

The Impact of Maize Morphology on Performance in

Organic Living Mulch and Effects on Soil Health

by

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A thesis submitted in partial fulfillment of

the requirements for the degree of

Master of Science

(Agroecology and Soil Science)

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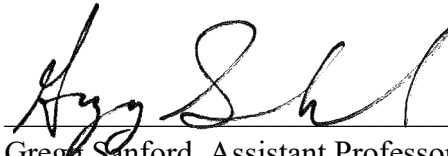
University of Wisconsin—Madison

2026

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DATE: June 10th, 2026

Acknowledgements

I would like to sincerely thank my advisors, Dr. Erin Silva and Dr. Gregg Sanford, for their guidance, support, and encouragement throughout this project and my graduate studies. Their mentorship has challenged me to grow as both a scientist and a thinker, and I am deeply grateful for the time, insight, and patience they invested in me.

I would also like to thank my committee members, Dr. Bill Tracy and Dr. Zac Freedman, for their expertise and support throughout the development of this work. Their perspectives strengthened both this thesis and my growth as a researcher.

A special thank you goes to Ben Brockmueller for his support in the field and willingness to help with the many challenges that arise during field research. I am also incredibly grateful to the undergraduate students who assisted with fieldwork and laboratory work over the course of this project. This research would not have been possible without their hard work and dedication.

I would also like to thank the statistical consultants who generously shared their time and expertise throughout the analysis process. Their guidance helped me navigate complex modeling decisions, grow my statistical skills, and strengthen the rigor of this work.

Finally, I would like to thank my parents and my partner for their constant encouragement, patience, and support throughout this journey. Their belief in me carried me through the most challenging moments and made this accomplishment possible.

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ABSTRACT

Organic farmers often rely on tillage for weed control, but frequent soil disturbance undermines the soil health goals central to organic farming philosophy. Living mulch systems, in which cover crops are maintained between cash crop rows rather than terminated prior to planting, offer a promising reduced-tillage alternative by keeping the soil continuously covered, suppressing weeds, and sustaining biological activity. However, competition from the living mulch has historically reduced yields, limiting farmer adoption despite well-documented ecosystem benefits. This study evaluated agronomic performance and soil health outcomes of organic maize (*Zea mays* L.) grown under a strip-tilled red clover (*Trifolium pratense* L.) living mulch compared to a fully tilled control over two years at Arlington, WI. Two grain maize varieties and one open-pollinated specialty flint variety were grown at three planting densities in a split-split plot design with four field replicates. The living mulch system imposed a significant yield penalty in both variety groups, with larger gaps and higher weed pressure in the drier 2025 season, consistent with intensified competition for soil moisture. In grain maize, morphological traits including leaf area index, leaf angle, and plant height were the strongest predictors of yield within living mulch plots, while in the flint variety, fall clover biomass was the dominant yield predictor and planting density played a more meaningful role. Weed suppression by the living mulch was greatest in the higher-weed-pressure year, with living mulch and control plots converging in fall weed biomass in 2025. Living mulch had a positive impact relative to the tilled control on four of the five soil health indicators evaluated during the 2025 growing season. This included significant accumulation of permanganate oxidizable carbon (POxC) and particulate organic carbon (POC), maintenance of particulate organic matter (POM), and sustained season-long increases in microbial respiration (24-hr C_{min}). These results reveal an environmentally conditioned yield tradeoff alongside

encouraging early gains in soil health and suggest that variety selection and plant morphology are key levers for optimizing organic living mulch system performance.

1. INTRODUCTION

Farmers, the ecosystems they manage, and the landscapes they influence face a host of growing challenges: soil degradation, biodiversity loss, and extreme and unpredictable weather (Yang et al., 2024). Across the globe, soil is being lost 10 to 40 times faster than it is regenerated (Borrelli et al., 2021; Pimentel, 2006; Thaler et al., 2021). Many farming methods, both conventional and organic, contribute to this environmental degradation. For most commodity crops, biomass removal at harvest, coupled with intensive land management, depletes soil organic carbon at a rate that crop residues cannot sufficiently replace (Boniface et al., 2025; Dietz et al., 2024; Yi et al., 2025), while excessive nutrient inputs exacerbate SOC loss (Ju et al., 2025), and compromise critical water resources (Chan et al., 2024; Khan and Mohammad, 2014; Whittinghill et al., 2023). Heavy tillage breaks up soil structure and disrupts the soil microbiome while bare fields leave the soil vulnerable to erosion, reducing agricultural productivity (Kraut-Cohen et al., 2020; Shaffer et al., 1995; Thaler et al., 2021). Meanwhile, widespread monocropping eliminates habitats for pollinators and decreases microbial biodiversity (Aizen et al., 2019; McDaniel et al., 2014). Addressing these grand and interconnected challenges facing food, feed, fuel, and fiber production in the 21st century requires farming systems that center soil health as an intentional goal.

Organic farming is grounded in this philosophy, promoting diversified cropping systems and practices designed to protect and regenerate on-farm resources. Soil health, for example, is a core tenet of organic farming from both a philosophical and regulatory standpoint. USDA National Organic Program practice standards specify that organic cropping systems must ‘maintain or improve the physical, chemical, and biological condition of [the] soil’ (§205.203(a)), and ‘maintain or improve soil organic matter’ (§205.203(c), (d)), through ‘rotations, cover crops, and

the application of plant and animal materials' (§205.203(b)). In practice, however, the widespread reliance on mechanical weed control in organic systems remains a major challenge to fully actualize these aspirational soil health goals. While tillage is an effective weed control strategy, it is also extremely disruptive to soil physical, chemical, and biological processes, undermining aggregate stability and weakening soil structure, enhancing oxidative enzyme activity, disrupting the soil microbiome, particularly fungal communities, leaving the soil vulnerable to erosion, and depleting organic carbon stocks (Dietz et al., 2024; Judd et al., 2025; Kraut-Cohen et al., 2020; Rui et al., 2022; Shaffer et al., 1995) .

Farmers are increasingly adopting management practices aimed at conserving soil and environmental quality, particularly in organic farming circles (Carlisle, 2016). Reduced-till management, when paired with cover-cropping, has become a promising avenue for meeting soil health goals (Farmaha et al., 2022; Yan and Arthur, 2025). “Living mulch” systems are one example of synergistically combining reduced tillage with cover crops in an effort to maintain or boost crop production while enhancing soil health (Hill et al., 2021). While cover crops are typically terminated and incorporated into the soil via tillage prior to planting a cash crop, living mulches are left to grow alongside the cash crop throughout the season. This is often accomplished by strip-tilling the living mulch to accommodate cash crop planting, then mowing semi-regularly until canopy closure of the cash crop to reduce competition between the living mulch and cash crop (Brockmueller et al., 2025; Dobbratz et al., 2019; Wiggans et al., 2012). This method keeps the soil covered continuously by maintaining living vegetation between the rows of the cash crop, with the added benefits of outcompeting and suppressing weeds; hence the term “mulch” (Llnicki and Enache, 1992; Pfeiffer et al., 2016; Westbrook et al., 2022). In organic systems, where herbicides are not permitted, this combination of benefits is particularly desirable.

The soil health benefits of reduced-tillage and cover cropping are well documented. Maintaining continuous soil cover reduces runoff and erosion by improving aggregate stability and protecting the soil surface from wind and rain (Siller, 2016; Baker, 2022). Beyond protection from erosion, the continuous presence of living roots, ground cover, and organic matter inputs fosters the formation of soil aggregates, and enhances porosity, which in turn improves infiltration and water holding capacity (Hill et al., 2021; Wiggans et al., 2012). These improvements in soil structure enhance system resilience to drought and other extreme weather events driven by global climate change (Bybee-Finley et al., 2024; Pretty, 2018; Sanford et al., 2021). Living mulches also absorb excess nutrients, particularly HPO_4^{2-} , H_2PO_4^- , and NO_3^- , that might otherwise runoff or leach to water systems, threatening human health (e.g., NO_3^- linked to cancer and methemoglobinemia) and water quality due to eutrophication of freshwater (primarily P) and marine (primary N) ecosystems (Andrews et al., 2020; Essien et al., 2022; Ochsner et al., 2010). Living roots support a diverse, active soil microbiome via root exudates and symbiotic associations, as demonstrated by increased microbial respiration and community diversity (Elhawat et al., 2024; Li et al., 2023; Sui et al., 2025). Aboveground, the additional biodiversity creates a habitat for pollinators, insects, and animals (Tamburini et al., 2020). Particularly useful in pesticide-free organic systems, increased biodiversity can improve pest and disease resistance (Dainese et al., 2019; Hartwig and Ammon, 2002; Peralta et al., 2018). A meta-analysis from Huang et al. (2026) found that plant mixtures reduce pathogen abundance and damage by roughly 31 %. Living mulches also have the potential to influence nutrient cycling; increasing soil organic matter through root exudates and decomposing biomass improves cation exchange capacity, and subsequently plant nutrient availability (Hill et al., 2021). What's more, leguminous living mulches (*Fabaceae*) fix atmospheric nitrogen through a symbiotic association with nitrogen-fixing

soil bacteria (e.g., *Bradyrhizobium spp.*, *Rhizobium spp.*) providing nitrogen to cash crops in current and future seasons via fine root turnover as well as above- and belowground biomass decomposition (Bhaskar et al., 2021; Leoni et al., 2024).

Despite these extensive benefits, living mulch systems have not been widely adopted by farmers because competition between the living mulch and cash crop typically results in yield loss, regardless of organic or conventional management (Andrews et al., 2018; Bartel et al., 2017; Brockmueller et al., 2025; Martin et al., 1999; Zemenchik et al., 2000). Yield reductions are most frequently attributed to competition between the living mulch and cash-crop for water, light, and nutrients, as well as delayed cash crop emergence, which can reduce crop yield potential (Andrews et al., 2018; Dobbratz et al., 2019; Sanders et al., 2018). Standard cover cropping systems, in which the cover is terminated via tillage prior to cash crop planting and high-density, high-quality biomass is achieved, provide many of the soil health benefits discussed above, with little to no yield loss, and in some cases, even yield increases (Juneja et al., 2026; Marcillo and Miguez, 2017). However, because living mulches persist in the field for much longer and are coupled with minimal tillage, they are theoretically capable of providing substantially greater soil health benefits than classic cover cropping systems. Understanding and managing the tradeoffs in ecosystem service of these systems (e.g., yield, soil health) is therefore central to the broader goal of reducing tillage in organic grain production via living mulch systems.

Managing living mulches in a way that limits competition with cash crop establishment and growth is critical if the practice is to achieve widespread adoption (Bhaskar et al., 2021). Conventional living mulch suppression strategies include use of synthetic herbicides like glyphosate, which, while effective, are not viable options in an organic system (McCauley, 2022). In organic maize (*Zea mays* L.) cropping systems, strip tillage and inter-row mowing have shown

some effectiveness at terminating the living mulch within a row prior to planting (strip till) and reducing above-ground growth between row (mowing), subsequently reducing vigor and competition with the cash crop (Brockmueller et al., 2025; Dobbratz et al., 2019). Beyond suppression strategies, Bhaskar et al. (2021) highlighted the importance of additional systems-level decisions such as selection of living mulch species, planting density of the cash crop, and exogenous nutrient inputs. Perennials and self-seeding annual varieties often provide better weed suppression as living mulches than annual species, resulting in higher cash crop yields (Bhaskar et al., 2021; Leoni et al., 2024). However, no practice or combination of practices has yet proven to completely reduce the risk of yield loss.

Bartel et al. (2017) emphasized the need for compatibility between maize variety and living mulch management in a conventional system, but little research has been done to investigate these complex competition dynamics in both commercial and specialty organic maize systems. Although plant architecture has not previously been analyzed in the context of living mulch, inferences about competitive traits can be drawn from evolutionary theory and integrated weed management research. Traits associated with weed competitiveness include plant height and canopy architecture measurements such as leaf width, leaf angle, and leaf area index (LAI), although the impacts of individual traits can be challenging to isolate and the combined effect of multiple traits may be more easily evaluated for practical use (Andrew et al., 2015; Boydston and Williams, 2016; Naruhn et al., 2025). Weiner et al. (2010) has also suggested that higher planting densities can provide a competitive advantage by creating an “offensive” shading strategy, limiting the access of weeds and living mulch to light, an interpretation corroborated by the findings of Mhlanga et al. (2016) and Youngerman et al. (2018).

Here, we evaluate the agronomic performance and soil health outcomes of Midwestern organic maize production under two contrasting management scenarios—one integrating red clover (*Trifolium pratense* L.) as a terminated green manure cover crop prior to planting corn into bare soil and subsequent cultivation for weed management (e.g., “standard organic management”), and a reduced-till system in which red clover serves as a living mulch—with the goal of optimizing living mulch system configuration as it relates to maize morphology and planting density. This research aims to identify the combination of morphological traits in maize and crop planting densities that contribute to reduced weed pressure and positive crop performance, providing best management guidelines for organic farmers and informing future research directions to select for maize traits that work well in organic, agroecological systems. Three maize varieties (two commercial hybrids and one open-pollinated flint), each with distinct morphological characteristics (e.g., height, leaf angle), were grown at three population densities in both cover crop and living mulch systems over two growing seasons (2024 and 2025) in south-central Wisconsin. Indicators of agronomic performance included the assessment of maize yield, morphology, and leaf area index, as well as living mulch and weed biomass. The soil health implications of organic living mulch systems were evaluated based on physical, biological, and chemical indices. These indices—aggregate stability, particulate organic matter (POM), POM carbon (POC), microbial respiration (24-hr C_{min}), and permanganate-oxidizable carbon (POxC)—were selected for their responsiveness to agricultural management, widespread use in soil health assessments, and biophysical relevance to the functioning of healthy agroecosystems (Cates et al., 2016; Culman et al., 2012; Nunes et al., 2020).

Based on previous work in living mulch systems, we hypothesized that: 1) weed pressure would be equivalent between living mulch systems and cover crop systems due to the weed

suppression effect of living mulch; 2) yields would be highest in medium density maize plantings as a result of reduced red clover competition balanced with reduced competition between maize plants; and that 3) traits that contribute to a denser maize canopy such as broader leaves, wide leaf angles, and tall plant height would correlate with greater yields and lower beneath-canopy biomass due to enhanced light interception of maize at the expense of the living mulch understory. From a soil health perspective we hypothesized that: 1) soil aggregate stability would be greatest in the living mulch plots as a result of reduced tillage, greater organic matter inputs, and the presence of living roots compared to the cover cropped systems; 2) microbial respiration would likewise be highest in the living mulch systems as greater belowground inputs (e.g., roots and root exudates) provide energy and other resources for the growth and activity of soil microbial communities, and 3) POM, POC, and POxC would be greatest in the living mulch systems as living biomass adds high-quality (low C:N) organic material to the soil via root exudates, root turnover, leaf litter, and subsequent elevated microbial biomass.

2. METHODS

2.1 Site Overview

This work was conducted at the University of Wisconsin-Madison's Arlington Agricultural Research Station (N695 Hopkins Rd., Arlington, WI 53911) on USDA certified organic land over 2024 and 2025. Fields for the two growing seasons were adjacent to one another with identical edaphic conditions. Soils at the site are classified as Plano Silt loam (fine-silty, mixed, superactive, Mesic Typic argiudolls). Mean annual temperature and precipitation normals between 1991 and 2020 were 7.8 °C and 902 mm, respectively (NOAA, 2023).

2.2 Experimental Design

A randomized complete block with a split-split plot design was employed to evaluate maize performance and soil health in living mulch systems. Two cover treatments, living mulch (LIV) and a control (CON) were randomly assigned to plots in each of four blocks. The CON treatment consisted of a tilled red clover cover crop and standard organic mechanical weed management. Six sub-plots were then randomly assigned within each plot to accommodate a factorial array of two maize varieties, and three planting densities. Each subplot was 4.6 m (6 maize rows at 76 cm) by 22.9 m long.

In the late summer preceding each field season, all fields were planted with a red clover cover crop. Red clover was selected as an ideal cover crop candidate for living mulch systems due to its short-lived perennial characteristics, and its ability to fix nitrogen (Cougnon et al., 2022). In the CON treatment, clover was fully terminated and incorporated via tillage the following spring, approximately 20-40 days before planting maize. In the LIV treatment plots, clover was strip-tilled approximately 20-40 days before planting maize at 76 cm spacing to create 25 cm-wide bare planting strips, leaving 51 cm of inter-row clover to persist as a living mulch.

The experiment design outlined above was applied to both grain maize varieties (GRM) and specialty maize varieties (SPM). The GRM and SPM varieties were evaluated separately to accommodate significant genetic, phenotypic, and morphological differences and to ensure appropriate fertility and planting density for each of these two maize groupings. Selection of maize variety was based on plant height and canopy structure, which were hypothesized to contribute to positive performance in living mulch systems given the competitive advantage they confer to the maize relative to understory growth (weeds and living mulch) (Boydston and Williams, 2016;

Cougnon et al., 2022; Mhlanga et al., 2016). Planting density gradients were based upon the typical planting density for the variety group and scaled by a factor of 12,355 seeds ha⁻¹.

Grain Maize Varieties (GRM). In both years, the Albert Lea (1414 W Main St, Albert Lea, MN 56007) Blue River organic varieties 44-88 and 58-85 were planted. The planting densities from low to high were 74, 86, and 99 thousand seeds ha⁻¹.

Specialty Maize Varieties (SPM). Two specialty maize varieties were planted in both 2024 and 2025. However, one of the varieties was replaced after 2024 due significant lodging. As a result, only a single polenta variety (Caribbean flint) was present in the trial in both growing seasons. Results for the SPM trial are therefore limited to the Caribbean flint. Results for the additional single-season SPM varieties can be found in the Appendix. The planting densities from low to high were 49, 62, and 74 thousand seeds ha⁻¹.

2.3 Field Operations

Operation	2024 Season	2025 Season
Red clover seeding	September 5, 2023	August 29, 2024
Strip tillage	April 30	May 9
	June 11	May 29
Tillage	May 1	May 12
	May 15	May 19
	June 10	May 29
	June 12	
Manure application	May 15	May 7
Planting	June 12	May 29
Starter fertilizer	June 12	May 29
Interrow mowing	June 17	June 10
	June 27	June 20
	July 9	June 30

Tine weeding	June 18	June 2
	June 20	June 6
Rotary hoe (CON only)	June 26	
Row cultivation (CON only)	July 1	June 21
	July 9	June 29
Harvest	November 17-18	November 6-7 (GRM)
		November 12 (SPM)

Table 1. *Field operations across all experiments in the 2024 and 2025 growing seasons. Planting was delayed in the 2024 season due to heavy spring rains.*

Equipment Type	Model	Manufacturer	Manufacturer Location
Grain drill	1590	John Deere	Moline, IL
Row cultivator	725	John Deere	Moline, IL
Rotary hoe	181 MT	Case IH	Racine, WI
Strip tiller	1tRIPr	Orthman	Kalida, OH
Planter	1750	John Deere	Moline, IL
Interrow mower	IRM-X4	R-Tech	Homewood, MB, Canada
Tine weeder	TS470	Treffler	Pöttmes-Echsheim, Germany
Plot combine	SPC20	Almaco	Nevada, IA
Sprayer	STS	Hagie	Clarion, IA

Table 2. *Equipment used for field operations.*

In spring, pelletized poultry manure (Crop Fertility Services, Cokato, MN) with a guaranteed nutrient analysis of 4-3-2 was broadcast at 196.1 kg N ha⁻¹ (3,923 kg manure ha⁻¹) in the GRM field and 84.1 kg N ha⁻¹ (1,681.3 kg manure ha⁻¹) in the SPM field. Shortly after fertilization with poultry manure, LIV plots received a first strip-till pass and CON plots received an initial tillage pass to terminate red clover biomass. CON plots were tilled two to three additional times prior to planting to manage weed pressure and prepare the seedbed, while LIV plots received

a second strip-till pass approximately two to four weeks after the first. Specific dates for all field operations are provided in Table 1 and equipment specifications are provided in Table 2.

Planting followed the second strip-till and final CON tillage passes. In 2024, heavy spring rainfall delayed planting by several weeks relative to the typical target date, while 2025 planting proceeded without significant delay. Pro-Booster starter fertilizer (10-0-0; North Country Organics, Bradford, VT) was applied in-furrow at planting at a rate of 33.6 kg N ha⁻¹ (336.3 kg fertilizer ha⁻¹) in both seasons.

Following planting, all plots were tine-weeded twice within the first two weeks to control small emerging weeds. In LIV plots, inter-row tines were raised to avoid disturbing the living mulch, while in-row tines remained down for weed control in the planted strip. CON plots received additional mechanical weed control via rotary hoeing and row cultivation through early July in 2024; in 2025, CON plots received one additional tine weeding pass followed by two row cultivation passes. Living mulch in LIV plots was mowed to a height of 10 cm three times following planting at weekly to biweekly intervals, through early July. In 2025 only, true armyworm (*Mythimna unipuncta* L.) pressure in late July necessitated a foliar application of Valent DiPel® DF at 2.24 kg ha⁻¹ (187 L solution ha⁻¹). Grain harvest took place in November of each season.

2.4 Agronomic performance

All data was collected from the center 3 m of each plot, or two of the middle four rows, leaving a 1.5 m buffer on each side to avoid edge effects. 3 details the dates of all data collection events across the 2024 and 2025 growing seasons.

Data Collection Event	2024 Season	2025 Season
Biomass	April 30	May 7
	June 11	May 29
	August 29	August 15
SPAD	July 8	June 26
	August 8	July 29
Morphological traits	August 22	August 12
LAI	August 21	August 18

Table 3. *Agronomic data collection event dates in the 2024 and 2025 growing seasons.*

2.4.1 Biomass

Red clover and weed biomass were sampled at three time points each growing season: (i) pre-tillage (spring), (ii) post-second strip till (summer), and (iii) pre-harvest (fall) (Table 3).

For spring sampling, prior to maize planting, two 0.5 m² quadrats were collected per maize grouping (GRM, SPM), management system (CON, LIV), and block (1-4) for a total of 16 samples per time point (2 systems x 2 variety groups x 4 blocks). Quadrats were tossed randomly within each block and then oriented so that the long axis was perpendicular to the crop row. All above-ground biomass was clipped flush with the ground and separated into red clover and weeds and bagged for drying. Samples were dried for one week at 60° C, or until weights had stabilized, and

then weighed to determine the mass of dry biomass. Summer and fall biomass were collected using the same sampling procedure, but at the level of sub-sub-plots ($n= 72$, 4 blocks x 2 systems x 3 maize varieties x 3 planting densities).

2.4.2 Relative chlorophyll content (SPAD)

To assess the potential impact of cover crops and living mulch on maize vigor a Spectrum (Aurora, IL) Soil Plant Analysis Development (SPAD) 502 Plus meter was used as a chlorophyll content proxy. SPAD measurements were taken twice each growing season, once at the V5/V6 growth stage for maize, and once at tasseling (VT) (“Corn Growth Stages,” 2020). Readings were taken from twenty consecutive plants in the sampling section described above. At the V5/V6 growth stage, measurements were taken from the midpoint of the uppermost fully-developed leaf, with visible collar, approximately 6 mm from the leaf edge. At VT, measurements were taken from the ear leaf.

2.4.3 Morphological traits

At the time of peak vegetative/early reproductive maturity for maize (~R2 stage), roughly two weeks after pollination, leaf angle, leaf width, and plant height measurements were taken. Measurements were collected from 10 consecutive plants per experimental unit and averaged. Leaf angle and width were measured from the ear leaf using a protractor and ruler. Angle was taken from the collar (ligule) with respect to the stalk and width was taken from the center of the leaf at the widest point. Plant height was measured from the base of the stalk to the base of the tassel using a measuring pole.

2.4.4 Leaf area index

Leaf area index (LAI) was measured using a LI-COR LAI-2200 Plant Canopy Analyzer (Lincoln, NE) approximately two weeks after pollination when weather conditions were optimal: high, thin clouds or uniform overcast conditions. In each plot, samples were taken at six random locations. Each sample consisted of four sensor readings taken diagonally across the row 30 cm above the soil surface to avoid shading from understory clover or weeds. Readings were avoided directly beneath leaves. Measurements were taken starting at the southern edge of each experimental unit and moving north. To account for light scattering due to the change in sun angle across the day, K calibrations were performed after approximately 24 plots.

2.4.5 Grain yield

Grain yield was measured at harvest using a two-row research combine (Table 2). Grain yield was harvested and measured from two of the center four rows in each plot. Grain weight, test weight, and moisture content were recorded, and grain weight values were corrected to 15.5 % grain moisture. Exact plot lengths were measured and used to calculate the final yield values in kilograms of grain per hectare based on area and moisture-corrected grain weight.

2.5 Soil health assessment

All data (agronomic and soil) outlined below was collected from the center 3 m of each plot, or two of the middle four rows, leaving a 1.5 m buffer on each side to avoid edge effects.

2.5.1 Soil sampling

Samples were collected with a 2.5 cm diameter soil probe at three time points over the course of the 2025 growing season: spring (April 24th), summer (July 15th), and fall (October

30th). Spring samples were collected per maize grouping (GRM, SPM) and management system (CON, LIV) and block (1-4) for a total of 16 samples per time point (4 blocks x 2 systems x 2 variety groups). Summer and fall samples were collected at the level of sub-sub-plots (n= 72, 4 blocks x 2 systems x 3 maize varieties x 3 planting densities). Analysis of summer samples was limited to 24-hr carbon mineralization (24-hr Cmin). In each plot, 15 cores were taken to a depth of 15 cm between the maize rows following a ‘zigzag’ pattern. Cores were aggregated to create one sample per plot. Samples were dried at 60° Celsius for three days (Stewart and Wolcott, 2026). Once dry, samples were sieved through a 2 mm sieve. Soils < 2 mm were labeled and set aside for POxC, POM, POC, 24-hr Cmin, and wet aggregate stability.

2.5.2 Permanganate oxidizable carbon

Permanganate oxidizable carbon (POxC) content of spring and fall soil samples was measured using the Ohio State University procedure (POXC Protocol-OSU Soil Procedure for the Determination of Permanganate Oxidizable Carbon, 2019). For each sample, 2.5 grams of soil was weighed into 50 ml reaction tubes and 18 ml of DI water and 1 ml of 0.2 M potassium permanganate solution was added. Each tube was shaken for 2 minutes at 180 oscillations per minute, then permitted to settle for 10 minutes. After settling, 0.5 ml of supernatant was pipetted into dilution tubes for analysis. Sample quantification was conducted using a single cuvette spectrophotometer. Four levels of working standards, DI water blanks, and a soil check were prepared and analyzed for each bunch of samples processed. Absorbance values were converted to moles of permanganate oxidized per kg soil via the following equation:

$$POxC \text{ (mole kg}^{-1}\text{)} = [0.02 \text{ mol L}^{-1} - (a + b \times Abs)] \times \frac{0.02 \text{ L}}{Wt}$$

0.02 mol/L = initial solution concentration

a = intercept of the standard curve (-0.00004)

b = slope of the standard curve (0.0502)

Abs = absorbance of unknown

0.02 L = volume of stock solution reacted

Wt = weight of air-dried soil sample in kg

Three analytical replicates were performed for each sample and averaged to obtain one value per sample in spring and fall.

2.5.3 Particulate organic matter

The particulate organic matter (POM) fraction of spring and fall samples was measured by separating the POM fraction by size using the procedure from Poeplau et al. (2018) and Cotrufo et al. (2019). Samples weighing 5.4-5.6 grams were dispersed by shaking at 180 oscillations per minute for 18 hours with a 0.5 % sodium hexametaphosphate solution, then washed with a wet sieving machine through a 53 μm sieve. POM remaining on top of the sieve was dried at 60° Celsius for three days and weighed, and the resulting POM weight was divided by the total sample weight to obtain the POM mass percent. Three analytical replicates were performed for each sample and averaged to obtain one value per sample in spring and fall.

2.5.4 Particulate organic carbon

The particulate organic carbon (POC) of spring and fall samples was measured by tinning approximately 25 mg of separated POM for each sample and combusting the samples on a Thermo Scientific FlashSmart elemental analyzer. Three analytical replicates were performed for each sample and averaged to obtain one value per sample in spring and fall.

2.5.5 24-hour carbon mineralization

24-hr C_{min} was measured by incubation of spring, summer, and fall samples. For each sample, 10 mg of soil was placed in an aluminum weighing dish and re-wetted to 60 % water-filled pore space (WFPS) (Linn and Doran, 1984). Re-wetted samples were immediately sealed in 1 L mason jars fitted with valves compatible with a LI-COR 820 infrared gas analyzer (IRGA) and left to incubate at room temperature (20° Celsius) (Creamer et al., 2014). After 24 hours, CO_2 concentration in the jar was measured with the IRGA, and concentration values were recorded when readings stabilized. The ambient CO_2 concentration in the jar prior to soil-rewetting was subtracted from the recorded readings, which were corrected for the added volume of the tubing between the jar valves and IRGA. Final results were converted to mg carbon per kg soil using the following formula:

$$C_m (mg\ C\ kg\ soil^{-1}) = \left(\frac{C_v \times M \times P}{R \times T} \right) \times \frac{V}{S} \times 1000$$

C_m = carbon mineralized in mg C kg soil⁻¹

C_v = ppm (volume) CO_2 - ambient CO_2 ppm

M = molecular weight of C (12 $\mu g / \mu mol$)

P = Barometric pressure (1 atm)

R = universal gas constant (0.0820575 L · atm · °K · mole)

T = incubation temp in °K

V = volume of the incubation chamber in L

S = weight of the soil used in the incubation (10 mg)

Three analytical replicates were performed for each sample and averaged to obtain one value per sample in spring, summer, and fall.

2.5.6 Aggregate stability

Aggregate stability was measured via an Eijkelkamp wet sieving apparatus (Eijkelkamp, 2024). Samples were sieved through both a 2 mm and 1 mm sieve, retaining the aggregates resting on top of the 1 mm sieve in order to isolate the 1-2 mm aggregates. For each sample, 4 grams of the 1-2 mm fraction was weighed into a 0.25 mm Eijkelkamp sieve, then pre-wetted with a hand mister and allowed to rest for 10 minutes to prevent premature slaking. Samples were then sieved in DI water for 3 minutes, and the dispersed (or “unstable”) aggregates that passed through the sieve into the water was placed in a 110 °C oven to dry. Samples were then sieved again in a 0.2 % sodium hexametaphosphate solution to disperse the remaining aggregates (the “stable” fraction). The machine was allowed to run until no aggregates remained on the sieves. Stable aggregates were dried overnight at 110 °C. Once dry, samples were weighed and the aggregate stability index was calculated by dividing the stable aggregate weight by the total aggregate weight (stable + unstable).

2.5 Statistical Analysis

All analyses were conducted in RStudio (version 2024.12.0+467). Unless otherwise noted, linear mixed-effects models were fitted using the lme4 package, estimated marginal means (EMMs) and pairwise contrasts were obtained using the emmeans package, and parametric bootstrapping was performed using the boot package. GLS models were fitted using the nlme package. Field replication, or block, was included as a random effect in all mixed-effects models to account for the split-split plot experimental design. In cases where block-level variance was estimated at or near zero, the random effect was nonetheless retained to respect the experimental design structure. Year, management system (LIV vs. CON), maize variety, planting density, and their interactions were included as fixed effects in all mixed-effects models, except for the

exclusion of system in LIV-only models and variety in flint-only models. Year was treated as a main effect in all models due to the low number of experimental years. Models for the GRM varieties (45-88 and 58-85 pooled) and the flint variety were fitted separately to avoid confounding differences in fertility inputs and planting density ranges between variety groups.

2.5.1 Agronomic experiment analysis

Main treatment effects on yield. The primary treatment yield model with yield as the response was fitted per the specifications above. Pairwise contrasts were used to interpret significant interactions. To further characterize the magnitude of yield loss in LIV plots, a percent yield difference was calculated as $(\text{CON} - \text{LIV}) / \text{CON} \times 100$ and modeled using a separate linear mixed-effects model.

Morphological and competition effects on yield. To analyze how morphological traits and biological competition (i.e., clover + weed biomass) influence yield (the response) within LIV plots, four linear mixed-effects model candidates were built and compared via Aikake Information Criterion (AIC): 1) an LAI-only model that excluded density and other morphological traits, given that LAI is a function of planting density and plant morphology; 2) a biological competition model including fall clover biomass and fall weed biomass; 3) a morphological model including leaf angle, plant height, and leaf width; 4) a full model combining all variables from models 1-3. A drop1 test (base R) was used within the full model to evaluate the contribution of each variable to model fit.

Treatment and morphology effects on clover biomass. To evaluate the effect of morphological traits on living mulch biomass in LIV plots, two linear mixed-effects models were fitted with fall clover biomass as the response: 1) a multi-trait model including leaf angle, leaf width, and plant height as covariates; and 2) a LAI-only model excluding density, for the biological

reasons described above. Both models included spring clover biomass as a covariate to account for pre-season establishment differences.

Weed biomass dynamics. Fall weed biomass was log-transformed prior to modeling in all analyses to address right skew and heteroscedasticity. Back-transformed EMMs are reported on the original scale for interpretation. To characterize weed dynamics across treatments, two linear mixed-effects models were fitted: 1) a model with fall weed biomass as the response to compare overall means, and 2) a delta response model with weed biomass change over time as the response, calculated as (fall weed biomass – spring weed biomass).

To assess the weed suppression effect of the clover in LIV plots, two linear mixed-effects models were fitted with weed biomass as the response and clover biomass as the primary predictor: 1) a model comparing summer biomass and 2) a model comparing fall biomass. An additional mixed-effects model was fitted with fall weed biomass as the response and plant height, leaf angle, and leaf width as covariates to examine the influence of measured morphological traits on weed biomass.

2.5.2 Soil health experiment analysis

POxC, POM, POC, aggregate stability. For all metrics, spring values were tested as baseline covariates for ANCOVA by fitting a linear mixed-effects model with spring values as the response. For several metrics, spring values differed significantly between management systems, likely reflecting pre-existing spatial heterogeneity in the field. Where this occurred, the ANCOVA assumption of covariate independence was violated and ANCOVA was not appropriate. Metrics that satisfied the independence assumption proceeded with ANCOVA using linear mixed-effects models with spring values as a covariate and fall values as the response, excluding year as a main effect due to the soil health experiment spanning a single year. Metrics that did not satisfy the

independence assumption proceeded with two alternate approaches: 1) a seasonal model including season (spring vs. fall) as a main effect, allowing within-system seasonal change to be estimated via pairwise contrasts; and 2) a delta score model with the seasonal difference, calculated as (fall – spring), as the response variable. Parametric bootstrapping with 2000 simulations was used to generate confidence intervals and p-values for mean comparisons in all soil health models, to account for the influence of both fixed and random effects on standard error.

24-hr Cmin. 24-hr Cmin was measured at three time points (spring, summer, fall) and the repeated-measures structure of the data required a different analytical approach than the two-timepoint metrics. Cmin data were log-transformed to address right skew and are reported back-transformed as ratios. Two generalized least squares (GLS) models with Cmin as the response and season, management system, and their interaction as fixed effects were compared for each variety group: a homogeneous variance model and a heterogeneous variance model that allowed residual variance to differ across sampling seasons. Model fit was evaluated via AIC and the better-fitting model was used for all inference. Pairwise comparisons were conducted across management systems within seasons and across seasons within management systems. Parametric bootstrapping with 2000 simulations was used to generate confidence intervals and p-values to verify mean comparisons.

3. RESULTS

3.1 Agronomic experiment

3.1.1 Treatment, morphological, and competition effects on yield

Main treatments effects on yield. Maize yields differed significantly between CON and LIV systems ($p < 0.001$) (Figure 1). Across all sub- and sub-sub plot combinations, maize yield was lowest in the LIV treatment, with a reduction of approximately 4.2 Mg ha^{-1} compared to the CON treatment. Yield was also significantly influenced by year ($p = 0.002$), with lower yields of approximately 0.85 Mg ha^{-1} across treatments in 2025 compared to 2024. A significant system:year interaction ($p = 0.023$) indicated that the yield difference between LIV and CON varied across years: 3.6 Mg ha^{-1} in 2024 versus 4.8 Mg ha^{-1} in 2025, a 1.2 Mg ha^{-1} difference. Weak evidence of a system:variety interaction ($p = 0.089$) suggested possible differences in response to management system between varieties, which was supported by a pairwise comparison showing a smaller yield difference between treatments in the 45-88 variety (approximately 3.7 Mg ha^{-1}) compared to the 58-85 (approximately 4.7 Mg ha^{-1}) across densities and years. As main effects, planting density and variety did not significantly influence yield (both $p > 0.3$).

Modeling yield in the flint variety saw significant system:year ($p = 0.002$) and year:density ($p = 0.016$) interactions, suggesting that the effects of management system and planting density on flint yield differed by year. Yield was significantly affected by all factors: primarily management system ($p < 0.0001$), followed by planting density ($p = 0.014$) and year ($p = 0.045$). The average yield difference was 1.1 Mg ha^{-1} between LIV and CON plots and 0.39 Mg ha^{-1} between 2024 and 2025 (Figure 1). Across densities, LIV and CON plots differed by 0.49 Mg ha^{-1} in 2024 and 1.7 Mg ha^{-1} in 2025. In 2024, no significant difference between LIV and CON plots was seen in the

medium ($p = 0.975$) and high ($p = 0.249$) planting densities while in 2025, differences between LIV and CON were significant at all planting densities.

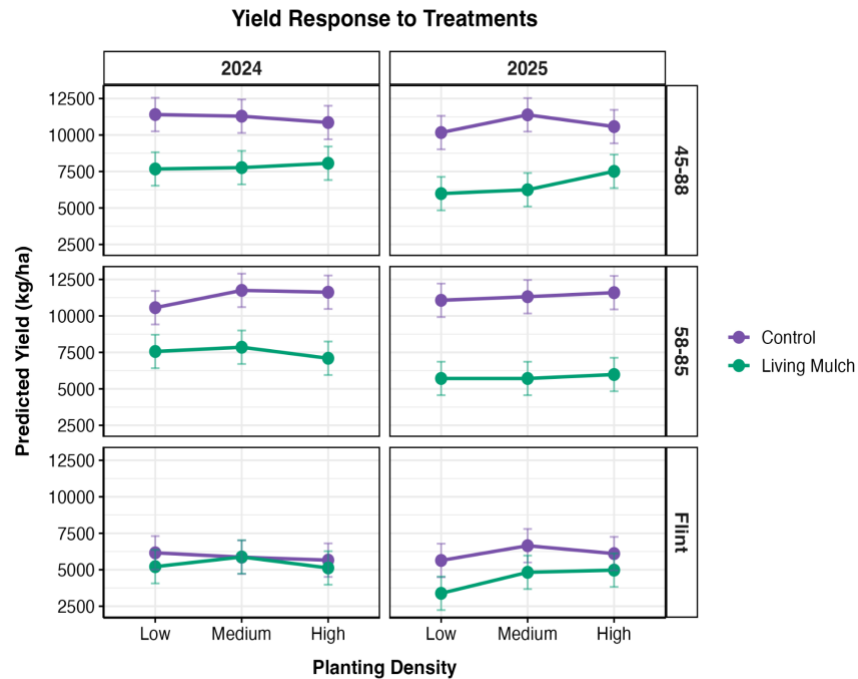


Figure 1. Predicted maize yield (kg ha^{-1}) as a function of planting density, management system, variety, and year. Points represent estimated marginal means from a linear mixed-effects model, and error bars indicate 95 % confidence intervals. Panels are faceted by year (columns) and variety (rows), with planting density levels (74, 86, and 99 thousand seeds ha^{-1} for GRM; 49, 62, and 74 thousand seeds ha^{-1} for flint) on the x-axis.

Living mulch yield penalty. Year had a significant influence on yield penalty in the GRM varieties ($p = 0.011$): differences were larger in 2025 compared to 2024, indicating that environmental conditions affected yields between management systems (Figure 2). Variety had a marginal effect on yield ($p = 0.087$) across years, with generally less pronounced differences between systems in the 45-88 variety. However, pairwise comparisons within density and year combinations were not statistically significant, suggesting that yield differences between varieties

are weak and inconsistent. Planting density did not have a significant impact on yield difference ($p = 0.72$), and no significant interactions were seen.

In the flint variety, year was also the most significant driver of yield difference ($p = 0.008$), while density and the year:density interaction were non-significant (both $p > 0.16$). In 2024, the difference between LIV and CON plot yields was not significant for any density; in 2025, all yield differences were significant except for the high planting density, which was marginally non-significant ($p = 0.055$). No significant differences were seen between any density levels, indicating that while the magnitude of the flint yield gap declined with increasing density in 2025, the trend was not statistically significant (Figure 2).

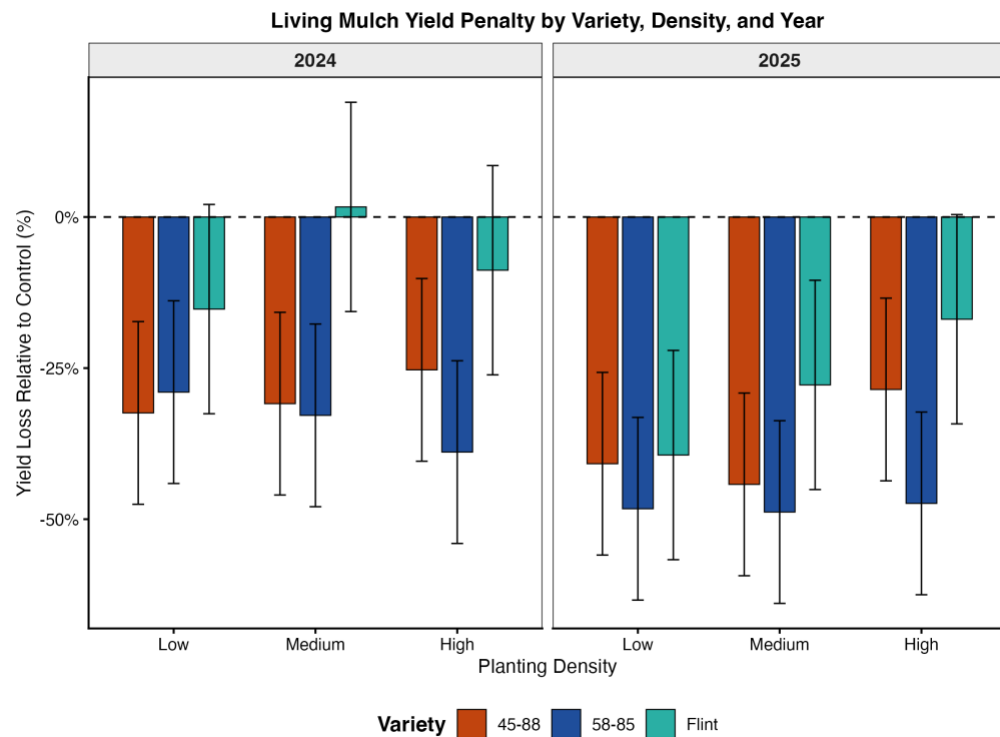


Figure 2. Modeled percent yield loss between management systems (%) as a function of planting density, variety, and year. Error bars indicate 95 % confidence intervals. Panels are faceted by year and planting densities increase across the x-axis. Yield loss magnitude was greater in 2025 than in 2024 across all variety-density combinations. Variety had a marginal effect between GRM varieties. Yield loss across all densities of the flint variety were non-significant in 2024.

Morphological and competitive influence on yield. In the GRM competition model, fall clover biomass was a significant negative predictor of maize yield ($p < 0.001$) while fall weed biomass was not significant ($p = 0.879$), indicating that the clover living mulch was the primary competitor of the maize over the weeds. Year was a significant predictor ($p = 0.014$), while variety, density, and interactions were not. In the flint competition model, fall clover biomass was again a significant negative predictor of yield ($p = 0.022$) and fall weed biomass was not significant ($p = 0.244$). In contrast with the GRM competition model, planting density was significant ($p = 0.010$) while year and interactions were not.

In the GRM morphology model, yield was significantly positively influenced by plant height ($p < 0.001$) and leaf angle ($p = 0.004$), while leaf width was non-significant ($p = 0.262$). Year was significant in this model ($p < 0.001$), indicating that seasonal yield variation was not fully captured by the morphological traits alone. Variety was significant as well ($p = 0.014$), with 45-88 outperforming 58-85, while density and all interactions were non-significant. In the flint morphology model, plant height was the only significant morphological predictor ($p = 0.006$), with taller plants associated with greater yield, along with a marginally significant year:density interaction ($p = 0.053$). Leaf angle and width were not significant ($p = 0.151$ and $p = 0.283$) while planting density was independently significant ($p = 0.034$). Combined with the non-significance of LAI in the full model and only marginal significance in the LAI-only model, these results suggest that the measured morphological traits influence flint yield only slightly (Figure 3).

In the GRM LAI model, LAI was a significant positive predictor of yield ($p < 0.001$), with increasing LAI corresponding to increasing yield (approximately 1.1 Mg ha^{-1} per LAI unit). Year and variety were not significant ($p = 0.728$ and $p = 0.165$), suggesting that between-season and between-variety yield difference was mostly explained after accounting for canopy light

interception. In the flint LAI model, LAI was only a marginally significant positive predictor of yield ($p = 0.069$) and year was not significant ($p = 0.293$) (Figure 3).

In the full GRM model, the most significant factors included LAI ($p < 0.001$) and leaf angle ($p < 0.001$), with plant height ($p = 0.045$) and leaf width ($p = 0.024$) also significant, while fall clover biomass was marginally significant ($p = 0.063$) and fall weed biomass was not. Variety remained significant ($p = 0.004$) alongside year ($p = 0.011$), and density and all interactions were non-significant. In the full flint model, density was significant ($p = 0.007$) and fall clover biomass was the most significant covariate predictor of yield ($p = 0.010$), while LAI, leaf angle, plant height, leaf width, fall weed biomass, and year were all non-significant.

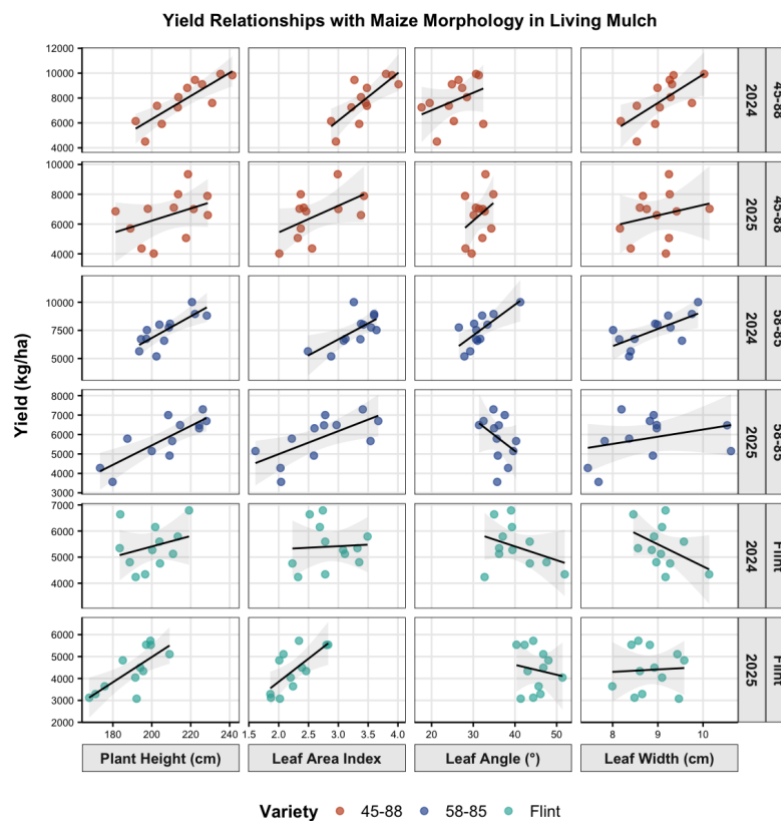


Figure 3. Relationships between morphological traits and maize yield. Scatterplots show observed relationships between yield and four traits (leaf angle, leaf area index, leaf width, and plant height), with linear regression lines ($\pm 95\%$ confidence intervals). Points are colored by maize

variety. Regression lines with wide confidence intervals reflect the limited within-group sample size and indicate directionality rather than effect magnitudes.

In the GRM varieties, model comparison by AIC revealed that the morphological model provided a substantially better fit than the competition model ($\Delta\text{AIC} = 26.0$) and the full model was significantly better than the morphological model ($\Delta\text{AIC} = 9.3$). The LAI-only model outperformed the competition model as well ($\Delta\text{AIC} = 22.0$), indicating that canopy light interception and plant morphology influence yield more than biological competition in the GRM plots. For the flint variety, model comparison by AIC showed that the competition and morphology models performed similarly ($\text{AIC} = 391.1$ and 390.2 respectively). The full model showed a significant improvement over the competition model and only a marginal improvement over the architecture model ($\Delta\text{AIC} = 3.0$). The LAI model provided the poorest fit ($\text{AIC} = 399.2$), a notable contrast with the GRM results.

Variety	System	Year	Height (cm)	Leaf Angle (°)	Leaf Width (cm)	LAI
			<i>Mean $\pm$ SD</i>	<i>Mean $\pm$ SD</i>	<i>Mean $\pm$ SD</i>	<i>Mean $\pm$ SD</i>
45-88	LIV	2024	216.43 $\pm$ 15.54	25.78 $\pm$ 4.66	9.1 $\pm$ 0.52	3.43 $\pm$ 0.34
45-88	LIV	2025	208.66 $\pm$ 15.6	31.43 $\pm$ 2.2	9 $\pm$ 0.53	2.64 $\pm$ 0.45
45-88	CON	2024	258.98 $\pm$ 6.4	29.33 $\pm$ 5.31	10.56 $\pm$ 0.31	4.72 $\pm$ 0.32
45-88	CON	2025	275.24 $\pm$ 8.53	33.31 $\pm$ 3.37	10.11 $\pm$ 0.54	4.67 $\pm$ 0.47
58-85	LIV	2024	207.06 $\pm$ 11.42	31.63 $\pm$ 3.77	8.92 $\pm$ 0.64	3.28 $\pm$ 0.34
58-85	LIV	2025	207.19 $\pm$ 18.57	36.07 $\pm$ 2.62	8.77 $\pm$ 1	2.68 $\pm$ 0.64
58-85	CON	2024	247.24 $\pm$ 11.32	37.58 $\pm$ 3.47	10.01 $\pm$ 0.39	4.45 $\pm$ 0.41
58-85	CON	2025	266.15 $\pm$ 7.29	35.33 $\pm$ 3.6	9.49 $\pm$ 0.55	4.65 $\pm$ 0.4

flint	LIV	2024	199.82 ± 11.46	40.13 ± 5.58	9.1 ± 0.44	2.87 ± 0.41
flint	LIV	2025	189.84 ± 12.53	45.05 ± 3.08	8.84 ± 0.48	2.26 ± 0.32
flint	CON	2024	215.44 ± 10.38	45.75 ± 5.32	10.1 ± 0.62	3.52 ± 0.42
flint	CON	2025	259.81 ± 6.8	37.85 ± 3.12	9.77 ± 0.32	3.64 ± 0.36

Table 3. Mean values ± SD of height (cm), leaf angle (°), leaf width (cm), and LAI for each maize variety, management system, and year.

3.1.2 Morphological effects on competition

In the GRM multi-trait model, plant height was the dominant morphological predictor of fall clover biomass ($p < 0.001$), with taller maize associated with lower clover biomass ($-21.89 \text{ kg ha}^{-1}$ per cm height). While variety alone had no significant individual influence along with density, the year:variety interaction was significant ($p = 0.006$): from 2024 to 2025, the 45-88 saw no change in clover biomass ($p = 0.935$), while the 58-85 had 515.5 kg ha^{-1} more clover biomass in 2025. Leaf angle, width and spring clover biomass were not significant predictors ($p = 0.595$; $p = 0.386$; $p = 0.236$). In the LAI-only model, LAI was a strong negative predictor of clover biomass ($p < 0.001$; $-541.47 \text{ kg ha}^{-1}$ per unit LAI), indicating that a denser canopy aligned with lower clover biomass. The year:variety interaction remained significant ($p = 0.022$), although year and variety were not significant individually. The heatmap of plant and LAI values illustrates this relationship, with high clover biomass values concentrated in plots with low LAI and shorter maize stature (Figure 4).

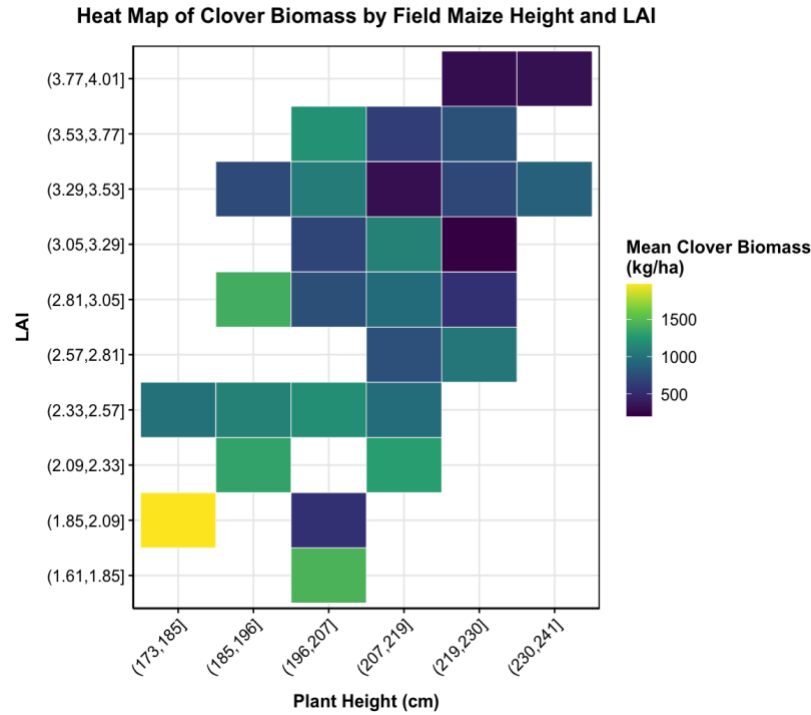


Figure 4. Mean fall clover biomass (kg ha^{-1}) by GRM plant height and leaf area index (LAI). Each cell represents the mean clover biomass for plots falling within the corresponding height and LAI bins. Color scale ranges from low (purple) to high (yellow) biomass.

In the flint multi-trait model, flint plant height showed a marginal negative correlation with clover biomass ($p = 0.084$) while leaf angle and width were not significant ($p = 0.930$ and $p = 0.377$). Density and its interaction with year were both not significant, although year was independently a significant predictor ($p = 0.004$) with greater clover biomass in 2025. In the LAI-only model, both year and LAI were significant predictors of clover biomass ($p = 0.007$ and $p = 0.005$), with higher LAI values associated with lower clover biomass. The heatmap of height and LAI values reflects this pattern (Figure 5).

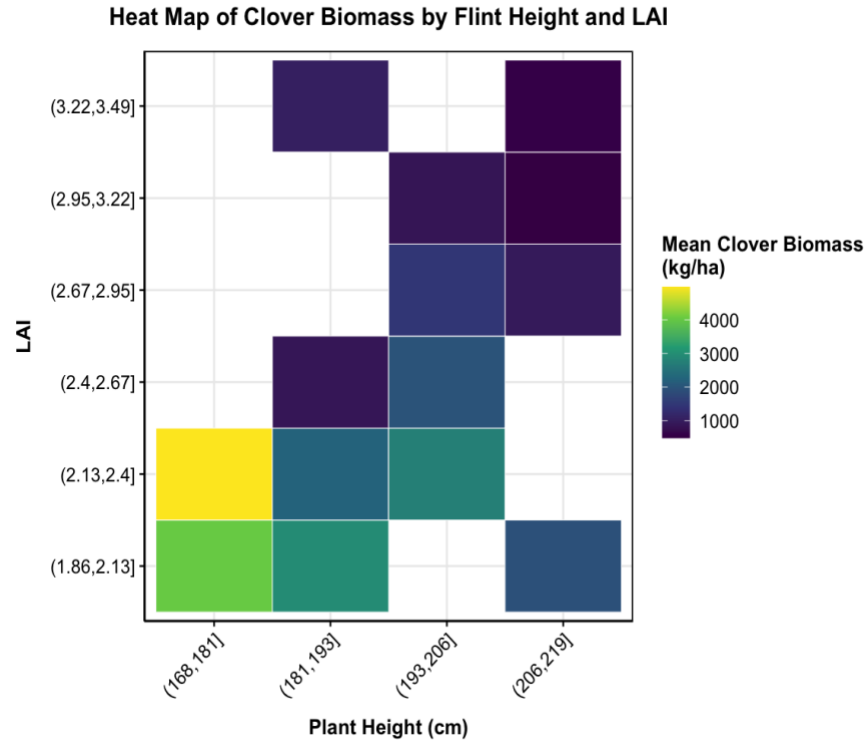


Figure 5. Mean fall clover biomass (kg ha^{-1}) by flint plant height and leaf area index (LAI). Each cell represents the mean clover biomass for plots falling within the corresponding height and LAI bins. Color scale ranges from low (purple) to high (yellow) biomass.

3.1.4 Weed suppression via living mulch

In the GRM varieties, a significant interaction between system and year ($p = 0.003$) indicated that differences in weed biomass between management systems were not consistent across years, with 148 kg ha^{-1} more weed biomass in LIV than CON plots in 2024 and 79 kg ha^{-1} more weeds in LIV plots in 2025 (Figure 6). Weed biomass was significantly influenced by management system ($p = 0.0001$) and year ($p < 0.0001$), with more weed biomass seen in LIV than CON treatments and more weed biomass seen in 2025 than in 2024. Variety ($p = 0.604$) and density ($p = 0.169$) had no significant effect. The 2024 difference was significant ($p = 0.012$), while the 2025 difference was not ($p = 0.538$). Comparing between years within treatments, CON plots saw

a significant difference of 229 kg ha⁻¹ across years ($p = 0.008$), while LIV plots did not see a significant difference between years (159 kg ha⁻¹, $p = 0.138$).

In the flint model, a three-way system:density:year interaction ($p = 0.050$) was seen, along with significant system:density ($p = 0.009$) and system:year ($p = 0.038$) interactions, suggesting that weed biomass varied in different ways across years by both system and density. Weed biomass was significantly influenced by management system ($p = 0.008$), density ($p = 0.006$), and year ($p = 0.001$). LIV plots had significantly greater weed biomass than CON plots in 2024 (370 kg ha⁻¹, $p = 0.023$), but the LIV versus CON difference was not significant in 2025 ($p = 0.642$). Comparing between years within tillage systems, CON plots saw a significant increase in weed biomass from 2024 to 2025 (472 kg ha⁻¹, $p = 0.017$) while LIV plots did not change significantly across years (241 kg ha⁻¹, $p = 0.387$). Adding density to the picture, no significant difference in weed biomass was seen between systems in any year-density combination except the high planting density in 2024 (-565 kg ha⁻¹, $p = 0.089$) (Figure 6).

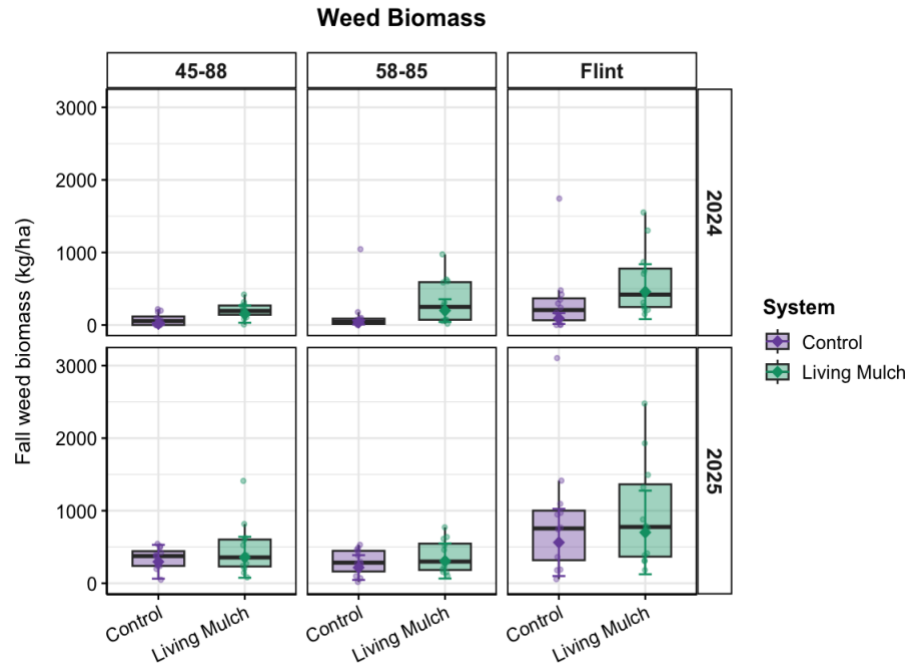


Figure 6. Fall weed biomass (kg ha^{-1}) by management system across 2024 and 2025. Boxplots show the distribution of observed values, while points and error bars represent model-estimated means $\pm$ 95 % confidence intervals (back-transformed from log scale). Weed biomass was greater in LIV than CON systems in 2024, but this difference closed and was no longer significant in 2025. Weed biomass increased significantly from 2024 to 2025 in CON systems, which was reflected in a significant system:year interaction. These results demonstrate a convergence of weed biomass between systems when weed pressure is higher.

In the GRM weed biomass delta response model, management system significantly influenced weed biomass change ($p = 0.002$) as well as year ($p = 0.012$), and a significant interaction was seen between year and management system ($p < 0.001$) (Figure 7). In 2024, no significant difference in weed biomass change was shown between LIV and CON plots ($p = 0.238$), indicating similar weed change dynamics that year. Consistent with the year:system interaction, the comparisons were different in 2025, with negative weed biomass change in LIV plots compared to positive in CON plots ($p < 0.0001$). ANCOVA models comparing spring, summer,

and fall weed biomass demonstrated that spring and summer weeds did not influence weed biomass in the fall (both $p > 0.2$).

In the flint variety, no significant main effects or interactions were seen, although density ($p = 0.086$) and the system:year interaction ($p = 0.098$) showed marginal significance. Within-year comparisons showed no significant difference in weed biomass change between LIV and CON in either year. Comparisons within tillage systems showed that CON plots saw a significant increase in weed biomass change from 2024 to 2025 ($p = 0.031$) while LIV plots did not ($p = 0.877$). The directionality of the flint results are consistent with the GRM experiment results; although effects were weaker and did not reach significance, the LIV flint plots saw a smaller mean weed biomass increase in 2025 than CON flint plots (Figure 7).

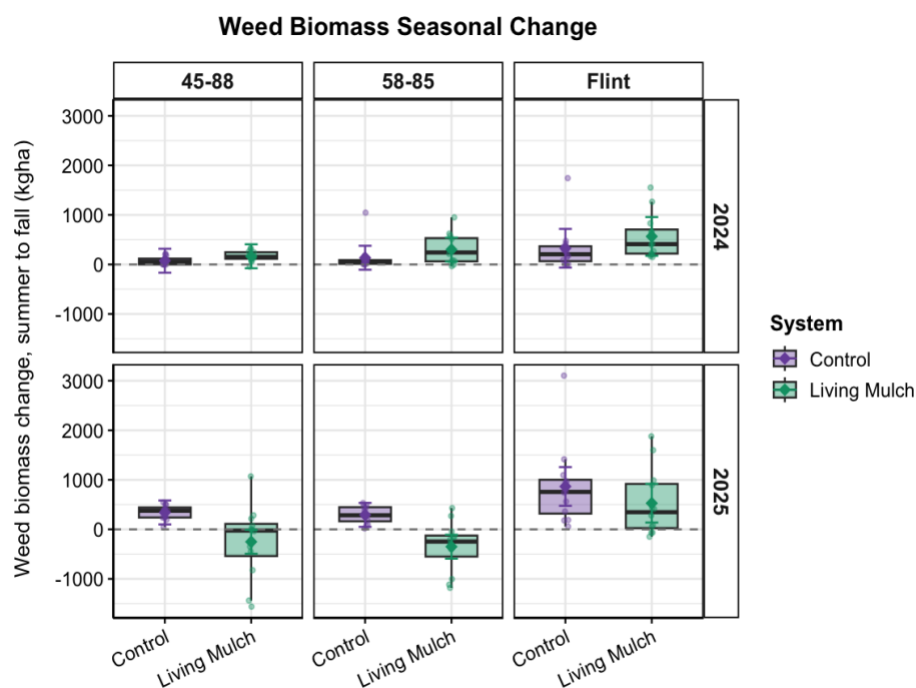


Figure 7. Seasonal change in weed biomass (fall – summer; kg ha⁻¹) by management system (CON, purple; LIV, green) in 2024 and 2025. Boxes and points show raw data; diamonds and error bars show model-estimated means $\pm$ 95 % CI. Change in weed biomass between management systems

varied across years; weed biomass increased slightly in both management systems in 2024, while weed biomass increased under CON but declined under LIV in 2025.

In the GRM varieties, fall clover biomass was negatively associated with weed biomass ($p = 0.045$). A significant negative interaction between 58-85 and a high planting density was seen ($p = 0.036$), but the overall variety effect was not significant in either model (both $p > 0.7$). Planting density and all main model interactions did not significantly influence fall weed biomass in either model. Year also significantly influenced fall weed biomass ($p < 0.02$), with substantially higher weed pressure in 2025 than in 2024. Model summaries demonstrated that the 58-85 variety was associated with significantly greater fall weed biomass than the 45-88 variety and low and medium planting densities ($p = 0.041$). For the flint variety, the fall model found that fall clover biomass was not a significant predictor of weed biomass ($p = 0.828$) (Figure 8). In contrast, summer clover was a significant negative predictor of summer weed biomass ($p < 0.001$), with year also strongly significant ($p < 0.001$).

Comparing morphological traits to weed biomass in GRM varieties, leaf angle and plant height both showed marginal negative associations with fall weed biomass (leaf angle: $p = 0.096$; plant height: $p = 0.083$). Leaf width, variety, density, and all interactions were not significant. In the flint variety, no morphological traits were significant predictors (all $p > 0.10$).

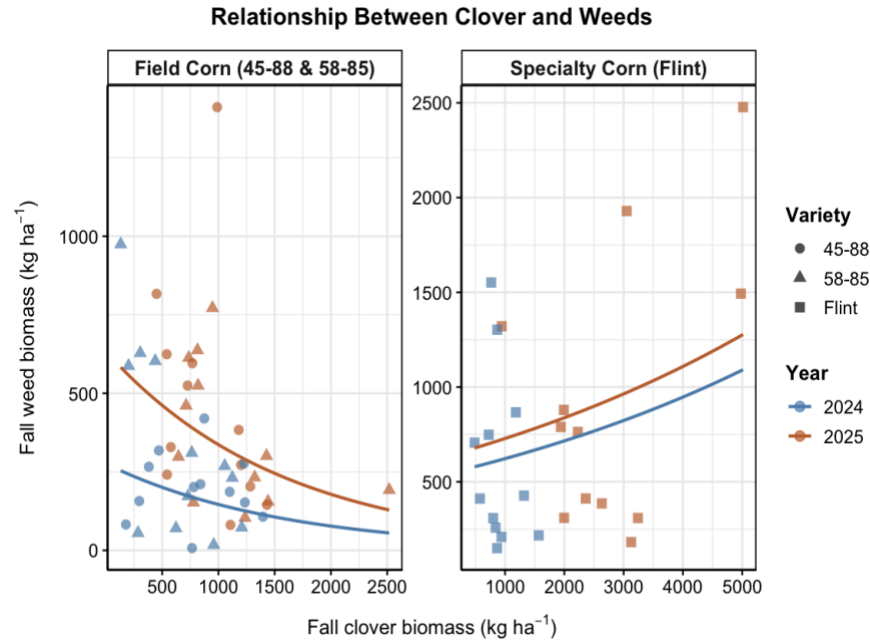


Figure 8. Relationship between fall clover biomass and fall weed biomass in LIV plots in 2024 (blue) and 2025 (red). Lines represent population-level model predictions from a linear mixed model; shapes represent variety (45-88: circle; 58-85: triangle; flint: square). Weed biomass was $\log(x+1)$ -transformed for model fitting; predictions are back-transformed to the original scale.

3.2 Soil health experiment

3.2.1 Permanganate oxidizable carbon

Testing spring POxC for ANCOVA suitability revealed that baseline spring POxC differed significantly between management systems before tillage ($p = 0.006$), with substantially higher POxC in CON plots than in LIV plots. Because ANCOVA assumptions were violated, we proceeded with the alternate modeling methods.

The first model revealed a significant interaction between management system and season ($p = 0.004$), suggesting that POxC changed differently across the growing season depending on management system. Both CON ($p = 0.009$) and LIV ($p < 0.0001$) plots saw a significant increase in POxC from spring to fall, but the increase was considerably larger in LIV plots (Figure 9). The

delta score analysis corroborated these results: LIV plots accumulated significantly more POxC across the season than CON plots ($p < 0.001$), with LIV plots accumulating 0.01129 moles kg^{-1} from spring to fall and CON plots accumulating 0.00436 moles kg^{-1} . Variety and planting density did not significantly influence POxC in any model (both $p > 0.69$). Bootstrapping confirmed these results, with a bootstrap mean difference of 0.00697 moles kg^{-1} (SD = 0.00186) and 95 % confidence interval of 0.00329 to 0.01053 (bootstrap $p < 0.001$). These results indicate that LIV plots increase POxC at a higher rate than CON plots, despite starting with less POxC than CON plots in the spring.

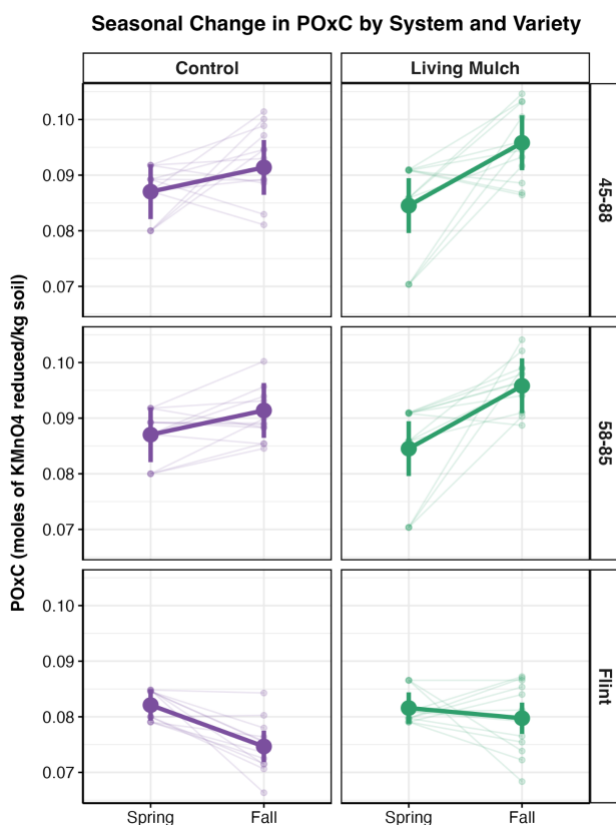


Figure 9. Seasonal change in POxC (reported as moles of permanganate reduced per kg soil) in CON and LIV plots in the flint and GRM varieties. Faded lines connect spring and fall observations for individual subplots and bold lines and points show model-estimated marginal means $\pm$ 95 % confidence intervals. In the GRM varieties, both treatments saw significant increases in POxC from spring to fall, but the increase in LIV plots was significantly greater than in CON. In the flint variety, CON plots saw a significant decrease, while LIV plots saw no significant decrease.

Spring POxC values in the flint variety did satisfy ANCOVA assumptions, so an ANCOVA model was built with fall POxC as the main response. A significant system:season

interaction was seen ($p = 0.031$), for which pairwise comparisons provided insight: a significant loss of $0.00744 \text{ moles kg}^{-1}$ of POxC across the season was seen in CON plots ($p = 0.0001$), while LIV plots saw a non-significant loss of $0.00183 \text{ moles kg}^{-1}$ ($p = 0.307$). Planting density and spring POxC had no effect, but management system significantly influenced fall POxC ($p = 0.038$) with significantly greater POxC in LIV plots than CON plots in the fall ($p = 0.038$). Bootstrapping confirmed this, with a bootstrap mean difference of $0.00498 \text{ moles kg}^{-1}$ and 95 % confidence interval of 0.0007 to 0.0093 ($p = 0.029$). In a long-data model with season as a main effect, season was the most significant factor ($p = 0.0006$) while system was only marginally significant ($p = 0.077$). These results indicate that in the flint plots, CON plots lost POxC across the season and LIV plots maintained it (Figure 9).

3.2.2 Particulate organic matter

In the GRM varieties, initial analysis identified substantially higher spring POM in CON plots than in LIV plots, violating ANCOVA assumptions. Similar to POxC, the two alternate modeling methods were used. The model using season as a main effect showed a significant system:season interaction ($p < 0.001$), with a significant POM decline in the CON plots 1.09 % from spring to fall ($p < 0.0001$) compared to a negligible change of 0.006 % in the LIV from spring to fall ($p = 0.976$) (Figure 10).

The delta score analysis corroborated these results, with LIV plots showing a significantly smaller decline in POM from spring to fall than cover crop plots ($p < 0.001$). Bootstrapping confirmed this finding: the bootstrap mean difference between delta scores was 1.085 % (SD = 0.267 %), with a 95% confidence interval of 0.557 to 1.597 % and a bootstrap p-value < 0.001 . Estimated difference means were consistent with those of the prior model, both in magnitude and direction. Maize variety and planting density did not significantly influence POM or POM change

in either model ($p > 0.35$ for all). These results verify the findings that reduced-tillage living mulch systems maintain particulate organic matter, while tillage-based cover crop systems result in a considerable loss of POM.

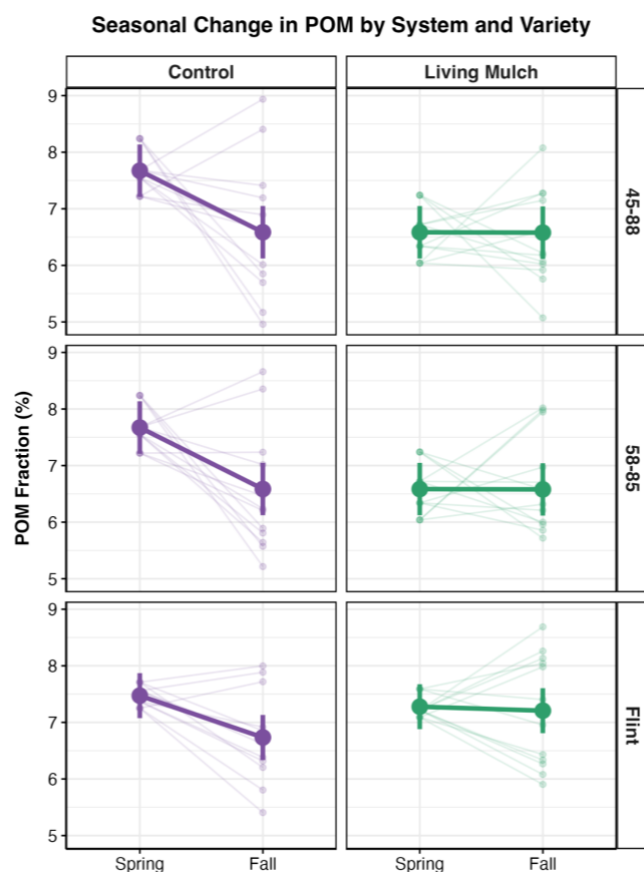


Figure 10. Seasonal change in POM in CON and LIV plots in the flint and GRM varieties. Faded lines connect raw spring and fall observations and bold lines and points show model-estimated marginal means $\pm$ 95% confidence intervals. CON plots saw a decline in POM from spring to fall ($\Delta = -1.09$ %), while LIV plots saw negligible seasonal change ($\Delta = -0.006$ %), suggesting that strip tillage preserved POM through the growing season.

Spring POM in the flint variety similarly did not satisfy ANCOVA assumptions, necessitating alternate model fitting. The model with season as a main effect saw a marginally significant system:season interaction ($p = 0.082$). Although density and system were independently non-significant (both $p > 0.39$), season significantly influenced fall POM ($p = 0.037$). In CON plots, POM decreased significantly by 0.783 % from spring to fall ($p = 0.008$) while in LIV plots, a POM loss of 0.071 % across the season was non-significant (0.790) (Figure 10). The delta score analysis also did not identify a significant difference between systems ($p =$

0.106); however, bootstrapped means saw a non-significant difference between seasonal deltas of 0.627 % (SD = 0.365) with a 95 % CI that included zero (-0.069 to 1.351), despite a marginally-significant p-value of 0.077. Despite weaker differences between systems in the flint variety, these results directionally align with those in the GRM varieties, indicating that tilled plots lose POM while strip-tilled plots maintain POM.

3.2.3 Particulate organic carbon

In the GRM varieties, spring POC met the ANCOVA assumption of covariate independence, allowing us to proceed with an ANCOVA analysis. The model revealed that management system had a highly significant influence on fall POC ($p < 0.001$), with more POC in LIV plots than in CON plots by the season end. Spring POC was not a significant predictor of fall POC ($p = 0.830$), nor was maize variety or planting density (both $p > 0.26$). LIV plots had a mean fall POC of 7.34 % and CON plots had a mean fall POC of 6.29 % (Figure 11). Bootstrapping showed the mean fall POC difference between cover treatments was 1.032 % (SD = 0.182 %), with a 95 % confidence interval of 0.675 to 1.383 % and a bootstrap p-value less than 0.001.

In the delta model, a significant interaction between management system and season ($p < 0.001$) indicated that POC changed differently across the growing season depending on the management system. While CON plots saw no significant change in POC across the season ($\Delta = -0.160$ %, $p = 0.334$), LIV plots saw a highly significant increase ($\Delta = 0.944$ %, $p < 0.0001$) (Figure 11). These results demonstrate that POC accumulates in reduced-tillage LIV plots, while tilled cover-crop systems see POC maintenance.

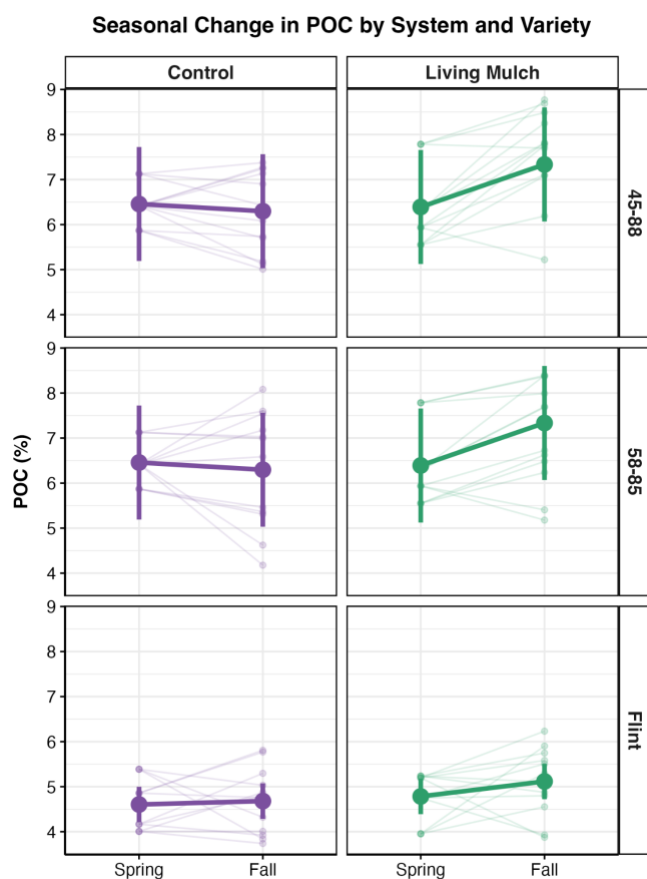


Figure 11. Seasonal change in POM carbon in CON and LIV systems in the GRM and flint varieties. Faded lines connect raw spring and fall observations and bold lines and points show model-estimated marginal means $\pm$ 95 % confidence intervals. In GRM varieties, CON plots saw no significant change in POC from spring to fall ($\Delta = -0.160$ %) while LIV plots saw a significant increase in POC ($\Delta = 0.944$ %), suggesting that the reduced tillage LIV system accumulates POC through the growing season while POC in tilled CON systems remains stable. No significant difference was seen in the flint variety, despite directional similarity to GRM results.

In the flint variety, ANCOVA assumptions were similarly met. The ANCOVA model with fall POC as the main response revealed that system, planting density, and spring POC all did not have a significant influence (all $p > 0.2$). Bootstrapped means showed 0.416 % greater fall POC in LIV plots compared to CON plots (SD = 0.320) with a CI that includes zero (-0.211 to 1.058), which was verified non-significant by a p-value of 0.2. A model with season as a main effect similarly saw no significant influence on POC by system, season, density, or any interactions. Pairwise comparisons showed no significant difference between spring and fall POC in either LIV or CON plots, although CON plots saw an increase of 0.076 % over the season ($p = 0.782$) and LIV plots saw an increase of 0.333 % ($p = 0.229$), 4.4 times bigger than that of CON (Figure 11).

Although not statistically significant, directional trends in the flint variety are similar to those of the GRM varieties, suggesting greater POC in LIV plots compared to CON.

3.2.4 24-hour carbon mineralization

24-hr Cmin was analyzed using generalized least squares to account for repeated measurements across the season (spring, summer, fall) within plots. 24-hr Cmin data was log-transformed prior to modeling to address right skew, and all EMMs are reported back-transformed to the original scale as ratios. Two GLS models were compared for best fit: a homogeneous variance model and a heterogeneous variance model.

Of the models analyzing the GRM varieties, the heterogeneous variance model was preferred based on lower AIC ($AIC = -177$) and all inference was based on the heterogeneous variance model. A highly significant system:season interaction was seen ($p < 0.001$), indicating that the effect of management system on 24-hr Cmin differed across the growing season. Pairwise comparisons across cover treatments within seasons showed that in spring, prior to any tillage, the two management systems did not significantly differ (CC/LIV ratio = 1.030, $p = 0.233$). In summer, LIV plots had significantly higher Cmin than CON plots (CON/LIV ratio = 0.803, $p < 0.001$). In fall, this pattern of higher Cmin in LIV plots compared to CON plots continued (CON/LIV ratio = 0.779, $p < 0.001$) (Figure 12).

Pairwise comparisons across seasons and within management systems revealed that in CON plots, 24-hr Cmin increased from spring to summer (spring/summer ratio = 0.892, $p < 0.001$), indicating a mid-season rise in Cmin, then declined below spring levels in fall (spring/fall ratio = 1.121, $p = 0.003$). LIV plots saw Cmin increase significantly from spring to summer (spring/summer ratio = 0.695, $p < 0.001$), a much greater increase than seen between spring and

summer in CON plots. Although fall Cmin levels dipped back down below summer levels in LIV plots (summer/fall ratio = 1.219, $p < 0.001$), fall Cmin levels were still higher than those in spring (spring/fall ratio = 0.848, $p < 0.001$). These results indicate that LIV plots see an overall increase in Cmin across the growing season, while CON plots see an overall decrease.

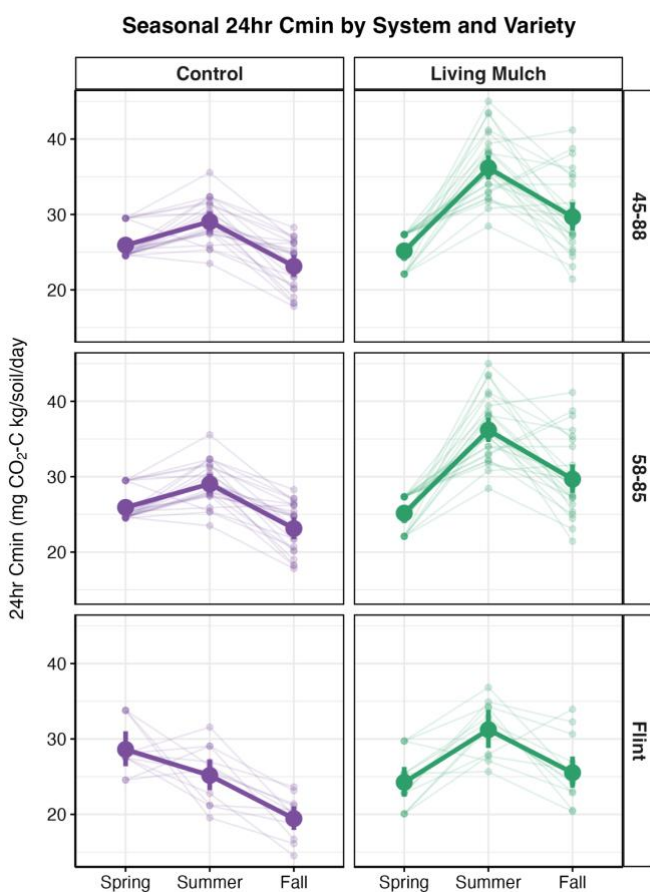


Figure 12. 24-hr Cmin in CON and LIV systems in the GRM and flint varieties across spring, summer, and fall. Faded lines connect raw observations across sampling points and bold lines and points show model-estimated marginal means $\pm$ 95 % confidence intervals.

For the flint variety, the homogenous variance model was preferred based on lower AIC (AIC= -41.3), and all inference was based on the homogenous variance model. A significant interaction was seen between management system and season ($p < 0.001$), indicating that the effect of management system on 24hr Cmin differed across the growing season. Pairwise comparisons across systems and within season showed that in spring, CON plots had significantly higher Cmin

than LIV plots (CON/LIV ratio = 1.180, $p = 0.005$), but in summer and fall, LIV plots had significantly higher Cmin than CON plots (both $p < 0.001$) (Figure 12). Pairwise comparisons across seasons and within systems revealed that in CON plots, Cmin declined significantly from spring to fall ($p < 0.001$), while in LIV plots, Cmin increased from spring to fall, albeit non-significantly ($p = 0.627$). LIV plots did see a significant spring-to-summer increase, however ($p = 0.0001$). These results are consistent with the summer peak and overall seasonal Cmin increase seen in the LIV plots of the GRM varieties, as well as the overall seasonal Cmin decrease seen in the CON plots, although the trajectory of the flint CON plots lacked a summer peak (Figure 12).

3.2.5 Aggregate stability

Spring aggregate stability differed significantly between management systems prior to tillage ($p < 0.001$) which violates the ANCOVA assumption of covariate independence, so seasonal and delta score linear mixed-effects models were fitted. Modeling aggregate stability with season as a main effect showed no significant interaction between management system and season, indicating that both systems saw aggregate stability decline at similar rates; a pairwise comparison confirmed that both management systems saw a significant decline in aggregate stability across the season (both $p < 0.0001$) (Figure 13).

The delta score model confirmed these results, as management system did not have a significant influence on aggregate stability ($p = 0.193$). Parametric bootstrapping to confirm summary statistics revealed a bootstrap mean difference of 0.0159 (SD = 0.0117), with a 95 % confidence interval of -0.00064 to 0.0391 with a bootstrap p-value of 0.180. These results consistently indicate that management system had no significant effect on seasonal change in aggregate stability.

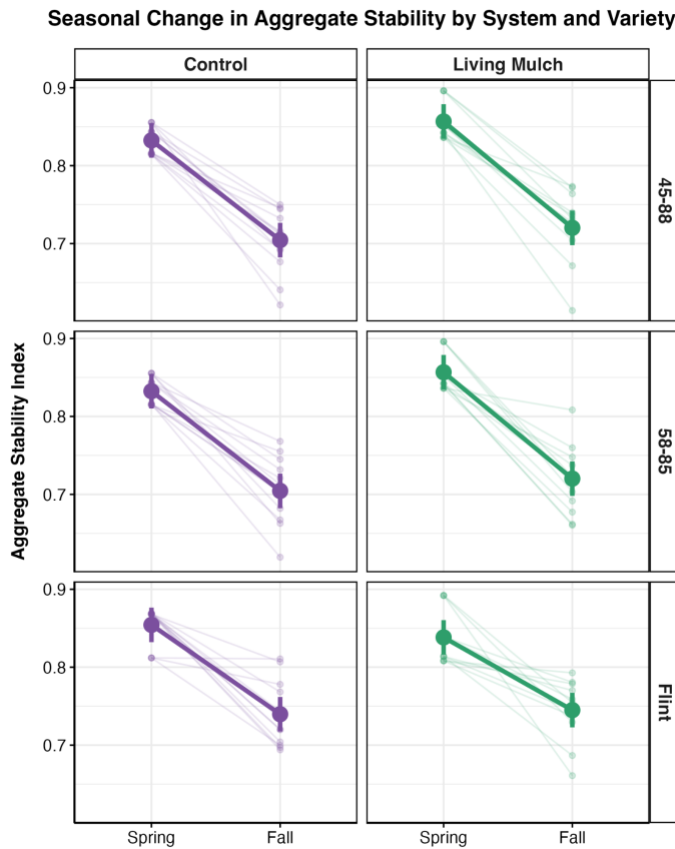


Figure 13. Seasonal change in aggregate stability in CON and LIV systems in the GRM and flint varieties. Faded lines connect raw spring and fall observations and bold lines and points show model-estimated marginal means $\pm$ 95 % confidence intervals. Both management systems saw a significant decline in aggregate stability from spring to fall (CC: $\Delta = -0.138$; LIV: $\Delta = -0.122$).

Spring aggregate stability in the flint variety similarly did not satisfy ANCOVA assumptions, necessitating the alternate models. In the model using season as a main effect, only season was a significant predictor ($p < 0.0001$), while system, density, and interactions were non-significant (all $p > 0.3$). EMMs revealed that CON plots saw a decrease of 0.1146 index units from spring to fall and LIV plots saw a decrease of 0.0933 index units; both of these losses were significant ($p < 0.0001$). The change score model supported these results, with no significant difference in seasonal trends seen between the CON and LIV plots ($p = 0.766$). These results indicate that aggregate stability decreased consistently throughout the season, regardless of management system or variety.

4. DISCUSSION

Before interpreting treatment effects, contextual features of the experimental design should be acknowledged. Environmental data from the Arlington Agricultural Research Station showed that the two growing seasons differed considerably in environmental conditions, particularly with regard to rainfall (Figure 14). Total precipitation differed substantially across years: 2024 received approximately 1,000 mm of cumulative precipitation through November compared to roughly 650 mm in 2025. Despite receiving more total precipitation, 2024 showed a mid-season soil moisture decline that was not present in 2025, particularly in the period between silking and harvest. This may be a result of differences in the seasonal distribution of rainfall as the 2024 season saw substantial rain in the early summer that dropped off quickly. It may also reflect potentially greater crop evapotranspiration demand during the 2024 grain fill period. The 2025 season appears to have experienced greater overall moisture stress relative to 2024, which likely contributed to the significant year effects regularly observed. These environmental differences should be held alongside the statistical results throughout the discussion below. It is also worth acknowledging that practical limitations of the experiment, including strip tiller alignment challenges that led to unintended clover termination in some 2024 plots and the statistical uncertainty inherent to four replications, introduce some noise into the picture.

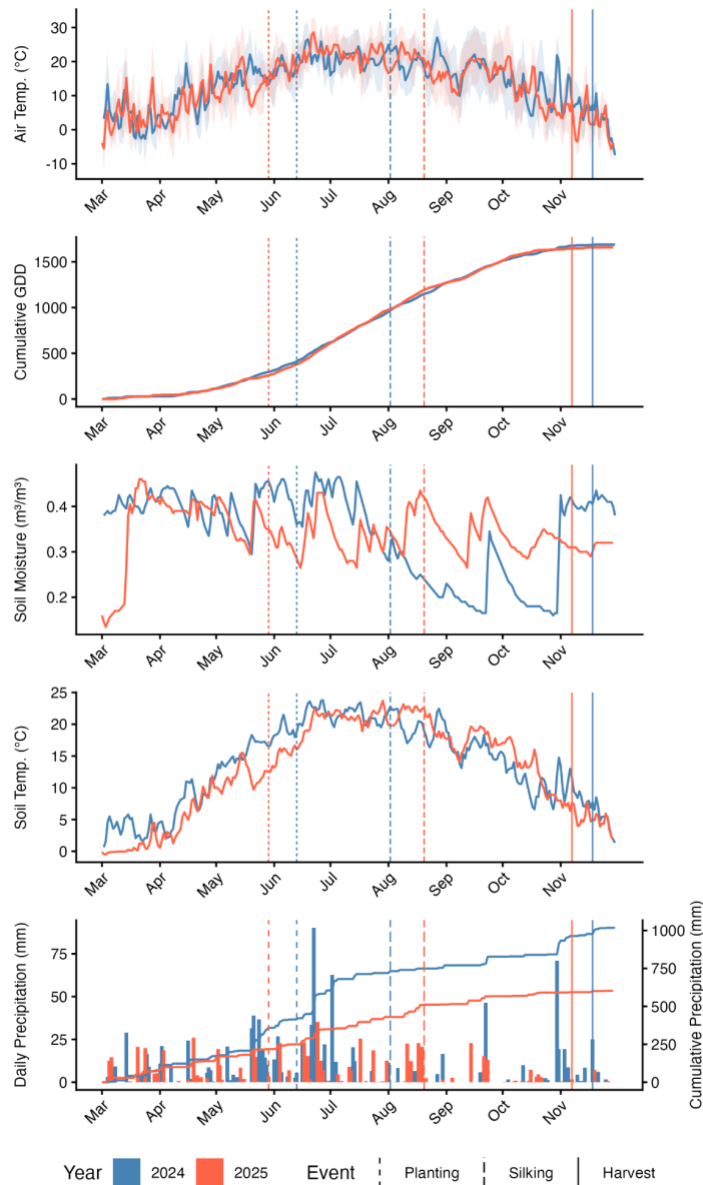


Figure 14. Seasonal environmental conditions at Arlington Agricultural Research Station during the 2024 and 2025 growing seasons (March–November). Panels show daily average air temperature with min/max range (°C), cumulative growing degree days (GDD, base 10°C), soil moisture at 10 cm depth (m^3/m^3), soil temperature at 10 cm depth (°C), and daily and cumulative precipitation (mm). Colored lines and bars represent 2024 (blue) and 2025 (red). Vertical lines indicate key events: planting (dashed), silking (long-dashed), and harvest (solid), colored by year. The 2024 season received substantially greater total precipitation than 2025, while cumulative GDD accumulation was similar between years. Soil moisture declined more steeply through the latter half of the 2024 season compared to 2025.

4.1 Morphology, density, and biological competition interact to influence maize performance in living mulch

4.1.1 Living mulch results in yield penalty for grain and specialty maize varieties

The living mulch system imposed a substantial yield penalty across both years and variety groups. In the GRM experiment, LIV plots yielded less than CON plots on total average, with a wider gap in 2025 compared to 2024. The system effect was smaller in the flint variety but directionally consistent: LIV plots yielded less than CON overall, and this difference was larger in 2025 than in 2024. These results confirm that strip-tilling and interrow mowing, while improving maize performance relative to no suppression, are not sufficient to fully eliminate the competitive effect of a living mulch in organic systems. This is consistent with prior research at this site and in comparable systems: Brockmueller et al. (2025) found that maize yields averaged only 4.61 Mg ha⁻¹ under a red clover living mulch at Arlington Agricultural Research Station even with strip-tillage undercutting, and Martin et al. (1999) documented yield reductions of 39–71 % relative to unmulched controls when strip-tillage was employed.

The significant year effect on yield and the significant system:year interaction observed in both GRM and flint analyses highlight that living mulch competition is conditioned by environmental context. The environmental data (Figure 14) shows that 2025 was a drier year overall, with substantially less total precipitation despite similar GDD accumulation. Under moisture-limited conditions, the water demands of a living clover mulch, which continues transpiring throughout the season, would intensify competition with the maize crop, particularly during reproductive growth stages. Sanders et al. (2018) documented a similarly environment-conditioned LIV yield penalty: across a two-year experiment, living mulch plots showed overall lower water-use efficiency (WUE) and grain yield compared to cover cropped controls, and these

results were exacerbated by the drought conditions of the second year, with a 2.5 Mg ha^{-1} lower yield and a 41% lower WUE seen in the drought year compared to the wet. The results here are consistent with that interpretation: in drier years, competing for soil moisture with a living mulch increases grain yield losses beyond what mechanical suppression can offset.

4.1.2 Canopy closure mediates competitive ability and differs by variety group

The central mechanistic finding of this experiment is that morphological traits influence LIV competitive ability, but only above a threshold of canopy closure, and whether that threshold is reached depends primarily on planting density. This distinction explains the different patterns observed between the GRM and flint variety groups across all analyses and provides support for our hypothesis that traits contributing to a denser maize canopy and greater light interception would correlate with greater yields and lower beneath-canopy biomass in LIV plots.

In the GRM varieties, planting density spanned a range (74–99 thousand seeds ha^{-1}) where all levels likely achieve canopy closure sufficient to tip the competitive balance by the time competition with the mulch is most consequential, neutralizing density-dependent effects on light interception. Consistent with this interpretation, planting density was not a significant yield predictor in either GRM variety, and the morphological model explained substantially more yield variation than the competition model. The LAI-only model also substantially outperformed the competition model, indicating that canopy light interception ability was the most informative predictor of GRM yield in LIV plots. In the full GRM model, the most significant predictors of yield were LAI and leaf angle, with plant height and leaf width also significant. These relationships are directionally consistent: larger canopies, wider leaf angles, and taller plants all contribute to a more light-intercepting, competitive morphology that limits light from reaching the inter-row clover, confirming the hypothesized mechanism for the GRM varieties. The suite of traits here

aligns closely with those identified by Mhlanga et al. (2016) to improve weed competitiveness: high LAI, greater plant height, and vertical leaf orientation. A recent ideotype review for living mulch-compatible cereals also identified tall stature, high LAI, and contrastingly, planophile leaf inclination, as primary selection criteria for competitive ability in LIV systems (Cougnon et al., 2022). The results of the experiment here provide field-based support for these recommendations in an organic maize production context.

The flint variety operated differently: at lower planting densities, (49–74 thousand seeds ha⁻¹) canopy closure is likely incomplete, meaning additional plants meaningfully increase the degree of light interception above the clover understory. Consistent with this, density was a meaningful yield predictor for the flint variety, and the higher density partially narrowed the yield gap between LIV and CON in both years. In 2024, the yield difference between LIV and CON plots was non-significant in the medium and high planting densities, with a nearly nonexistent gap seen in the medium density. In 2025, yield differences were significant across all densities, but the differences decreased as planting densities increased. These results provide evidence that higher densities improve competitive ability and suggest that medium planting density may be optimal depending on environmental circumstances. Sanders et al. (2017) evaluated planting densities of maize in a living mulch system and found that the low planting density yielded 20% lower than the high. These results are consistent with Weiner et al.'s (2010) conceptual framework in which higher planting densities create an “offensive” canopy-shading advantage over competitors, which Marín et al. (2014) tested and verified. Marín found that higher densities reduced weed biomass and increased yield; additionally, a density:variety interaction demonstrated that genetic differences influence the response of certain varieties to planting densities, and reduced phenotypic plasticity is advantageous under high densities.

Below the canopy closure threshold, the influence of individual morphological traits on yield is overridden by the degree of clover competition, which becomes the primary yield driver. This is consistent with the flint full-traits model, where fall clover biomass was the most significant predictor of yield while morphological traits were largely individually non-significant. Sanders et al. (2018) found that clover biomass did not decrease until canopy closure had reached 40% light interception, which supports the interpretation that morphological traits cannot drive meaningful competition management until sufficient canopy density is achieved. At higher planting densities like those of the GRM varieties, when all density levels achieve closure sufficient to shade the clover zone, morphological differences can noticeably drive further competition management and yield differences. At the lower flint density range, density itself is the predominant mechanism because the degree of canopy closure can vary meaningfully across treatments. The hypothesis that morphological traits influence yield and suppress beneath-canopy biomass is therefore supported for the GRM varieties but only partially supported for the flint, where density is the prerequisite mechanism that morphology alone cannot substitute for.

Despite mechanistic differences between variety groups, plant height was a consistent signal across both. Height was a significant predictor of GRM yield in LIV plots, and the most significant negative predictor of fall clover biomass. Height was also a significant positive predictor of flint yield in LIV plots. Taller stature likely predicts yield independently of leaf morphology because it contributes to canopy dominance over the clover regardless of whether full closure has been achieved. LAI showed a similarly consistent pattern in the clover biomass models: high clover biomass was concentrated in plots with both low LAI and shorter maize stature, while the lowest clover biomass was found in tall, high-LAI plots for both variety groups. These results are consistent with Boydston and Williams (2016), who found that taller sweet corn varieties with

greater LAI suppressed weed biomass more effectively than shorter plants in three of four experimental years, and with Naruhn et al. (2025), who found that LAI and leaf area as a function of leaf width and length were critical for greater absorption of photosynthetically active radiation and subsequent weed suppression.

The role of leaf angle sits at a point of disagreement in the literature. Leaf angle was a significant positive yield predictor in the GRM morphological model, but despite the generally smaller yield gap seen in the 45-88 variety compared to the 58-85 in LIV plots, the 45-88 had a narrower leaf angle. The broader ideotype literature offers competing recommendations here. Naruhn et al. (2025) and Cougnon et al. (2022) identified planophile leaf inclination as a selection criterion for competitive ability in LIV systems, reasoning that horizontal leaves maximize light interception at the canopy surface and reduce radiation reaching the inter-row mulch. Mantilla-Perez and Salas Fernandez (2017), however, note that erect upper-canopy leaves are more advantageous at high planting densities because they allow more even light distribution through the canopy, improving whole-canopy photosynthetic efficiency. At the GRM planting densities used here, where all treatments achieved near-complete canopy closure, an erectophile canopy may have provided a competitive advantage through greater photosynthetic efficiency and avoiding self-shading, consistent with the 45-88 variety's performance. That said, the system:variety interaction was only marginal in the fixed-effects yield model, and variety was only marginal in the yield difference model, so the more planophile leaves of the 58-85 variety may have contributed to minimizing the performance difference given the evidence that broader leaf angle improves yield. The more likely explanation for the dominance of the 45-88 variety lies in the combination of height, leaf width, and LAI, traits for which the 45-88 variety held a marginal advantage over the 58-85.

4.1.3 Living mulch provides weed suppression in high-pressure years

Weed biomass dynamics between management systems were conditioned by year in both variety groups, providing only partial support for our hypothesis that weed pressure would be equivalent between LIV and CON systems. In the GRM experiment, LIV plots had significantly more weed biomass than CON plots in 2024, directly contradicting the hypothesis in a low-pressure year. In 2025, however, the gap closed and was non-significant, with weed biomass declining in LIV plots while rising in CON plots. The hypothesis is therefore better understood as conditionally supported: LIV and CON systems converge in weed suppression, but only in years with sufficient weed pressure for the competitive and allelopathic effects of the clover to provide a measurable advantage over cultivation alone. The seasonal delta model provided further insight into this dynamic: across all varieties in 2025, LIV plots saw less weed biomass growth across the season than CON plots, with weed biomass even decreasing in the 58-85 LIV plots. This pattern is consistent with the broader cover crop suppression literature, where relative advantages of living cover over conventional cultivation are most apparent when baseline weed pressure is high (Osipitan et al., 2019): in low-pressure years, cultivation manages weeds adequately and the LIV offers no comparative advantage; whereas in high-pressure years, the continuously growing clover limits the light and resource availability that would otherwise allow late-season weed establishment.

The negative relationship between fall clover biomass and fall weed biomass in LIV plots provides evidence that greater clover biomass quantity enhances weed-suppressive benefits. Higher clover biomass in the inter-row area provides greater light interception at the soil surface and more competitive demand for soil nitrogen and water, outcompeting and suppressing weeds (Osipitan et al., 2019). Additionally, mowed red clover residue releases phytotoxic isoflavone

compounds that can inhibit germination of small-seeded weed species for as long as 30 days (Liebman and Sundberg, 2006; Lou et al., 2016). Repeated interrow mowing throughout the season continuously deposited cut clover as a surface mulch, meaning both active resource competition from the growing clover and allelopathic inhibition from decomposing mowed residue contributed simultaneously to suppression.

Results for the flint variety mirrored this pattern directionally and were also conditioned by planting density. Except for the high density in 2024, no significant difference in weed biomass was seen between LIV and CON plots in any combination of density and year, providing support for the hypothesis that weed biomass would be similar between systems. It is important to note that system differences were considerably lower in 2025 than in 2024, and CON plots had a significant increase in weed biomass from 2024 to 2025 while LIV plots did not change significantly across years, supporting the interpretation that clover biomass provides a suppressive effect in years with greater weed pressure, consistent with Menalled et al. (2022). Unlike the GRM experiment, fall clover biomass was not a significant predictor of fall weed biomass in the flint, though it was marginally negatively associated. Spring clover biomass did significantly reduce spring weed biomass, suggesting that earlier in the season, actively-growing clover provides the main suppressive effect. Because the lower densities of the flint variety did not reach full canopy closure, weeds were able to establish later in the season when the clover began to senesce and no longer provided a suppressive effect.

4.1.4 Variety selection influences living mulch outcomes

Variety selection shapes LIV performance through two distinct pathways in this experiment: within the GRM varieties through morphological differences, and in the flint through characteristics associated with adaptation to low-input, organic systems.

Between the GRM varieties, the 45-88 showed a smaller yield gap than the 58-85 across both years. Mean trait values indicate that the 45-88 was marginally taller than the 58-85, and height was the most important yield-determining trait in the morphological model, so even a small difference in stature likely conferred a competitive advantage. The 45-88 also had slightly wider leaves, and while leaf width was only significant in the full model, it contributes to LAI, which was a highly significant factor in both the full and LAI-only models. These results align with Boydston and Williams (2016), Mhlanga et al. (2016), and Naruhn et al. (2025), who found that plant height and LAI together give maize a competitive advantage over weeds, and with Cougnon et al. (2022), who characterized a competitive maize ideotype with tall stature and dense canopy. Bartel et al. (2017) and Ziyomo et al. (2013) similarly demonstrated that genetic background is a key determinant of maize performance in living mulch systems with respect to both general LIV tolerance and drought tolerance, and the present results further corroborate that conclusion.

For the flint variety, the near-equivalent performance of LIV and CON plots at medium planting density in 2024 provides evidence that a variety-density combination can substantially offset LIV competitive pressure. Of the measured morphological traits, height was the only one with a significant influence on yield, but that influence was non-significant when modeled alongside weed and clover biomass. The flint had more planophile leaves than the GRM varieties, but leaf angle did not significantly influence its yield. These results indicate that the flint variety

is more equipped to thrive in a living mulch system for reasons beyond the morphological traits measured here. Ares et al. (2021) argues that open-pollinated and heirloom varieties are preferentially adapted to organic, low-input systems, which may explain a competitive advantage of the flint over the GRM modern hybrids in LIV plots that is not fully captured by morphology alone. This finding has practical implications for variety screening in organic LIV systems: morphological traits may not fully predict performance in living mulch for every variety.

4.2 Soil health metrics are greater in living mulch than in control

Across the majority of the soil health metrics measured, spring values differed significantly between CON and LIV plots before any in-season management had occurred. Because spring sampling preceded spring field operations (tillage in CON plots and strip-tillage in LIV plots), these differences cannot be attributed to treatment effects. The most likely explanation is pre-existing spatial heterogeneity in residual organic inputs across the field; in particular, patches of standing dead biomass from prior field experiments and areas of volunteer rye that were observed during sample collection, which would create localized spikes in various soil health indicators such as microbial respiration and soil organic matter. With only four field blocks, even randomized split-plot designs cannot guarantee balance across this kind of spatial variation.

In order to contextualize differences in results between GRM variety plots and flint variety plots, it is important to note management differences between the experiments. As described in the Methods, the flint variety plots received less than half of the amount of poultry manure applied to the GRM variety plots, meaning they received significantly fewer nutrients. The flint variety planting density range was also significantly less than that of the GRM varieties, with the “high” planting density in the flint variety being equivalent to the “low” planting density in the GRM varieties. Beyond these management differences, there are significant varietal differences between

the modern hybrid GRM varieties and the specialty-bred, open-pollinated flint variety that likely produce differences in how the plant interacts with the microbial community in the soil. The combined effect of differences in fertility, planting densities, and varieties would necessarily lead to differences in the chemical, biological, and physical soil dynamics in each experiment's plots.

4.2.1 POxC increases in living mulch

POxC is widely considered a measure of labile or “active” carbon in the soil because it is assumed that permanganate oxidation closely mimics microbial oxidation. Although this interpretation is popular, recent proof-of-concept research has demonstrated that POxC is sensitive to lignin and lignin-derived aromatic compounds (Woodings and Margenot, 2023). These results directly contradict the idea of POxC as a measure of labile carbon, since lignins are considered part of the slow-degrading “recalcitrant” carbon pool (Wander, 2004). This indicates that POxC is chemically reactive but biologically slow, and increases in POxC indicate retention or accumulation of oxidizable, usually plant-derived, biomass.

Despite starting with lower spring POxC values than GRM CON plots, GRM LIV plots accumulated considerably more POxC across the season. These results are consistent with findings from comparable systems: Ginakes et al. (2020) documented that POM and POxC were greater in inter-row regions of a zone-tilled kura clover living mulch than within tilled crop rows and Hill et al. (2021) documented higher POxC after four years of living mulch compared to four years of cover crops. The mechanism behind this differential accumulation lies in the combination of tillage, perennial roots, and contrasting biomass inputs of the two systems across the season. In CON plots, young, nitrogen-rich, low-lignin clover tissue was terminated and incorporated via tillage in May, stimulating decomposition and leaving relatively little plant-derived carbon in the POxC-reactive pool by fall. In LIV plots, living clover persisted between maize rows throughout

the growing season, slowing mineralization and contributing continuous inputs through root exudates, root turnover, and above-ground biomass. This interpretation is supported by previous work showing that POxC increases under reduced tillage (Veum et al., 2022), in more perennial systems (Martin and Sprunger, 2022), and in systems with greater annual biomass inputs (White et al., 2020). Beyond simply greater biomass inputs in LIV plots, as the clover matures and senesces, its tissue shifts toward higher lignin content; because POxC is sensitive to lignin, the progressive accumulation of senescing, lignin-enriched clover tissue in LIV plots directly drives the observed POxC gains.

In the flint variety, CON plots saw a significant loss of POxC across the season, while LIV plots saw POxC maintenance across the season. This result is not incompatible with those of the GRM varieties; it reflects similar dynamics, only to a lesser degree. The lower magnitude of POxC dynamics in the flint variety is likely due to experimental differences: the flint plots were fertilized at a considerably lower rate and planted at a lower density range. This means that the flint plots and the living mulch biomass in them received less than half the amount of poultry manure and thus less than half the amount of applied nutrients as the GRM varieties, as well as lower maize biomass inputs. Reduced fertility leads to decreased clover biomass growth throughout the season, and combined with reduced maize biomass inputs, flint plots had overall fewer POxC-sensitive biomass inputs. The biomass inputs in the LIV plots still exceeded those in the CON plots to a degree capable of replenishing any POxC lost across the season, while CON plots saw an overall loss.

4.2.2 POM is maintained in living mulch

Spring baseline POM fraction values differed significantly between LIV and CON plots in the GRM varieties, likely due to spatial variation of volunteer rye and legacy effects of prior

experiments across the field. Despite this initial imbalance, POM declined significantly over the season in CON plots while LIV plots maintained POM. Although the magnitude was smaller, the flint variety saw similar POM dynamics between LIV and CON plots. The primary mechanism driving POM loss in CON plots is physical disruption of aggregates by tillage. A substantial amount of soil POM is physically occluded within macroaggregates, where it is protected from microbial decomposition; when macroaggregates are disrupted by tillage, this previously-protected POM is exposed and mineralized (Six et al., 2000). Reduced-till systems, on the other hand, see slowed macroaggregate turnover and increased occluded POM.

In this experiment, CON plots were tilled via chisel plow and field cultivator and cultivated twice more before canopy closure to manage weed pressure. Such a tillage intensity would substantially disrupt macroaggregates and expose aggregate-protected POM to decomposition within the season. In LIV plots, strip-tillage disturbed only about one-third of the soil surface, leaving inter-row aggregates and their physically protected POM largely intact. The near-zero seasonal change in LIV POM fraction reflects preservation through physical protection rather than net accumulation from new inputs: if new biomass addition from the living mulch were the primary driver, one would expect POM fraction to increase rather than simply stabilize. The balance between new organic inputs and ongoing microbial decomposition of existing POM likely produces near-zero net change under LIV management, consistent with the results observed.

4.2.3 POC increases in living mulch

POC results offer an important complement to the POM fraction findings. While GRM variety LIV plots maintained POM quantity, they simultaneously accumulated POC while CON plots showed no significant seasonal change. While results for the flint variety were not statistically significant, POC dynamics were directionally aligned with those in the GRM varieties, with a

larger POC increase in LIV plots compared to CON. This pattern indicates that the POM collected from each system is compositionally distinct, with more carbon-enriched POM found in LIV plots.

Two mechanisms likely contribute to this. First, root-derived POM tends to be more chemically recalcitrant than shoot-derived POM: the mean residence time of root carbon is 2.4 times that of shoot C (Rasse et al., 2005). LIV plots maintain active clover roots throughout the growing season, contributing a continuous supply of root-derived organic matter to the POM pool via sloughing and fine root turnover. In the CON plots, POC stays relatively level because spring-incorporated clover decomposes quickly, replacing the POM exposed by tillage and prone to rapid mineralization. Prairie et al. (2023) saw similar results, with POC gains of 19.7 and 33.3 % in no-till and cover-cropped systems; Khosa et al. (2025) found no-till systems to accumulate POC as well. Rui et al. (2022) also suggests that the presence of a legume cover played a role, finding higher POC in systems with perennial legumes compared to monoculture maize. Second, as red clover matures and senesces through the season, its tissue lignifies, shifting from nitrogen-rich, rapidly decomposable material to more carbon-rich, recalcitrant material (Balk et al., 2023). Labile nitrogen compounds are mobilized and repurposed, leaving a carbon skeleton of lignified cell walls and a carbon-enriched residue in the POM fraction of the LIV plots.

4.2.4 Microbial respiration increases in living mulch

24-hr C_{min} results revealed a consistent seasonal pattern: while CON and LIV plots did not differ significantly in spring, by summer, LIV plots had significantly higher C_{min} than CON plots, and this pattern persisted through fall. While GRM CON plots saw C_{min} increase from spring to summer, the pattern diverged from that of the LIV plots as C_{min} declined below spring levels by fall. C_{min} dynamics in the flint variety followed a nearly identical pattern to the GRM

varieties; aside from a consistent, overall decline in CON plots instead of a summer peak, the LIV plots saw a similar summer peak, fall decline, and overall spring-to-fall increase.

24-hour carbon mineralization is a somewhat limited assessment of microbial activity given it is conducted *in vitro* under controlled laboratory conditions, but it can be effectively described as measuring the amount of labile, or active, carbon that is microbially-accessible upon rewetting. The spring-to-summer C_{min} increases generally seen in both CON and LIV plots (except for flint CON plots) are due to a flush of additional organic carbon entering the system. In CON plots, tillage incorporates young, nitrogen-rich clover residues and breaks up aggregates occluding organic matter from decomposition, providing a mid-season pulse of accessible carbon before being exhausted (Duval, 2020; Fiedler et al., 2016). By fall, minimal additional organic inputs after clover incorporation leads to nutrient depletion and a net seasonal decline below spring levels.

In contrast, the substantially larger peak seen in LIV plots is the result of aboveground biomass inputs from mowing. Regular mowing throughout the early season deposits nitrogen-rich biomass for microbes to break down, providing both more overall carbon and increased carbon accessibility. Living clover roots also supply a continuous stream of labile carbon in the form of root exudates secreted throughout the growing season, while additionally supplementing the carbon pool with root turnover biomass (Martin and Sprunger, 2022; Zhu et al., 2014). This creates a larger and more sustained C_{min} response in LIV plots than in the maize-only rhizospheres of CON plots, supported by the results of Peters et al. (2020), who similarly found elevated CO_2 fluxes in living mulch systems compared to cover cropped. The minimal tillage in LIV plots also maintains soil structure and protects occluded soil organic matter, aligning with the findings of Rieke et al. (2022), who found that reduced-tillage systems see considerably higher C_{min} than

conventionally-tilled systems. The combination of clover inputs and reduced tillage replenishing labile carbon and protecting existing pools thus explain the net seasonal increase seen in LIV plots.

4.2.5 Aggregate stability decreases in all systems

Across both the GRM varieties and flint, both management systems showed a significant decline in aggregate stability from spring to fall, and no significant difference in the magnitude of this decline was detected between CON and LIV plots for either experiment. This null result warrants careful interpretation given an important methodological consideration.

Soil samples in this study were dried in a 60°C oven for three days, a more intensive protocol than the room-temperature air-drying for which the Eijkelkamp wet-sieve aggregate stability method was designed. Room-temperature drying standardizes the slaking effect and the intermolecular bonding that occurs between organic compounds and mineral surfaces during drying, while oven-drying has been demonstrated to artificially increase wet aggregate stability, potentially compressing the range of detectable differences between systems (Kemper and Rosenau, 1986). However, both CON and LIV samples were processed identically, so relative treatment comparisons remain internally valid even if absolute stability values were affected. The aggregate stability findings should be interpreted with this methodological caveat in mind.

The universal seasonal decline across both management systems is most likely explained by the physical weathering effects of wetting and drying cycles through the growing season, which operate similarly across both systems. Repeated wetting-and-drying events cause air trapped in aggregates to compress and escape as the dry soil rapidly absorbs and releases water, breaking apart the aggregates and producing aggregate stability declines within a single growing season; (Denef et al., 2001) found that a single wet-dry cycle reduced macroaggregates from 30 to 21% of total soil weight. Furthermore, the absence of a system effect is likely temporal. Macroaggregate

formation is a slow process mediated by fungal hyphal networks, living root growth, and the progressive stabilization of organic matter within aggregate hierarchies (Six et al., 2004). The biological mechanisms by which reduced tillage and continual cover in LIV plots would likely eventually improve aggregate stability by preserving fungal networks and accumulating and protecting organic matter through living roots and inputs, but such mechanisms may require multiple seasons to produce detectable structural changes. The results of this experiment provide evidence that one growing season of strip-tilled living mulch is insufficient to produce measurable aggregate stability improvements. As such, multi-year monitoring of aggregate stability is recommended.

5. CONCLUSION

Across both the agronomic and soil health components of this experiment, a consistent narrative emerges: the living mulch system operated as a biologically richer but more competitively demanding environment for the maize, with outcomes shaped by the interactive effects of variety-level morphological traits, environmental conditions, and the continuous presence of living clover roots.

The agronomic results make clear that not all maize varieties are equally equipped to handle living mulch competition, and that tall stature, planophile leaf angle, broad leaves, and overall denser canopy are the traits most consistently associated with maintaining yield under a living mulch. The practical implications of these findings are that organic producers adopting living mulch systems should prioritize varieties with these characteristics, and future breeding programs targeting organic or reduced-tillage contexts should treat living mulch competitive ability as a

selection criterion. Competition management remains one of the central challenges of this system, however, and the right combination of variety traits, suppression practices, and management timing will require further investigation before clear prescriptive guidance can be offered.

The soil health results provide encouraging evidence that the living mulch system preserves organic matter pools, builds soil organic carbon, and sustains biological activity more effectively than the tilled control even within a single growing season. Four complementary metrics converge directionally on this conclusion, strengthening confidence in a genuine biological signal despite the modest statistical power of four field replications. To further contextualize the soil health and productivity tradeoffs, the ecological benefits of living mulch-driven soil health improvements could even help mitigate the yield gap over time given evidence that POxC and carbon mineralization are effective predictors of yield (Hurisso et al., 2016).

Taken together, these results document an agronomic tradeoff that is environmentally conditioned. The yield penalty is meaningful, but so are the early soil health gains. In terms of future research opportunities, several directions seem promising for managing living mulch competition and expanding what the system can achieve. Measuring root morphology would help clarify the belowground mechanisms driving the competition and soil health results observed here. Band-applying fertilizer in the planting strips rather than broadcasting could weaken clover while strengthening the maize at a critical early-season window (Mhlanga et al., 2016). Building on Ziyomo et al. (2013), testing whether drought-tolerant varieties confer competitive advantages in an organic living mulch context is a relevant research question. Studies across multiple seasons remain essential to determine whether the early soil health signals documented here translate into long-term soil health gains and ecosystem service benefits that motivate living mulch adoption in the first place.

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APPENDIX

In 2024, a masa maize (Chilean Choclo) bred by Dr. Bill Tracy's lab at UW-Madison was planted. Due to excessive lodging seen in the Chilean Choclo, the variety was replaced in 2025 with another polenta maize from the Tracy Lab, Garish's Dent.

Given the significant root lodging seen in the 2024 season, a root lodging assessment was performed on August 23rd (R3 growth stage) by measuring the angle of the plant stalk with respect to vertical. Plants with an angle greater than or equal to 30 degrees from vertical were considered lodged, and the lodging rate was calculated as the average degree of lodging per plot.

In 2024, SPM grain yield was significantly influenced by management system and variety (cover: $p = 0.002$, variety: $p < 0.0001$), while planting density and all interactions were not significant (all $p > 0.2$). CON plots yielded 0.776 Mg ha^{-1} more on average than LIV plots ($p = 0.002$), and the Caribbean flint variety yielded 1.543 Mg ha^{-1} more than the Chilean Choclo on average across all treatment combinations ($p < 0.001$).

Management system effect was most significant in the Choclo plots, where CON plots yielded on average 1.043 Mg ha^{-1} more than LIV plots ($p = 0.002$). The flint variety, on the other hand, saw a smaller and non-significant difference of 0.490 Mg ha^{-1} on average ($p = 0.131$). This pattern was consistent when analyzing yield differences between density and variety combinations; at low density, the yield difference between systems in the Choclo was significant ($p = 0.011$) and decreased in significance as the planting density increased, becoming non-significant at high density ($p = 0.290$). For flint maize, no combination of density and management system saw a significant yield difference, with nearly identical yields seen at the medium planting density ($p = 0.979$). These results suggest that the flint maize may be somewhat more tolerant of living mulch

competition than the Choclo, although the interaction between management system and variety was not significant ($p = 0.225$).

In 2025, the estimated variance between field replications was effectively zero, suggesting negligible between-block variation, but field replication as a random effect was retained to respect the experimental design structure. Maize grain yield was significantly influenced by management system, with CON plots yielding more than LIV plots by an average of 2.518 Mg ha^{-1} across all treatment combinations ($p < 0.0001$). Variety and density were not independently significant (both $p > 0.44$), but significant cover:variety ($p = 0.002$) and variety:density interactions were seen ($p = 0.007$). These interactions suggest that the yield response to management system and density differed by variety.

The system:variety interaction was supported by EMMs results that revealed a considerably larger yield difference between systems for the Dent variety than for the flint. CON Dent plots yielded 3.299 Mg ha^{-1} more on average than Dent LIV plots ($p < 0.001$). The yield difference in flint plots was also significant ($p < 0.001$) but considerably smaller, with flint CON plots yielding 1.736 Mg ha^{-1} more than flint LIV plots. These results are consistent with the 2024 results that indicate flint maize shows a greater tolerance for living mulch competition. Examining contrasts within each density:variety combination showed a significant difference between management systems across all densities for Dent maize (all $p < 0.001$). In flint maize plots, there was a significant yield difference in low ($p < 0.001$) and medium densities ($p = 0.004$), but the yield difference at high density was just short of significant ($p = 0.059$), which somewhat suggests that higher planting densities may partially improve the competitiveness of the flint variety (Figure A).

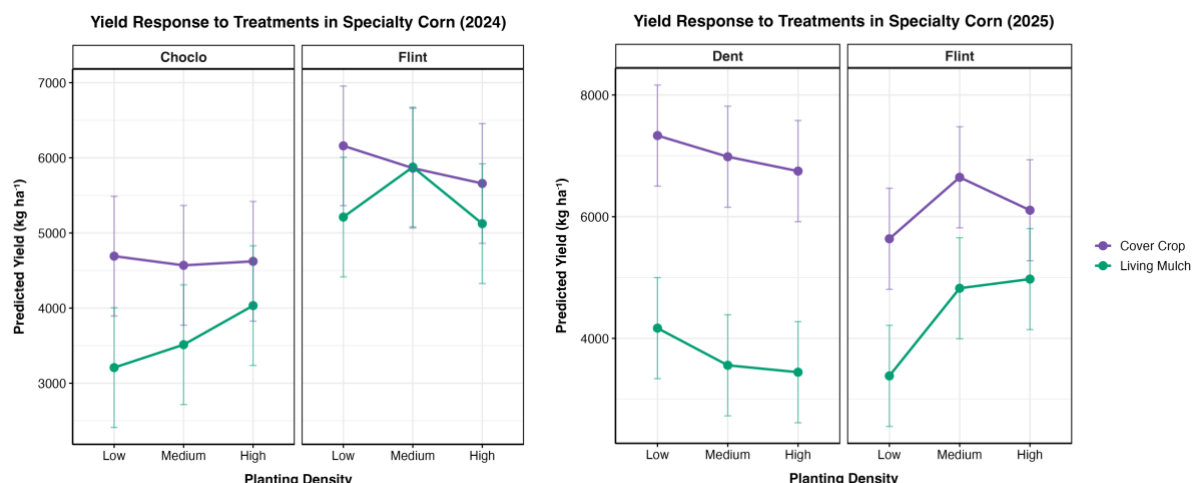


Figure A. Predicted grain yield of two SPM varieties in 2024 (Chilean Choclo and Caribbean flint, left) and 2025 (Garish's Dent and Caribbean flint, right) in CON and LIV systems across three planting densities. Points represent estimated marginal means and error bars represent 95 % confidence intervals. In 2024, the Choclo variety saw higher yields in CON plots across all densities, while yields in the flint variety converged between management systems, particularly at medium density. In 2025, the CON system consistently yielded more than the LIV system across all varieties and densities, with a considerably larger difference between systems in the Dent variety. In the LIV system, the flint yield increased with planting density while Dent yield decreased with increasing planting density.

To investigate the competitive effect of the LIV system on yield from another perspective, a model was fitted for the yield gap between CON and LIV plots of the same variety, density, and field replication combination. In 2024, neither variety, density, nor interaction were significant predictors of the yield gap (all $p > 0.29$), and field replication as a random effect explained no variance. EMMs showed that the yield gap in the Choclo variety was greatest at low density (1.484 Mg ha^{-1}) and decreased as planting density increased, with a gap of 0.589 Mg ha^{-1} at high density, suggesting that Choclo may handle competition in living mulch more effectively at higher planting densities. In the flint variety, the yield gap was generally smaller across all densities, with essentially equivalent yields seen between management systems at the medium density. This result

corroborates that of the yield model represented in Figure 8, which indicates that the flint variety may be generally more resistant to living mulch competition than the Choclo (Figure B).

In 2025, the yield gap between management systems was significantly influenced by variety ($p = 0.006$), while planting density and the interaction were not significant (both $p > 0.58$). EMMs showed that Dent maize exhibited consistent and significantly larger yield gaps than flint maize by an average of 1.563 Mg ha^{-1} across all densities ($p = 0.006$). The flint variety saw that yield gap decrease as planting density increased, with a gap of 2.254 Mg ha^{-1} at low density and 1.132 Mg ha^{-1} at high densities (Figure B). Although the contrast between low and high planting densities was not significant ($p = 0.404$), these results tentatively suggest that higher planting densities may help flint maize compete with living mulch more effectively.

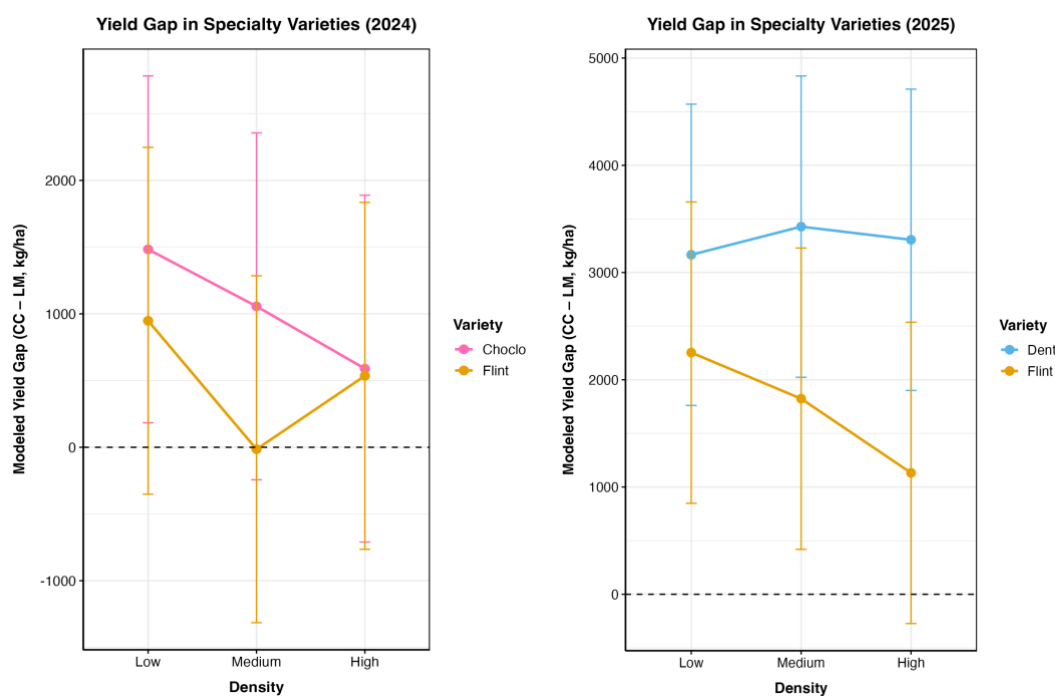


Figure B. Modeled yield gap ($\text{CON} - \text{LIV}$, kg ha^{-1}) across three planting densities for Choclo and flint in 2024 (left) and Dent and flint in 2025 (right). Points represent estimated marginal means and error bars represent 95 % confidence intervals. Positive values indicate higher yield in the CON system, while values at or below zero indicate equivalent or higher yield in the LIV system.

In 2024, the Choclo yield gap declined consistently with increasing density, while the flint yield gap was near zero at medium density and more variable across the density range. In 2025, the Dent variety saw a consistently large yield gap across all densities, while the flint yield gap declined with increasing density.

Substantial root lodging was seen in the Choclo variety, prompting inclusion of lodging ratings in the statistical analysis. Management system was shown to significantly influence lodging severity ($p = 0.009$), with consistently lower lodging ratings in LIV plots compared to CON plots. Pairwise contrasts revealed that at the low planting density, LIV plots averaged a lodging rating of 39.2 degrees compared to 65.2 degrees in CON plots, a marginal difference of 26 degrees ($p = 0.082$), likely due to high variability ($SD = 27.2$). As planting density increased, the lodging difference between systems decreased and was no longer significant, with a difference of 11 degrees at medium planting densities and 7.7 degrees at high planting densities (both $p > 0.23$). Across both LIV and CON plots, lodging rating increased with planting density, with the highest lodging rating in LIV plots (61 degrees) still lower than the lowest lodging rating in CON plots (65.2 degrees). These results suggest LIV management provides a mediating effect on lodging, which may potentially be further influenced by planting density.

Models were also fitted to assess the impact of lodging on yield. In a model including management system, density, and lodging rating as fixed effects, no effects were independently significant. A significant three-way interaction between management system, planting density, and lodging rating ($p = 0.027$) indicates that the relationship between lodging and yield is conditioned by both planting density and management system simultaneously. A large negative coefficient of -473.8 for the LIV – high density combination ($p = 0.009$) suggests that high planting density in LIV plots was associated with substantial yield loss, supporting the results that indicate higher planting densities see increased lodging.

To examine if and how the measured morphological traits influenced yield in LIV plots, linear mixed-effects models were fit for the 2024 and 2025 years with LAI, leaf angle, leaf width, and plant height as covariates. In 2024, after controlling for variety, no individual trait significantly influenced yield in the all-trait model (all $p > 0.48$). When modeling individual traits without controlling for variety, however, plant height and leaf angle were demonstrated to be significant predictors of yield. Taller plant height was positively associated with yield ($p < 0.001$), while leaf angle was negatively associated with yield ($p = 0.004$). LAI showed a marginally significant positive relationship ($p = 0.067$), while leaf width was not significant ($p = 0.943$) (Figure C). In 2025, the all-trait model showed variety as the strongest predictor of yield ($p < 0.001$), and of the traits, LAI ($p = 0.002$) and leaf width ($p = 0.021$) were both significant positive predictors of yield. Leaf angle and plant height were non significant (both $p > 0.21$). Individual trait models showed a different story than in 2024, with only LAI retaining near-significance ($p = 0.066$), while all other traits were non-significant (Figure C).

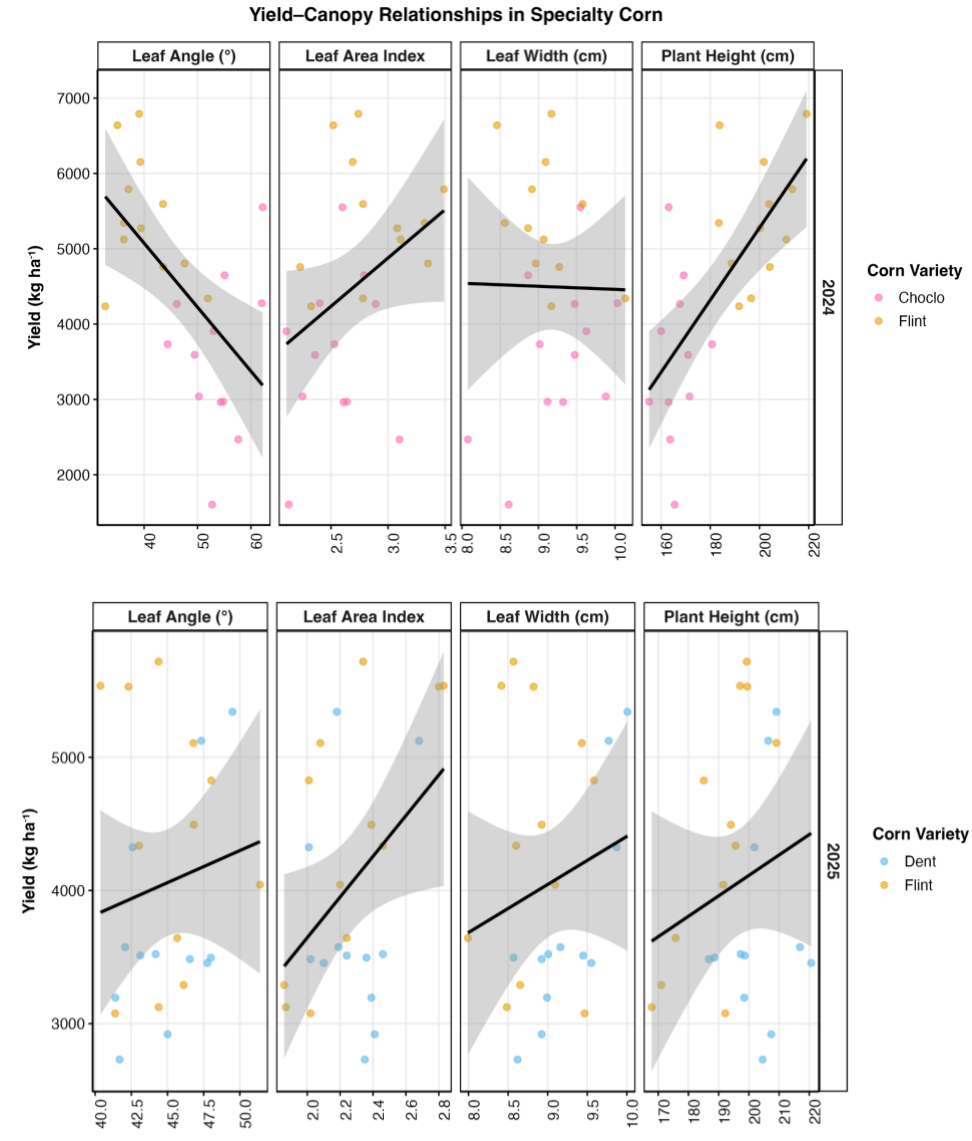


Figure C. Relationships between canopy traits and maize yield. Scatterplots show observed relationships between yield and four canopy traits (leaf angle, leaf area index, leaf width, and plant height), with linear regression lines ($\pm 95\%$ confidence intervals). Points are colored by maize variety. In 2024, leaf angle showed a negative relationship with yield and plant height showed a positive relationship with yield, though neither was significant after controlling for variety. In 2025, all traits were positively associated with yield.

Variety	Management System	Mean Plant Height (cm)	Mean Leaf Angle (°)	Mean Leaf Width (cm)
flint	LIV	195	42.60	8.97
flint	CON	237	41.80	9.94
Choclo	LIV	166	53.50	9.25
Choclo	CON	176	59.40	10.4
Dent	LIV	203	44.9	9.24
Dent	CON	285	37.8	9.93

Table 5. Mean values of morphological traits for each SPM variety and management system

To evaluate whether canopy architecture or biological competition had a greater impact on yield in SPM, we fit several mixed-effects models for each year and compared them using AIC model selection. In 2024, the competition model was significantly more effective (AIC = 386.12, $p = 0.019$) than the full model (AIC = 393.03) including all covariates. The architecture model did not improve over the full model (AIC = 396.2), although only marginally. These results suggest that biological competition, in particular weed biomass, had a greater effect on yield in 2024, with canopy architecture providing little explanatory power. In 2025, differences were again marginal. The competition model (AIC = 396.69) did not provide more explanatory power than the full model (AIC = 392.83), while the architecture model did (AIC = 391.86). These results tentatively suggest that canopy architecture was somewhat more impactful on yield than competition (Figure D).

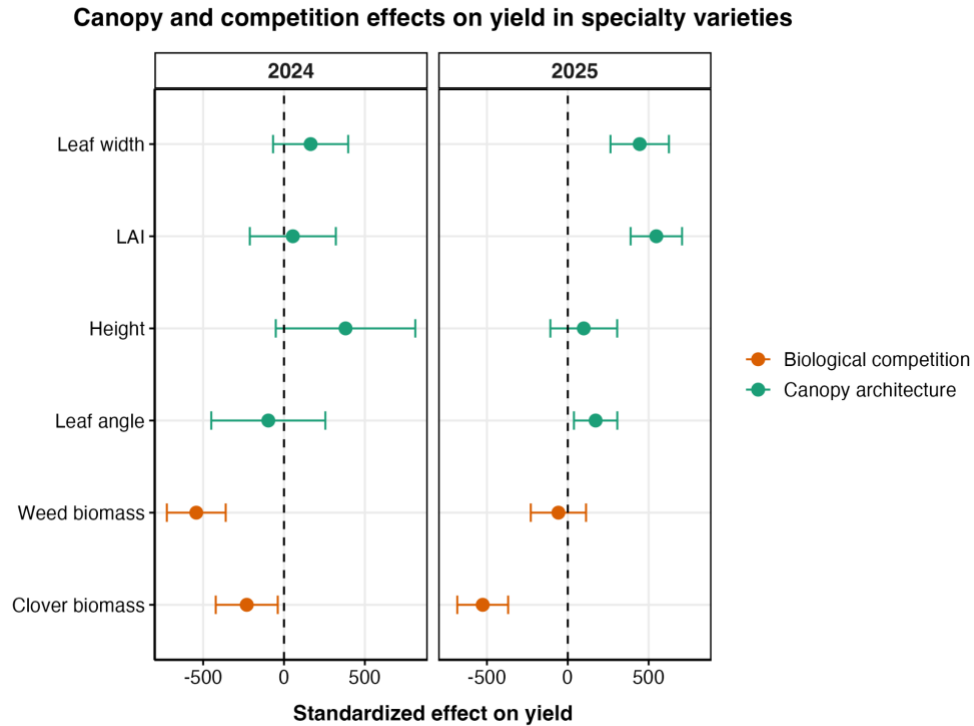


Figure D. Standardized effects of canopy architecture and biological competition on SPM yield in 2024 and 2025. Points represent standardized coefficients from mixed-effects models and error bars indicate ± 1 SE. Positive values indicate traits associated with increased yield, while negative values indicate yield reductions. Confidence intervals overlapping zero indicate non-significant effects. Weed biomass showed a negative effect on yield in both years, while canopy architecture traits were more variable in direction and magnitude. Model selection results showing that neither trait group substantially improved on a model containing only variety and density effects.

Across all models analyzing fall weed biomass, weed biomass was log-transformed to address heteroscedasticity. EMMs were back-transformed to the original scale for interpretation and presentation. In 2024, weed biomass was significantly influenced by management system ($p < 0.001$) and planting density ($p = 0.036$), and a significant interaction between density and management system was observed ($p = 0.013$), indicating that the effect of planting density on weed biomass differed by management system. Variety and all other interactions were not significant (all $p > 0.24$). EMMs provided more insight into the system:density interaction: at low density, a non-significant difference of -72 kg ha^{-1} was seen between CON and LIV plots ($p =$

0,839), while at high density, the difference was -487 kg ha^{-1} and still non-significant ($p = 0.196$), likely due to the high variability in weed biomass in high-density CON plots. As planting density increased, weed biomass decreased in CON plots, but stayed relatively the same in LIV plots (Figure E).

In 2025, management system no longer significantly influenced weed biomass ($p = 0.106$), while planting density remained significant ($p = 0.015$). Variety and all interactions were non-significant (all $p > 0.21$). Pairwise comparisons showed that while LIV plots tended to have higher weed biomass than CON plots when averaging across varieties and densities, this difference was non-significant ($p = 0.158$). Comparing within density levels, contrasts between management systems was still non-significant across all densities (all $p > 0.28$). However, weed biomass decreased with higher planting densities across each management system. These results suggest that higher planting density provides weed suppression regardless of management system (Figure E).

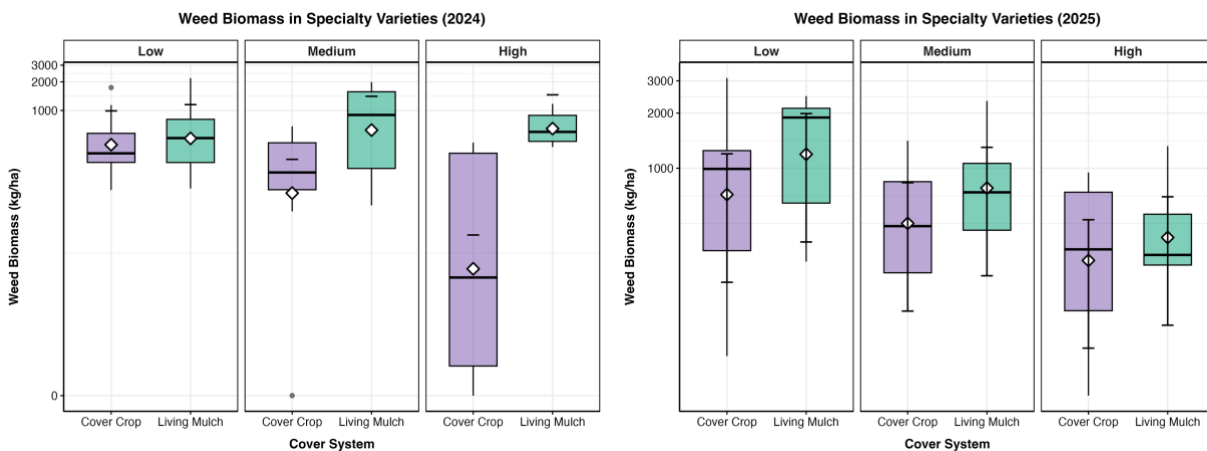


Figure E. Fall weed biomass (kg ha^{-1}) by management system across 2024 and 2025. Boxplots show the distribution of observed values, while points and error bars represent model-estimated means $\pm 95\%$ confidence intervals (back-transformed from log scale). Weed biomass was greater in LIV than CON systems in both 2024 and 2025, but this difference was typically non-significant.

To further explore weed biomass dynamics, we calculated a weed biomass delta (the change in weed biomass over the season from summer to fall) and fitted mixed-effects models for each year with the weed biomass delta as the main response. In 2024, management system significantly influenced weed biomass accumulation ($p = 0.006$) while variety, density, and all interactions were non-significant (all $p > 0.15$). Pairwise comparisons between management systems and averaged across varieties and densities revealed that LIV plots accumulated 412 kg ha^{-1} more weed biomass over the season than CON plots ($p = 0.006$), indicating that the LIV system was associated with greater net weed growth between summer and fall (Figure F).

In 2025, management system, along with variety and all interactions, did not significantly influence weed biomass change (all $p > 0.31$). Planting density did influence weed biomass change ($p = 0.003$), with higher densities associated with lower weed biomass accumulation. Pairwise comparisons within densities showed that across all densities, no significant difference in weed biomass accumulation was seen between management systems (all $p > 0.29$). ANCOVA models comparing spring, summer, and fall weed biomass demonstrated that spring and summer weeds did not influence weed biomass in the fall (both $p > 0.2$). These results indicate that planting density may suppress weed growth regardless of management system, but these effects are not consistent across years (Figure F).

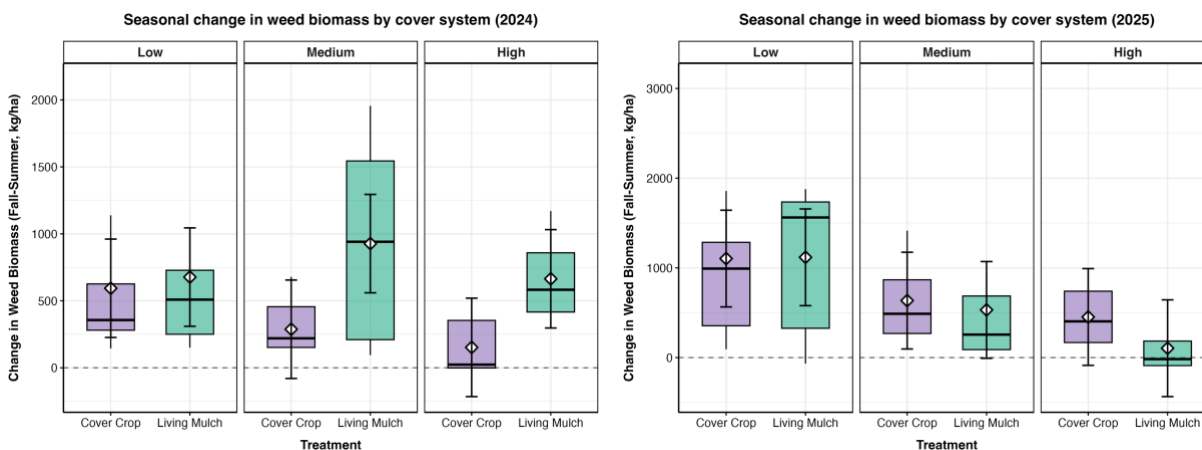


Figure F. Seasonal change in weed biomass (fall – summer; kg ha^{-1}) by management system in specialty varieties across 2024 and 2025. Boxes and points show raw data; diamonds and error bars show model-estimated means $\pm$ 95 % CI. In 2024, management system significantly influenced weed biomass change, with LIV plots showing greater weed growth than CON plots at medium and high densities. In 2025, management system did not significantly influence weed biomass change while planting density did, with weed growth declining with increasing density regardless of management system.

To evaluate how living mulch biomass contributed to weed suppression, we modeled the relationship between clover and weed biomass in LIV plots for each year. Weed biomass was log transformed to address heteroscedasticity. Unlike the grain maize experiment, clover biomass in the specialty varieties experiment did not significantly influence fall weed biomass in both years, although both years saw a negative relationship between weed and clover biomass (2024: $p = 0.306$; 2025: $p = 0.743$). The wide confidence intervals seen in Figure G reflect that considerable uncertainty in the estimated relationship influenced these results.

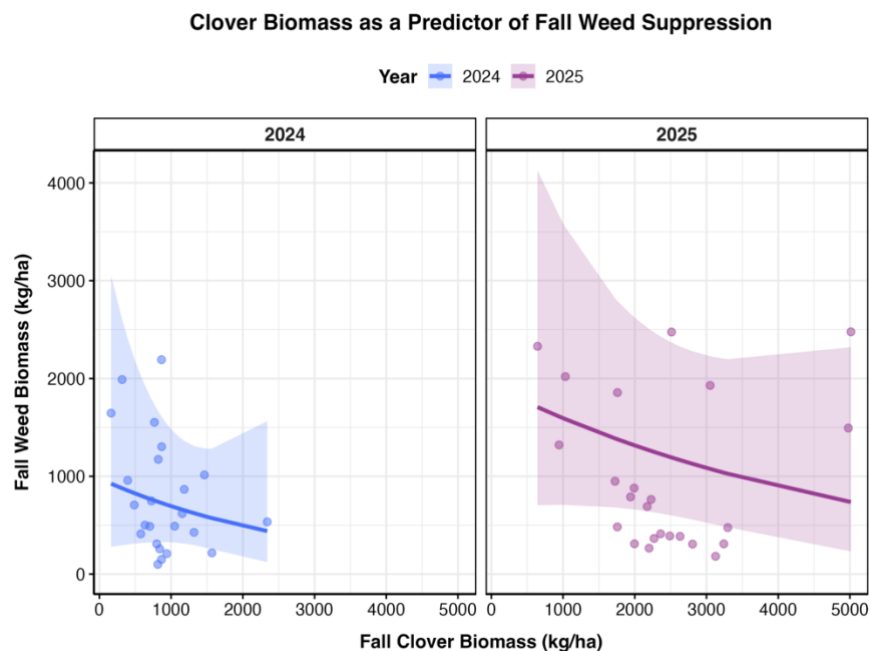


Figure G. Relationship between fall clover biomass and fall weed biomass in SPM LIV plots in 2024 and 2025. Lines represent population-level model predictions from a linear mixed model; shaded ranges indicate 95 % confidence intervals. Weed biomass was $\log(x+1)$ -transformed for model fitting; predictions are back-transformed to the original scale. The negative slopes in both years show a suppressive effect of clover biomass on weeds, but wide confidence intervals reflect that neither relationship was statistically significant.