary zone tillage was consistently ahead of corn grown in seedbeds prepared using traditional strip tillage.
- Rotary zone tillage led to higher grain yields than strip tillage in one of our two study years.
- Although promising, the rotary zone tillage system requires more capital input for implement purchase. Living mulch systems in general use might be best suited for more sensitive environments and those at high risk for water quality challenges, due to these increased management costs and potentially higher labor needs.

CHAPTER 1. SOIL CARBON AND NITROGEN DYNAMICS UNDER ZONE TILLAGE OF VARYING INTENSITIES IN A KURA CLOVER LIVING MULCH SYSTEM

Peyton Ginakes, Julie Grossman, Thanwalee Sooksa-Nguan, Michelle Dobbratz, and John Baker

1. Introduction

Zone tillage, a reduced tillage approach where only crop rows are tilled, has been proposed as a way to maintain ground cover while disturbing soil where necessary to prepare seedbeds and incorporate nutrients (Brainard et al., 2013). Tillage has detrimental effects on soil structure (Kabiri et al., 2015), soil biological activity (Sapkota et al., 2012), and soil water capture and holding capacity (Haramoto & Brainard, 2012; Alliaume et al., 2014). Zone tillage approaches can warm soil seedbeds in areas with cold and wet spring seasons (Licht & Al-Kaisi, 2005), clear crop rows of competing vegetation, and localize nutrient incorporation (Liebman & Davis, 2000).

Living mulches are crops maintained year-round or perennially, typically for the purposes of erosion protection, soil-improvement or nutrient enhancement. Their use necessitates zone tillage approaches for crop production, as the living vegetation must be removed from crop rows prior to sowing. Living mulches are generally biennial or perennial legumes such as clovers and alfalfa that can withstand frequent mowing as well as winter conditions. Living mulches have been examined for their potential to reduce between-row weeds (Enache & Ilnicki, 1990; Hiltbrunner et al., 2007; Gibson et al., 2011), reduce excess soil nitrogen (N;

Brandsæter et al., 1998; Ochsner et al., 2010), and provide localized N to crop rows (Berkevich et al., 2008; Sawyer et al., 2010; Deguchi et al., 2014).

Kura clover, a long-lived and rhizomatous perennial, has been studied for its use as an effective living mulch in the Upper Midwest. It is more winter hardy than many other perennials owing to its origin in the Caucasus region (Sheaffer & Marten, 1991; Zemenchik et al., 2000), and has been found to accumulate up to 276 kg N ha^{-1} via biological nitrogen fixation (BNF; Seguin et al., 2000). Kura clover can reduce soil and nutrient runoff by increasing water infiltration and protecting the soil surface (Siller et al., 2016), as well as reduce nitrate loading in water bodies through its extensive root system (Qi et al., 2011b).

Living mulches also benefit soils via their ability to contribute soil nutrients. Typical kura clover living mulch management includes suppression (spraying or tilling, or both), followed by crop planting, then kura clover recovery in the fall, post-harvest. A hardy stand will encroach into rows by the following spring. As a legume, kura clover can repeatedly supply fixed N to subsequent crops, provided enough biomass accumulates in the tillage zone between growing seasons (Zemenchik et al., 2000), and nitrogen fixation is effective. Perennial roots like those of kura clover have been found to enhance soil structure and contribute to soil microbial activity (Bissett et al., 2011; DuPont et al., 2010; Anderson & Coleman, 1985) via continuous root growth, sloughing, rhizodeposition, and turnover (Abdollahi et al., 2014). These N and soil organic matter (SOM) additions provide easily accessible, labile nutrient pools that may enhance soil nutrient cycling. Soil indicators of interest to measure contribution to such labile pools include soil microbial biomass (MB) and inorganic N, potentially mineralizable N (PMN), and permanganate oxidizable C (POXC), a methodologically defined SOM pool that determines the amount of soil C that is easily available to microbes for respiration (Weil et al., 2003), due to their sensitivity to soil disturbance and management (Culman et al., 2013; Culman et al.2012; Larsen et al., 2014; Idowu et al., 2009).

Challenges to kura clover living mulch management in row crop systems also exist. First and most importantly, reduced yields are often observed with the use of conventional strip tillage equipment that utilizes a coulter-driven shank of relatively narrow width, generally due to living mulch encroachment and subsequent competition with cash crops for water, sunlight, and nutrients (Grabber et al., 2014; Qi et al., 2011a; Sawyer et al., 2010). To this end, a novel zone tillage implement consisting of a PTO-driven rotary tiller to create a wider planting zone has been proposed as an approach to reduce competition between the living mulch and the cash crop. To date, most studies investigating living mulches in agronomic systems have examined the system capacity to increase yields or environmental services, rather than the impact of kura clover living mulch on soil nutrient cycling (Ochsner et al., 2010; Qi et al., 2011b; Sawyer et al., 2010).

Our goal in this study was to compare the effects of tillage approaches that vary in intensity on soil carbon and nitrogen cycling in a corn-kura clover cropping system. Specifically, our objectives were to examine the effect of tillage intensity on 1) soil N contributions, and 2) belowground nutrient cycling as mediated by microbial activity and easily accessible SOM pools.

2. Materials and methods

Study site

This experiment was conducted in Rosemount, MN (44°71'N, 93°7'W) at the University of Minnesota's Rosemount Research and Outreach Center in 2015 and 2016. Soil at the site is a Waukegan silt loam (fine-silty over skeletal mixed, super active, mesic Typic Hapludoll). Kura clover was planted in the field in 2006, and a corn/soy rotation had been planted into it since 2008, with the year immediately prior to the experiment in 2015 cropped to soybean. Due to kura clover slug damage after the 2015 corn harvest, the 2016 field plot was moved to an adjacent area, such that the year prior to the 2016 experiment was a rest year for the kura clover in which the clover was managed as a hay crop. Thus, in 2015 the experiment followed eight years of row crop production, while in 2016, the experiment was planted into clover that was given a year to recover. Plot management history is provided in Table 1.

Management and sampling details	2015	2016
Previous crop	soybean	KC forage
Spring field preparation	--	flail mowed
Pre-till KC sampling	30-Apr to 1-May	9-May
Pre-till soil sampling	30-Apr	11-May
NT spray	1-May	18-May
Tillage date	4-May	18-May
Fertilizer application	5-May	18-May
Corn planting	5-May	18-May
Sidedress fertilization	mid-June	mid-June
Post-till soil sampling	18-May	29-May
Mid-season KC sampling	21-Aug	26-Jul
Harvest KC sampling	--	4-Oct
Harvest soil sampling	9-Oct	4-Oct
Corn harvest	9-Oct	24-Oct

Table 1. Schedule of field operations and samplings in 2015 and 2016, in Rosemount, MN. KC = kura clover.

Experimental design

This study was designed as a randomized complete block, with four replications of four tillage treatments. Tillage treatments included NT (spray-down no-till), ST (shank-till, traditional strip till unit), ZT (zone-till, PTO-driven rotary zone tiller), and DT (double-till, ST+ZT). No-till used 1 kg ae ha^{-1} glyphosate (*N*-(phosphonomethyl)glycine) to suppress kura clover before planting. A conventional strip tillage unit (1tRIPr, Orthman Mfg., Lexington NE) was implemented for ST, consisting of a shank with ground-driven coulters. The ZT implement was a PTO-driven 6-row rotary tiller (Northwest Tillers, Yakima WA), in which each set of rotary tines tilled a zone 30 cm wide and approximately 10 cm deep. Plot management details and dates are in Table 1. Plots had six rows, each 76 cm wide and 37 m long. All samples were collected from the two central rows. Plots were fertilized with 224 kg ha^{-1} of 18-46-0, 224 kg ha^{-1}of 0-0-60, and 84 kg ha^{-1} of 21-0-0-24 (N-P-K-S), resulting in 58 kg N ha^{-1}, 103 kg P_2O_5 ha^{-1}, 135 kg K_2O ha^{-1}, and 20 kg S ha^{-1} at planting in both years. Additionally, plots were side dressed with 41 kg N ha^{-1} in 2015 and 34 kg N ha^{-1} in 2016.

Left: Rotary Zone Till implement (ZT), Right: Shank Till (ST) implement. Double Till (DT) treatments combined ZT and ST.

Kura clover and soil sampling

Kura clover sampling using a quadrat sampling frame

Each year, kura clover biomass was collected at three time points: immediately prior to spring tillage (pre-till), middle of the growing season (mid-season), and at corn harvest (harvest; Table 1). Aboveground biomass was collected from in-row areas where crops were to be planted using a 0.1 m^2 quadrat. Biomass was then transferred to a 60ºC oven for at least 48 hours before being ground to 1 mm and analyzed for C and N content on a combustion analyzer (Elementar VarioMAX CN analyzer, Elementar Americas).

Soils were also collected from within crop rows at three time points per year: pre-till, post-till, and at harvest (Table 1). A composite of ten samples from the top 15 cm were collected in a bucket, homogenized, and divided into two subsamples. One subsample was dried at 35ºC for at least 48 hours before removing them, grinding and sieving to 2 mm, and setting aside for inorganic N extractions, POXC analysis, and C/N analysis (Elementar VarioMAX CN analyzer, Elementar Americas). The other subsample was sieved to 2 mm and kept field-moist at 4ºC for PMN and MB determination.

Permanganate oxidizable C

Permanganate oxidizable carbon was measured according to Weil et al. (2003). Briefly, 2.5 g of dry soil were reacted with $KMnO_4$, a strong oxidizing agent. Diluted supernatants were transferred to 96-well plates and measured on a spectrophotometer at 540 nm. Absorbance was fitted to a standard curve, and calculated to determine C oxidation by $KMnO_4$ reaction.

Soil inorganic N and potentially mineralizable N

Inorganic N (NH_4^+ and NO_3^-) was extracted from in-row soils using 1 M KCl and filtered through #42 Whatman papers (Robertson et al., 1999). Extractions were frozen in scintillation vials until N analysis on a Shimadzu TOC and TN analyzer (Kyoto, Japan).

In 2016, post-till soils were analyzed for PMN using a 28d aerobic incubation method (adapted from Scott et al., 1998; Prescott et al., 2005) with the goal of elucidating kura clover N contribution and subsequent mineralization. Briefly, 10 mL water was added to dry soil samples until water holding capacity was reached. Tubes were loosely capped and incubated in a bin at 37ºC for 28 days. Tubes were weighed three times per week to ensure consistent soil moisture. At the end of the incubation, PMN was extracted with 40 mL 1.3 M KCl, and extracts were analyzed on a Shimadzu TOC and TN analyzer (Kyoto, Japan). Final values were calculated by subtracting soil inorganic N from values for the 28 d incubation.

Microbial biomass C and N

A simulated chloroform slurry extraction (simCSE) method was used to determine MB (Fierer et al., 2003). Briefly, field-moist soil samples were divided into two test tubes; 40 mL 0.5 M K_2SO_4 was added to each, and one also received 0.5 mL $CHCl_3$ to lyse microbial cells. After shaking for 4 h and settling, samples were filtered through #42 Whatman papers and frozen until analysis on a Shimadzu TOC and TN analyzer (Kyoto, Japan). Final MB C and N values were calculated by subtracting baseline values from values for fumigated samples.

Statistical Analysis

All statistical analyses were performed in SAS (Cary, NC). All data met assumptions of normality and equal variances. An ANOVA was used to detect the effect of tillage treatments, sampling times, and interactions of tillage treatment by sampling time within year using PROC MIXED, with only block as a random effect (Table 2). Years were analyzed separately due to spatial and environmental differences between 2015 and 2016 plots. Where ANOVA data showed significance, data were then analyzed using Tukey's Honest Significant Difference.

3. Results and discussion

Kura clover biomass

In 2015, there was an interaction effect of tillage by sampling time for kura clover biomass (p = 0.0672; Table 2, Fig. 1). While at the pre-till sampling no trends for tillage treatments were apparent, by mid-season shank till (ST) treatments had greater kura clover biomass present in crop rows than did DT (490 and 115 kg ha^{-1}, respectively), indicating that the greater soil disturbance of the DT treatment reduced kura clover encroachment into rows when compared to ST (Dobbratz, 2017; Fig. 1). By harvest, little to no kura clover biomass remained in rows, thus biomass data was not collected. These trends were supported by kura clover N content data, where by 2015 mid-season and 2016 harvest samplings both trended toward having more kura clover biomass N in ST compared to DT (data not shown). Biomass N was relatively constant across all treatments in 2015, ranging from 6 to 22 kg N ha^{-1} with a mean C:N of 8.4 ± 1.05). Since % N of kura clover stands across the experiment was not expected to vary, quantity of kura clover biomass present following application of tillage treatments at this mid-season sampling point drove the final overall biomass N contributions. Kura clover biomass in rows at the 2015 harvest time point was negligible, likely due to corn canopy closure that shaded kura clover and suppressed growth. Biomass production differed by nearly an order of magnitude between 2015 and 2016 growing seasons, where mean spring biomass in 2015 was 261 kg ha^{-1}, versus 2361 kg ha^{-1} in 2016 (Fig. 1). Seguin et al. (2001) reported a similar kura clover aboveground biomass yield (2678 kg ha^{-1}) in a seeding year when 100 kg N ha^{-1} N fertilizer was applied incrementally, similar to our experiment's split N application. In 2015, the lower biomass can be attributed to a previous year of soybean cropping (2014) followed by limited kura clover regrowth into the permanent crop row regions, leaving sparse kura clover vegetation within rows. Still, tillage effects on biomass were similar in both years in that more intensive tillage served to reduce kura clover. Reductions in kura clover biomass over the course of the growing season occurred in 2016 for all tillage approaches, where spring biomass decreased after tillage or no-till herbicide application, and further diminished by corn harvest ($p < 0.0001$; Table 2, Fig. 1). Work by Peterson et al. (1994) on repeated kura clover forage harvests supports the recovery of kura clover, where biomass of a third harvest in the season was reduced to approximately one-third of the first harvest.

Soil inorganic N

Soil inorganic N values varied greatly between years. While in 2015, inorganic N values ranged from 6 to 125 mg N kg $soil^{-1}$, the range in 2016 was much narrower, from 6 to 24 mg N kg $soil^{-1}$ (Fig. 2). No effect of tillage on inorganic N was observed in 2015, although all treatments were collectively associated with a decreasing trend in soil inorganic N over the course of the growing season. Across treatments, soil inorganic N in pre-till > post-till > harvest ($p < 0.0001$; Table 2). In 2016, there was also an effect of time, such that pre-till < post-till ≈ harvest ($p = 0.0084$; Table 2). We also observed differences between tillage approaches (Fig. 2, $p = 0.0254$), where at the post-till sampling, soil inorganic N was greater in double-till compared to no-till, and by harvest, DT had more soil inorganic N than both NT and ST.

There was an inverse relationship between inorganic N and kura clover biomass in crop rows, suggesting kura clover was accumulating N from existing soil pools. Work by Seguin et al. (2000) has shown kura clover fixes only 57% of its N from the atmosphere, suggesting a relatively high soil N scavenging ability, and available soil N is known to decrease biological N-fixation in legumes. Indeed, Grabber et al. (2014) cited kura clover's nitrate uptake as a factor leading to low corn yields due to excessive nutrient competition. Qi et al. (2011a) found that kura clover took up more soil nitrate than either winter rye or an orchardgrass-clover mix at $p < 0.05$.

In 2015, there was a consistent reduction in soil N with each sampling time across all tillage treatments, despite kura clover biomass incorporation following tillage, and an additional influx of N via side dressed N application (123 kg N ha^{-1}) in mid-June between the post-till and harvest soil sampling (Fig. 2). As both were expected to increase soil N, this was unexpected. Likely explanations for this decrease were the uptake of plant-available soil N by the corn crop, as well as N uptake by the living kura clover. These possibilities are confirmed by findings of Sawyer et al. (2010), who found in a study on a corn-kura clover system's response to fertilizer N that kura clover neither influenced soil nitrate at any point in the growing season, nor reduced corn fertilizer N need. These results contradict findings in previous studies, which found that corn intercropped with kura clover needed 0-20 kg N ha^{-1} (Affeldt et al., 2004; Berkevich, 2008), suggesting a high capacity for N supply from incorporated kura clover. These opposing findings may be reconciled by considering the priming effect frequently stimulated by high quality residue additions (Kuzyakov et al., 2000). These additions of SOM are often not considered in mass balance estimations, resulting in underestimated N mineralization where labile N additions spur mineralization of organic N (Wu et al., 2008). Therefore, it is possible that results showing no positive effect of kura clover on soil fertility may stem from spatial plant-available N competition, rather than an overall lack of N mineralization. Different results in 2016 where soil inorganic N was higher after kura clover suppression most likely stems from the greater amount of kura clover biomass in 2016 relative to 2015 (Fig. 1).

Permanganate oxidizable C

Post-till soils in 2015 had greater POXC than pre-till across all tillage treatments ($p = 0.0228$). In 2016, POXC decreased by harvest compared to pre- and post-till soil samplings across all tillage treatments ($p < 0.0001$). There was also an effect of tillage treatment in each time point, where NT and DT had greater POXC than ZT ($p = 0.0053$). A concurrent study in organically managed kura clover zone till systems found that when kura clover biomass production was high, POXC was greater under living kura clover compared to where it was incorporated via tillage (Ginakes, 2017). One possible explanation for the differences in POXC between years is the quantity of kura clover biomass incorporated into tilled areas. In 2016, kura clover biomass was nearly five times greater than in 2015 (Fig. 1). Tillage is generally expected to reduce POXC via an N-coupled carbon loss mechanism (Panettieri et al., 2013; Plaza-Bonilla et al., 2014). The high quantity of kura clover inputs in 2016 may have led to N-coupled carbon loss in crop rows via oxidation, with consequent POXC decreases over the growing season. Moldboard plow management has been found to be consistently lower than POXC values associated with reduced-till and no-till practices across all aggregate fractions in work by Panettieri et al. (2013), and Hurisso et al. (2016) highlight POXC as an indicator of practices that accumulate and stabilize SOM. In contrast, biomass contributions in 2015 may have lacked sufficient N to stimulate decomposition and instead stored labile soil C, resulting in a temporary increase in POXC by the post-till sampling.

Potentially mineralizable N

Labile organic N, measured as PMN, was measured only in 2016 post-till soils to capture the effect of tillage approaches on microbially-available nutrient pools (Fig. 4). The lack of differences found between treatments might be explained by our finding that spring kura clover biomass did not vary between tillage treatments (Fig. 1), and in-row soil sampling was guided by visual observations of where tillage occurred. Thus, despite differences in size of the disturbed area impacted by tillage approach (Dobbratz, 2017), our soil sampling appears to have captured a relatively homogenous range of disturbed soils (excluding NT) with approximately similar amounts of labile N inputs. It may be that kura clover biomass was mineralized by post-till sampling, with N already mineralized into available N, as in the DT treatment (Fig. 2), rather than organic N, as was expected to be captured in the PMN assay. In a study on N delivery from various legume cover crops, Parr et al. (2011) found that vetch had the lowest C/N (12) of all species and cultivars, which resulted in the highest corn yield. This supports our finding that a low C/N (10.5 in our 2016 pre-till kura clover) results in a rapid mineralization rate, driving decomposition and the shift from organically-bound PMN to available inorganic soil N. Overlapping trends of PMN with pre-till kura clover biomass (Fig. 1), though not significant, appear to support this.

Microbial biomass C and N

Microbial biomass was measured only in 2016. No differences between tillage approaches or across time points were observed for MBC at $p < 0.10$, although several trends were apparent (Fig. 5), with decreases between pre-till and post-till MBC (mean = 30 versus 18 mg MBC kg soil^{-1}), as was an increase by the time of a harvest sample (mean = 25 mg MBC kg soil^{-1}) with the exception of ST. Microbial biomass N, however, was affected by both time and treatment. Post-till MBN was greater than pre-till (mean = 13 and 8 mg N kg soil^{-1}, respectively; $p = 0.0211$), and ST and DT treatments had greater MBN than no-till ($p = 0.0506$). While MBC and MBN are usually expected to have similar trends on different scales, this was not this case in our study. It is possible that dominant microbial groups shifted over the course of the growing season, as bacteria and fungi are known to have different C/N values. Recent stoichiometric analyses of 87 bacteria and fungi showed that bacteria biomass have a lower C/N than fungi, 4.6 and 8.3 respectively (Mouginot et al., 2014).

4. Conclusions

The quantity of aboveground kura clover biomass appears to have played a critical role in governing C and N cycling in the living mulch systems studied. In 2015, where little biomass was present before tillage, the addition of labile residues did not impact subsequent N availability. However, incorporation of much greater kura clover biomass in 2016 contributed sufficient labile C and N to likely increase coupled N mineralization/C oxidation microbial processes, resulting in increased N availability and ultimately higher crop yields, especially in high intensity zone tillage approaches that incorporated the most kura clover biomass such as the DT treatment (Dobbratz, 2017).

Our double-till approach, which was the most intensive and resulted in the greatest soil disturbance and kura clover biomass incorporation, provided more soil inorganic nitrogen than no-till at the 2016 post-till sampling and more than both no-till and shank-till at the 2016 harvest sampling. Double-till also was more effective than shank-till at reducing kura clover encroachment into crop rows, and may have additional capacity to mitigate weed competition with a corn crop. For the purpose of crop nutrient provisioning, DT may thus be a more reliable approach for delivering easily mineralizable N and reducing kura clover living mulch competition with a cash crop. Applicable future work might further elucidate these dynamics by measuring the amount of kura clover actually incorporated by tillage approach, as well as more frequent soil sampling coupled with crop sampling in order to assess nutrient availability and crop uptake.

Table 2. Analysis of variance significance for kura clover biomass, soil inorganic N, permanganate oxidizable C (POXC), potentially mineralizable N (PMN), and microbial biomass C and N (MBC, MBN) in 2015 and 2016

	2015						2016					
Source	kura clover biomass	inorganic N	POXC	PMN	MBC	MBN	kura clover biomass	inorganic N	POXC	PMN	MBC	MBN
Block	NS	NS	NS	--	--	--	NS	NS	*	NS	NS	NS
Tillage	NS	NS	NS	--	--	--	NS	**	***	NS	NS	*
Time	NS	****	**	--	--	--	****	***	****	--	NS	**
Tillage x Time	*	NS	NS	--	--	--	NS	NS	NS	NS	NS	NS

"NS" indicates no significance

"*" indicates significance at 0.10

"**" indicates significance at 0.05

"***" indicates significance at 0.01

"****" indicates significance < 0.0001

"--" indicates data were not tested

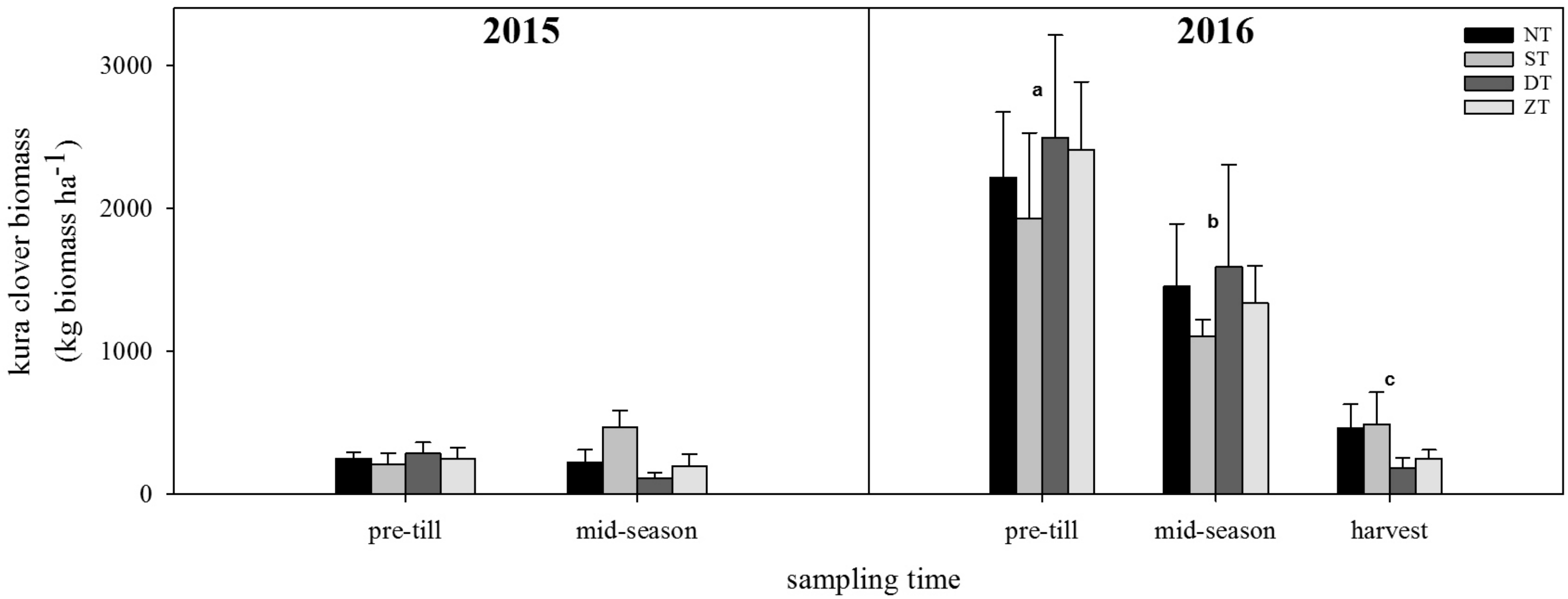


Fig 1. Mean aboveground kura clover biomass before spring tillage, and the effect of tillage treatment on regrowth by mid-season and corn harvest in 2015 and 2016. Error bars represent one standard error. Different lowercase letters over sampling time groupings represent a significant effect of sampling time on kura clover biomass in all treatments ($p < 0.0001$).

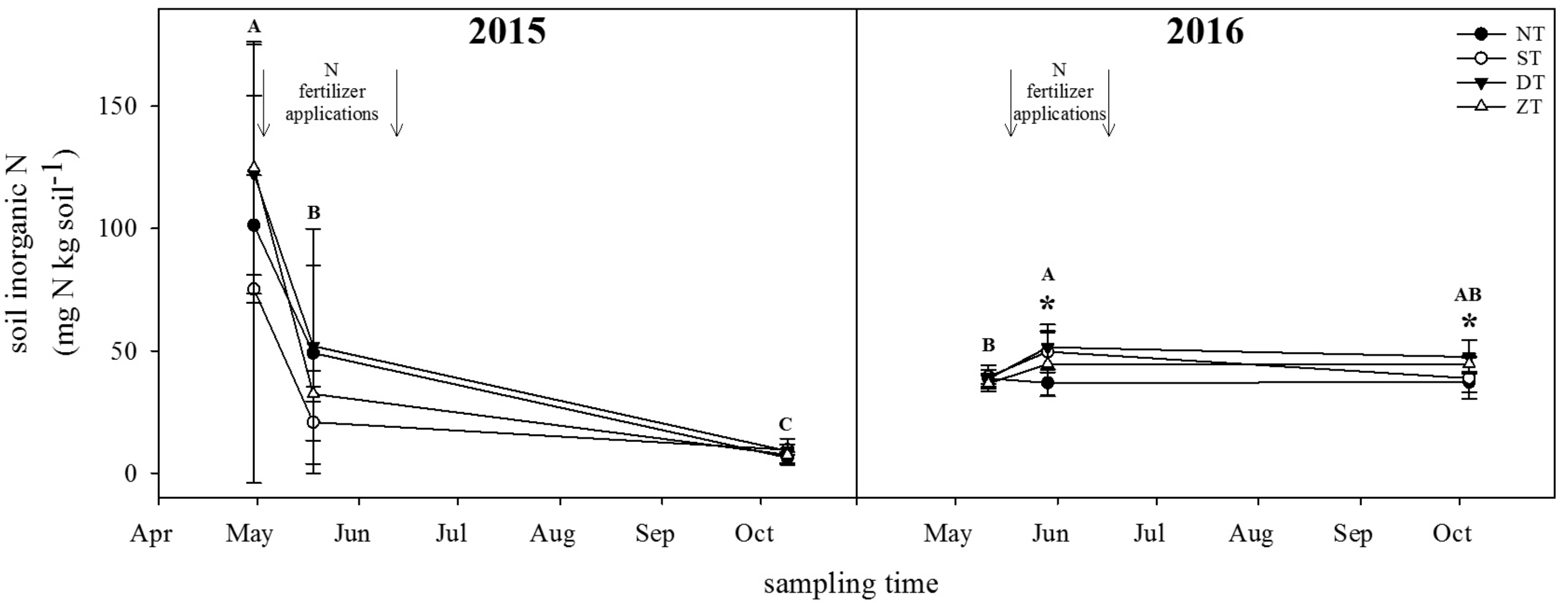


Fig 2. Mean soil inorganic N before spring tillage (pre-till), and the effect of tillage soil inorganic N at post-till and harvest sampling times in 2015 and 2016. Error bars represent one standard error. Asterisks (*) over a sampling time represents a difference in inorganic N between treatments within a sampling time ($p < 0.05$), and different letters indicate a difference between sampling times across treatments ($p < 0.01$).

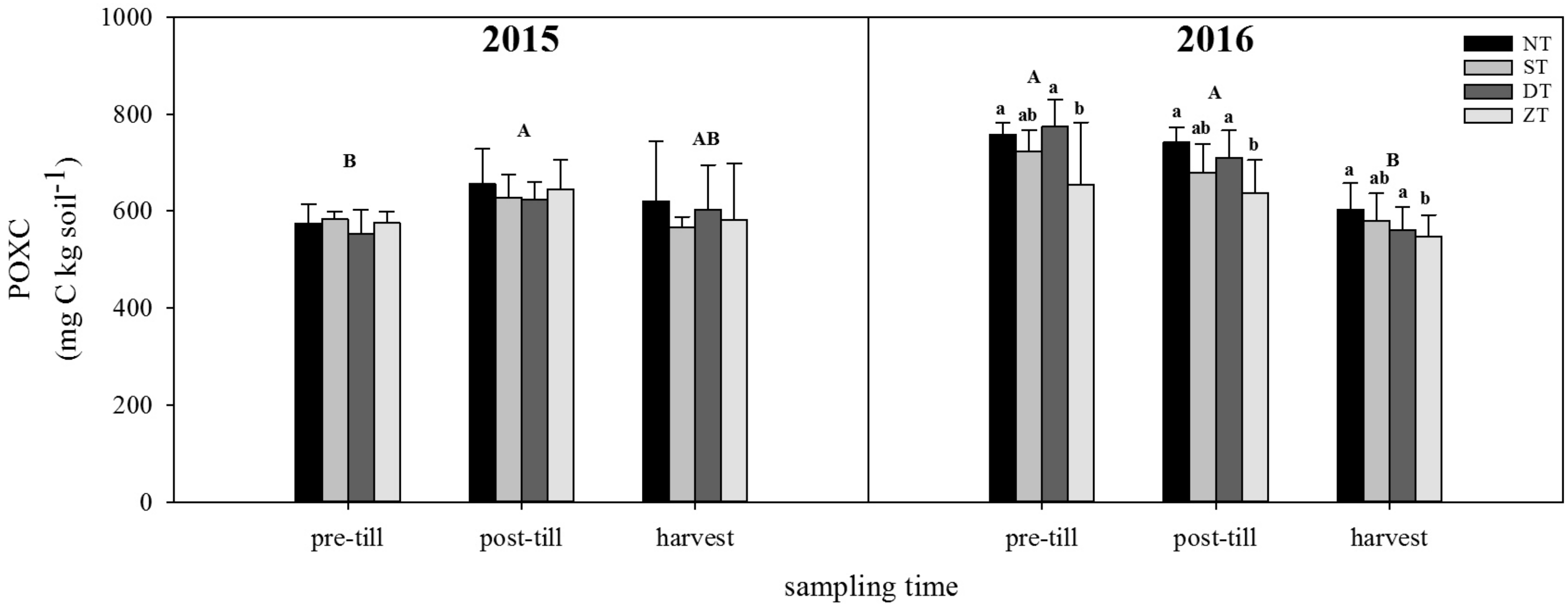


Fig 3. Mean POXC before spring tillage (pre-till), and the effect of tillage on POXC at post-till and harvest sampling times in 2015 and 2016. Error bars represent one standard error. Different capital letters over sampling time groupings represent a difference in POXC between sampling times ($p < 0.05$). Different lowercase letters over bars represent a significant difference between treatments' effects on POXC within a sampling time ($p < 0.01$).

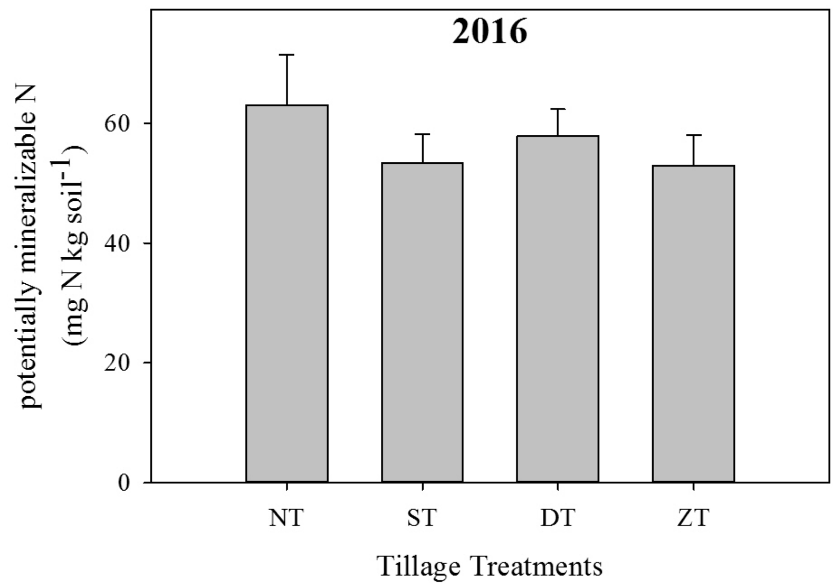


Fig 4. Mean PMN after spring tillage and planting (post-till) in 2016. Error bars represent one standard error.

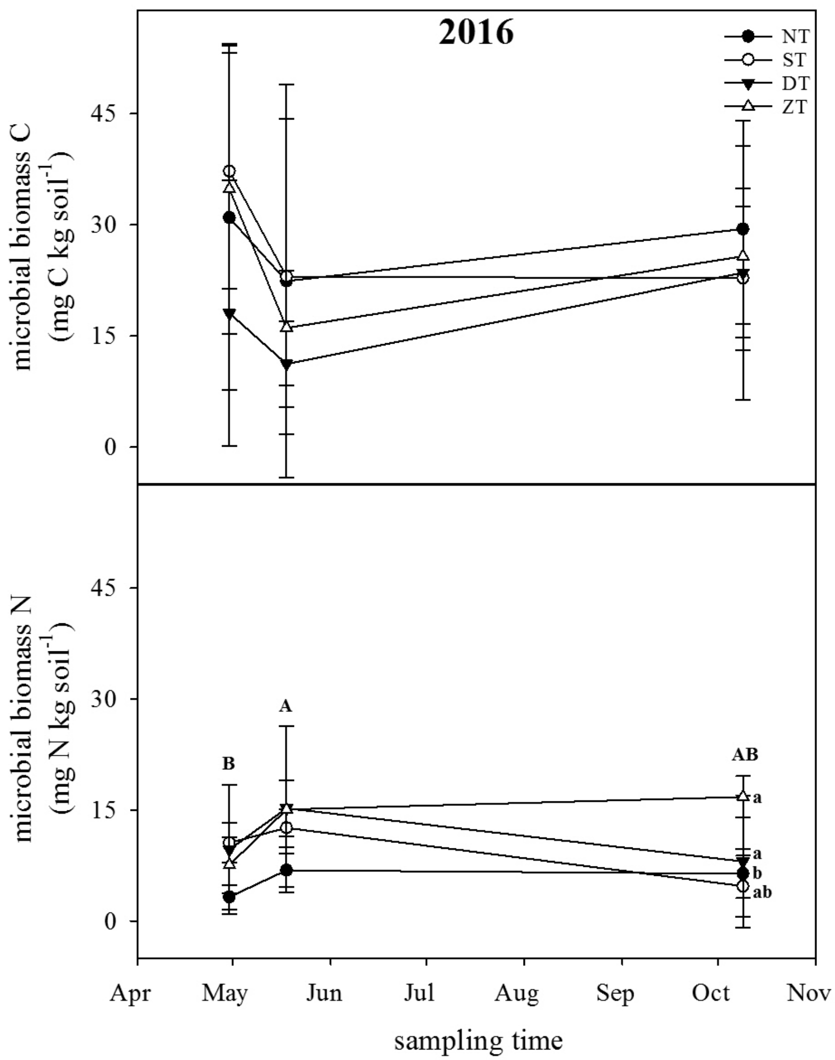


Fig 5. Mean microbial biomass C and N before spring tillage (pre-till), and the effect of tillage on MB by post-till and corn harvest (harvest) in 2016. Error bars represent one standard error. Different capital letters over scatter dots represent a difference in MBN between sampling times ($p < 0.05$), and different lowercase letters represent a difference between treatments over all sampling times ($p < 0.10$).

CHAPTER 2: ROTARY ZONE TILLAGE IMPROVES CORN ESTABLISHMENT IN A KURA CLOVER LIVING MULCH

1. Introduction

Nutrients exported from row crop production contribute to the eutrophication of local surface water, pollution of subsurface drinking water, and global environmental challenges such as the hypoxic dead zone in the Gulf of Mexico (Rabalais et al., 2002; Wolfe and Patz, 2002; Minnesota Polution Control Agency, 2015). As states in the Upper Midwest focus on reducing negative water quality impacts of grain corn production, there is a need for innovative production strategies that maintain profitability while minimizing the export of nutrients, agrochemicals, and soil. Maintenance of surface vegetative cover is probably the most effective means to protect soil, but it is challenging to do so in annual row crops (Carlson, 2017).

Perennial living mulches are one management option that could play a role in preventing runoff, leaching, and erosion in these systems. Living mulches are permanent cover crops grown alongside row crops remaining on the landscape during the fallow season. Living mulches have been shown to reduce surface runoff by 86-98%, soil erosion by 98-99% (Hall et al., 1984), and nitrate leaching by 86% (Liedgens et al., 2004) when compared with conventional practices. Living mulches also can benefit soil health indicators by increasing microbial biomass (Alvarez and Steinbach, 2009), organic matter content (Duda et al., 2003), and aggregate stability (Raimbault and Vyn, 1991). They have also been shown to suppress weeds (Teasdale, 1996) regulate pests and disease (Ramert, 1996; Ntahimpera et al., 1998), and increase water infiltration (Singh et al., 2009). Finally, leguminous living mulches have the capacity to fix nitrogen, which can reduce the fertilization requirements of the row crop (Hall et al., 1984; Grubinger and Minotti, 1990; Duiker and Hartwig, 2004). Living mulches thus have the potential to positively impact the landscape if applied to even a modest portion of the 36 million ha of corn production in the U.S., particularly if focused on the most vulnerable lands.

However, for corn producers to capitalize on these benefits, economically feasible management strategies must be developed that mitigate the risks and costs of adopting this system. Living mulches can reduce cash crop germination rates (Martin et al., 1999), and lower soil temperatures by 0.5-2.8ºC compared to monocrop production (O'Connell and Snyder, 1999; Singer and Pedersen, 2006), which can reduce row crop yields when compared to conventional production systems (Ochsner et al., 2017). The challenge in living mulch management is to optimize cash crop yield while maintaining the health of the perennial. To this end, the perennial living mulch is broadly suppressed and/ or selectively killed in rows. Before planting the cash crop, living mulches can be suppressed through mowing, grazing, or sub-lethal herbicide. Cash crop rows can be prepared in the spring through some form of partial width tillage or with banded herbicide. During the growing season, living mulches can be further suppressed by mowing before the cash crop gets too high, or by applying a selective herbicide.

Kura clover (*Trifolium ambiguum)*, a perennial forage legume, has demonstrated potential as a living mulch for corn production (Zemenchik et al., 2000; Affeldt et al., 2004; Pearson et al., 2014). Row preparation and suppression strategies have been tested that have sometimes produced grain yields similar to monocrop corn production (Affeldt et al., 2004). However, in that system corn was only planted for two consecutive years, allowing the clover to recover as a hay crop every third year, a system that is viable for producers with animals. Pearson et al. (2014) found that grain corn yields in furrow-irrigated kura clover with a combination of strip tillage and banded herbicide were comparable to that of monocrop corn, implying tillage is a promising management strategy in this system.

We propose that more aggressive zone tillage prior to planting might be a viable alternative to band herbicide applications, providing for more thorough control of competition and higher corn yields, while maintaining the health of the kura stand. This experiment was conducted to evaluate the effects of varying zone tillage intensity in kura clover living mulch for grain corn production, and to compare the effects of tillage in general with herbicide band kill.

2. Methods and Materials

Site and Experimental Design

Field studies were conducted in 2015 and 2016 at the Rosemount Research and Outreach Center (44°43' N, 93°05' W) on a Waukegan silt loam (fine-silty over sandy or sandy-skeletal, mixed, superactive mesic Typic Hapludoll) with good natural drainage and low erosion potential. The experimental location was within an unirrigated field of 'Endura' kura clover (*Trifolium ambiguum* Bieb.) established in 2006 and used as a living mulch for corn and soybean production since 2008. In 2015, the experiment followed soybean production, while in 2016 the experiment followed kura clover forage production. Four replications were arranged in a randomized complete block design. Experimental units comprised six 38.7m rows of corn with 76.2cm row spacing.

Agronomic Management

Seedbed preparations were performed 5 May 2015 and 18 May 2016, according to one of the following row preparation treatments: 1) band kill herbicide burn down (BK) with 4 kg a.i. ha^{-1} of glyphosate [N-(phosphonomethyl) glycine] with a standard tractor-mounted boom with nozzles set to a 30.5cm spray width, 2) shank-till (ST), also commonly known as strip-till, using an Orthman 1tRIPr shank-tillage implement with ground-driven wavy coulters (Orthman Manufacturing Inc., Lexington, NE), and 3) rotary zone tillage (ZT) using a custom PTO-driven rotary tine implement (Northwest Tillers, Yakima, WA). Immediately following seedbed preparation, a corn hybrid with glyphosate resistance (2015- Golden Harvest GO1O52; 2016- Dekalb DKC 45-65) was seeded at 79,000 seeds ha^{-1} with a six-row John Deere 7000 planter (John Deere, Moline, IL). A shorter season hybrid was chosen in the second year due to the later planting date. Kura clover was 10-20 cm tall at the time of planting and was mowed to a height of 5cm prior to corn emergence. Starter fertilizer of 9-18-9 at 56 L ha^{-1} was applied at planting, and in mid-June side dressing of 28% liquid N occurred (145 kg ha^{1} in 2015 and 123 kg ha^{1} in 2016). To control weeds and broadly suppress, but not kill, the kura clover, 1.04 a.i. kg ha^{-1} of glyphosate was broadcast on 8 June 2015 and 2 June 2016.

Data Collection and Analysis

Kura clover health was assessed pre-treatment, mid-season, and post-harvest. Percent cover was visually scored by estimating the portion of plot covered by living kura clover. Biomass of kura clover was assessed in between corn rows by harvesting two 0.1 m^2 samples per plot.

Installation of soil sensors occurred within two days of planting. In each plot, two calibrated matric potential and temperature sensors were installed at the 5cm depth in the seed bed. Soil volumetric water content and temperature sensors were placed at the 45cm, 35cm, 25cm, 15cm, and 5cm depths in each treatment to characterize temperature and moisture dynamics within the soil profile. Half-hour means were logged for the entire growing season. Specific instruments used were MPS-6 soil water potential and temperature sensors, and 5TM soil moisture and temperature sensors, logged with Em50 loggers (Decagon Devices, Pullman, WA).

Daily counts of emerged corn plants per 2 m unit of row length were recorded at four locations in each of the row preparation treatment plots. Corn height was recorded weekly by measuring the distance between the soil surface and the arch of the uppermost unfurled leaf (Hager and Sprague, 2002), beginning at emergence and continuing through vegetative maturity. Development was characterized by counting the number of visible leaf collars (Abendroth et al., 2011) from emergence through the seven-leaf stage (V7). Corn stover yield was determined by hand sampling 3 m of row down to 15 cm on 9 October 2015 and 24 October 2016. Grain was harvested mechanically in 2016 with a plot combine and scale on 24 October 2016 and adjusted to 15.5% moisture content. In 2015, whole-plot mechanical harvest was precluded by raccoon damage in a small portion of the field. Hand sampling was conducted by removing cobs from two 3m lengths of row per plot and drying to a consistent weight before adjusting to 15.5% moisture.

Mixed model analysis ($P = 0.05$) was conducted using the anova() function of R (R Core Team, 2016) to test the effects of year, treatment, and treatment by year interactions for all measurements. We failed to generalize across years, so years were analyzed separately for all parameters. When analyzing emergence rates and vegetative development, days after planting (DAP) were also included in the model. Linear models were constructed using the lme function of the nlme package (Pinheiro J., Bates D., DebRoy S., 2016), considering year, treatment, and DAP fixed effects and blocks as random effects. When significant differences were found ($P = 0.05$), post-hoc analysis was conducted using Fisher's Protected LSD.

3. Results and Discussion

Overall, both growing seasons had warmer temperatures and greater precipitation than the most recent climate normals for the site (Table 1; Midwestern Regional Climate Center and NWS Cooperative Observer Program). In 2015, total rainfall from May to September was 99 mm above the 30-yr long-term average. Monthly precipitation in 2015 was consistently above average through July, which was the wettest month. Precipitation in 2016 was overall 33% higher than average. In 2016, the month of May had a deficit of 24 mm, and moisture stressed corn was noted in late June through early July. Surplus rainfall and heat in September of both years extended the corn growing season and delayed corn harvest. Monthly average temperatures were at or above 30-year averages throughout the duration of the experiment, with September 3 and 2 degrees above average in 2015 and 2016 respectively.

Kura Clover Health

In 2015, kura clover pre-treatment dry matter ranged from 933-1365 kg ha^{-1}, while in 2016, kura clover pre-treatment dry matter ranged from 1660-1866 kg ha^{-1} (Table 3). Pre-treatment kura clover biomass was not different between treatments in either year. Compared with BK, ST lowered kura clover percent coverage by 7% at the post-harvest observation in 2015, but these differences did not persist through the spring. It is worth noting that the robust kura clover growth in 2016 - an average of 630 kg ha^{-1} greater biomass and 31% greater coverage - was likely a factor in the greater variability in soil and corn related parameters discussed below.

Early Season Seedbed Microclimate

In 2015, mean early season (0-50d) soil water potential in ZT plots was 4.5 kPa greater (wetter) than in BK plots (Table 4). This is contrary to the widespread finding that more intensive tillage lowers soil water availability (Xu and Mermoud, 2001; Alletto et al., 2011; Salem et al., 2015), but in agreement with findings by Schwartz et al. (2010) who found in a stubble mulch system, greater evaporation in tilled plots was offset by greater infiltration in no-till plots. In this case, tillage disturbed kura clover roots, which reduce seed depth soil moisture through both transpiration and percolation (Ochsner et al., 2010).

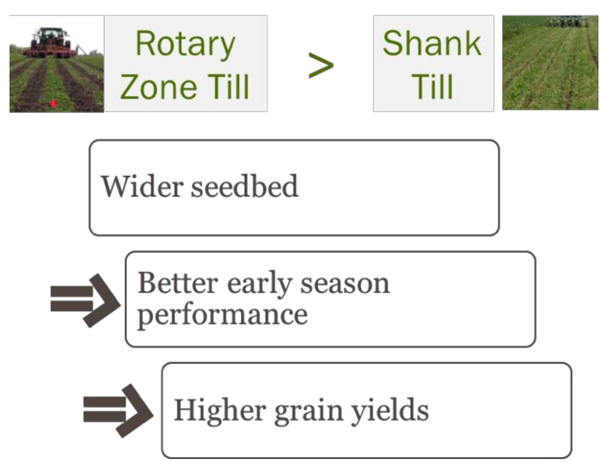
In 2016, ZT increased seedbed temperature by 0.7ºC during the period of emergence (0-17d) and 0.9ºC during the first 50 days after planting (Table 4). These results are comparable to values reported by Licht and Al-Kaisi (2005). More intensive tillage increases early seedbed temperature by removing insulative plant material from the soil surface and lowering the soil albedo, providing for more absorption of solar radiation (Johnson and Lowery, 1985). Burrows and Larson (1962) found that applying mulch at a rate of 2.25 Mg ha^{-1} lowered soil temperature at 10cm by 0.4ºC. In 2015, there was 1.2 Mg ha^{-1} living kura clover biomass at the time of row suppression, which did not perceptibly insulate the soil.

Corn Emergence and Early Season Development

Corn in ZT plots reached 95% emergence one day faster than corn in ST plots in 2015 and three days faster in 2016 (*P* < 0.05). Since corn emergence and early season development is largely a function of soil temperature and available water (Cutforth et al., 1985; Schneider and Gupta, 1985), the altered seedbed microclimate in ZT plots (Table 4) likely contributed to faster corn emergence and development. The emergence and development of BK corn kept pace with ZT corn in 2015, however in 2016 the BK was consistently behind ZT corn. This could be explained by the 50% greater productivity of kura clover in 2016 than 2015. Previous work on kura clover living mulch for corn production has found that in years when the kura clover is highly productive, glyphosate alone may not fully terminate kura clover (Affeldt et al., 2004). It is likely that the BK treatment was more effective in 2015 than 2016, providing for better conditions for corn emergence. Because of the need for a multi-faceted herbicide strategy to chemically control kura clover, rotary zone tillage may be a better, or at least simpler, strategy for managing perennial living mulches on vulnerable lands for water quality benefits.

Corn Grain and Stover Yield

In 2016, corn grown in ZT treatments produced 4.0 Mg ha^{-1} more grain and 3.5 Mg ha^{-1} more stover than corn grown in ST plots (Table 5). Both years had favorable corn production conditions in southern Minnesota, and no pests or diseases were observed in either year. However, slight leaf curling was observed in corn around the V6 stage in 2016. Cox et al., (1990) found that reduced tillage only impacted ultimate corn yields in seasons with some degree of moisture stress. This is in accordance



with our findings that more intensive rotary zone tillage had no effect on corn yields in a consistently wet year and improved corn yield when mid-season moisture was limiting. Rotary zone tillage appears to be a more reliable strategy for producing grain corn in a kura clover living mulch.

Rutto et al. (2014) observed that for each day corn emergence was delayed, grain yields were reduced by 122 kg ha^{-1}, suggesting that the three-day difference in emergence we observed in 2016 was responsible for a tenth of the yield difference observed. While the faster corn emergence in the RZT almost certainly contributed to the yield difference in 2016, the magnitude of this difference suggests additional factors, such as warmer soil, also contributed to yield gain. It is possible that corn grown in RZT plots developed stronger root systems capable of more effectively competing for soil water during critical periods later in the growing season, i.e.-anthesis.

Conculsion

Our results indicate that ZT is a promising strategy for managing kura clover living mulch in corn systems in the Upper Midwest. Living mulch management requires careful attention, particularly during the early season. During this critical period, corn grown in seedbeds prepared with ZT was consistently ahead of corn grown in seedbeds prepared using ST. As well, ZT led to higher grain yields than ST in 2016, probably due to warmer early season seedbed temperatures and faster emergence. It is likely the additional kura clover biomass exacerbated the negative effects of minimum tillage, delaying emergence and development of corn grown in ST plots. Further research across multiple environments and years, particularly dry years, is needed to fully assess the consistency of ZT and possible applications across the landscape.

Acknowledgements

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Figure Captions

Fig. 1- Corn emergence as a percentage of target population in 2015 (a) and 2016 (b) in a kura clover living mulch, Rosemount, MN. Error bars represent standard error of mean. BK = band kill, ST = shank tillage, and ZT = rotary zone tillage row preparation treatments.

Fig. 2- Mean vegetative stage of corn plants, as determined by number of visible leaf collars in 2015 (a) and 2016 (b) in a kura clover living mulch, Rosemount, MN. Error bars represent standard error of mean. BK = band kill, ST = shank tillage, and ZT = rotary zone tillage row preparation treatments.

Tables

Table 1- Monthly precipitation and mean monthly temperatures for Rosemount, Minnesota. Minneapolis/ St. Paul Airport Global Historical Climatology Network data obtained from the Midwestern Regional Climate Center.

Month	**Normal†**	**2015**	**2016**
Total monthly precipitation, mm			
May	85	90	61
June	108	112	114
Jul.	103	186	129
Aug.	109	76	199
Sept.	78	118	139
Sum	483	582	642
Average monthly temperature, °C			
May	15	15	16
June	20	21	22
Jul.	23	23	24
Aug.	22	22	23
Sept.	17	20	19

† Normal precipitation and temperature are based on 30-yr means.

Table 2- Significance of *F* tests for fixed effects of treatment (Trt), days after planting (DAP), and their interaction on kura clover biomass and percent cover, soil water potential and temperature to a depth of 5cm, corn emergence, corn vegetative stage, stover yield, and grain yield in a kura clover living mulch – grain corn system in Rosemount, MN in 2015 and 2016.

		Kura clover				Soil				Corn			
		Biomass		Percent cover		Water potential		Temperature				Yield	
Year	Source of variation	Mid-season	Post-harvest	Mid-season	Post-harvest	0-17 DAP	0-50 DAP	0-17 DAP	0-50 DAP	Emergence	Stage	Stover	Grain
		--- *P>F* ---											
	Trt	NS†	NS	NS	**	NS	*	NS	NS	***	***	NS	NS
2015	DAP					**	***	NS	***	***	***		
	Trt:DAP					NS	NS	NS	NS	***	***		
	Trt	*	NS	NS	NS	NS	NS	**	***	***	***		
2016	DAP					***	NS	NS	***	***	***	*	*
	Trt:DAP					NS	NS	NS	NS	**	NS		

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level

† NS, nonsignificant.

Table 3- Kura clover biomass and percent cover pre-treatment, mid-season, and post-harvest.

Year	Treatment	Biomass			Percent cover		
		Pre-treatment	Mid-season	Post-harvest	Pre-treatment	Mid-season	Post-harvest
		------------------ kg ha^{-1} ------------------			------------------ % ------------------		
2015	BK	933	258	154	44	49	18 a†
	ST	1140	277	159	46	53	11 b
	ZT	1365	141	138	47	50	15 ab
2016	BK	1801	1131	563	75	90	80
	ST	1866	922	556	81	98	81
	ZT	1660	735	463	74	93	80

BK = band kill, ST = shank tillage, and ZT = rotary zone tillage row preparation treatments.

†Within columns and years, different letters represent different values per Fisher's Protected LSD ($P < 0.05$).

Table 4- Average soil temperature and soil water potential in seedbed (5cm depth) through 17 and 50 days after planting.

Year	Treatment	Temperature		Soil water potential	
		0-17d	0-50d	0-17d	0-50d
		------------ °C ------------		------------ kPa ------------	
2015	BK	13.7	17.3	-21.7	-25.5 b†
	ST	13.6	17.0	-18.7	-22.3 ab
	ZT	13.7	17.2	-19.5	-21.0 a
2016	BK	17.7 b	20.0 b	-64.5	-90.5
	ST	17.8 b	19.7 b	-70.7	-147.9
	ZT	18.5 a	20.6 a	-93.6	-102.5

BK = band kill, ST = shank tillage, and ZT = rotary zone tillage row preparation treatments.

†Within columns and years, different letters represent different values per Fisher's Protected LSD ($P < 0.05$).

Table 5- Yield of corn stover and grain by row preparation treatments in Rosemount, MN.

Year	Treatment	Grain yield‡	Stover yield§
		------------- Mg ha^{-1} -------------	
2015	BK	14.2	8.6
	ST	13.2	7.5
	ZT	13.7	8.7
2016	BK	6.9 b†	5.3 ab
	ST	6.9 b	4.8 b
	ZT	10.9 a	8.3 a

BK = band kill, ST = shank tillage, and ZT = rotary zone tillage row preparation treatments.

†Within columns and years, different letters represent different values per Fisher's Protected LSD (P < 0.05).

‡Corn grain yields are adjusted to moisture content of 155 g kg^{-1}

§Stover yields are reported on a dry matter basis

Figures with captions for convenience

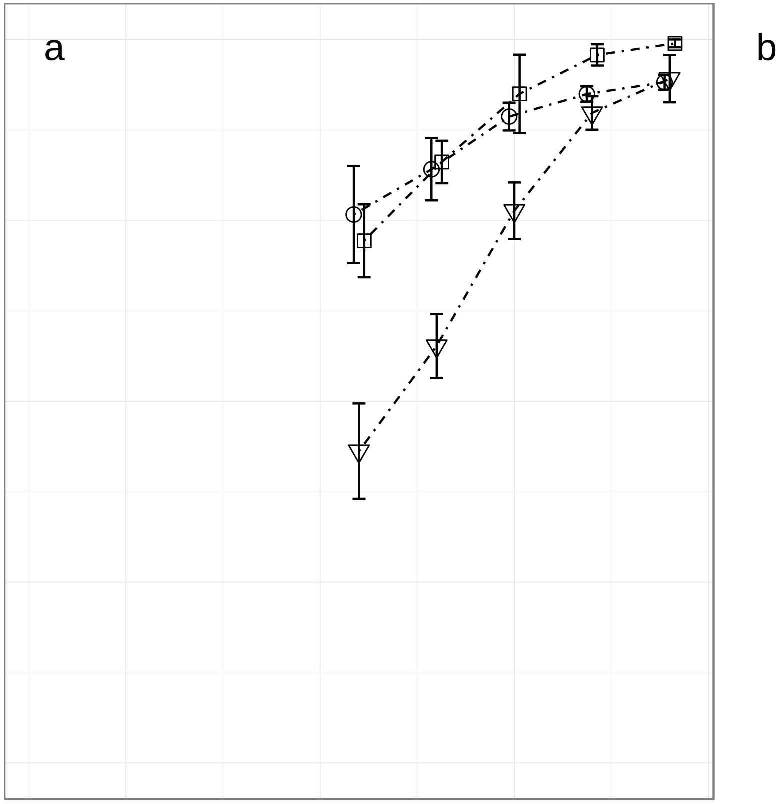
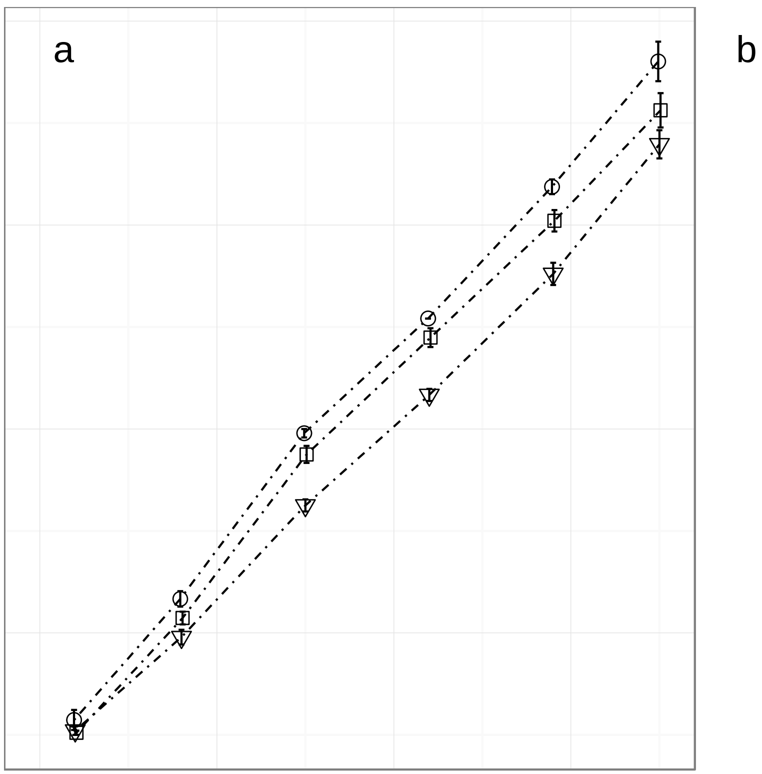


Figure **Captions**

Fig. 1- Corn emergence as a percentage of target population in 2015 (a) and 2016 (b) in a kura clover living mulch, Rosemount, MN. Error bars represent standard error of mean. BK = band kill, ST = shank tillage, and ZT = rotary zone tillage row preparation treatments.



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