

CROP PRODUCTION AND SOIL HEALTH TRADEOFFS OF BETWEEN-ROW WEED
AND SOIL MANAGEMENT STRATEGIES IN ORGANIC PLASTICULTURE VEGETABLE
PRODUCTION

By

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ABSTRACT

CROP PRODUCTION AND SOIL HEALTH TRADEOFFS OF BETWEEN-ROW WEED AND SOIL MANAGEMENT STRATEGIES IN ORGANIC PLASTICULTURE VEGETABLE PRODUCTION

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Strategies organic producers employ to manage weeds between plastic mulch beds vary in their efficacy and in important systems-level influences on the cropping system. The goal of this study was to identify tradeoffs of commonly used between-row weed-soil management strategies in organic plasticulture vegetable production. Between-row management strategies evaluated included cultivation, rye residue dead mulch, mowed weeds, and three living mulch species: rye (*Secale cereale* L.) monoculture, Italian ryegrass (*Lolium multiflorum*) monoculture, and a rye-Dutch white clover (*Trifolium repens*) mixture. Cultivation and dead mulch provided greater in-season weed control and reduced weed seedbank contributions more than living mulches. Despite physical separation, plants growing between plastic mulch beds showed the potential to compete for in-row resources. However, these results were only observed in bell pepper in a relatively dry year, with yellow summer squash yields unaffected across years and treatments. Plants growing between plastic mulch beds also showed the potential to reduce inorganic nitrogen leaching. Additionally, vegetative covers and rye residue dead mulch increased extracellular enzyme activity compared to cultivation, suggesting potential improvements in nutrient cycling. In a separate cover crop screen to evaluate species suitable as living mulches in plasticulture production, a significant negative correlation between weed and living mulch biomass was observed, with teff producing the most biomass and suppressing in-season weeds more than any other species in the screen.

This thesis is dedicated to
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KEY TO ABBREVIATIONS

BG	β -1,4-glucosidase
CBH	cellobiohydrolase
DW	Dutch white clover
EEA	extracellular enzyme assay
H	Shannon diversity index
LAP	leucine amino hydrolase
MBC	microbial biomass carbon
MC	methyl coumarin
MSU	Michigan State University
MUB	methylumbelliferone
N	nitrogen
NAG	β -1-2-N-acetylglucosaminidase
NZ	New Zealand white clover
PHOS	phosphate-monoester phosphohydrolase
SOM	soil organic matter
SWMREC	Southwest Michigan Research and Extension Center

INTRODUCTION

Since its introduction to agriculture in the 1950's, the use of polyethylene plastic mulch films has become standard practice in high-value crop production (Lamont, 2017). Plastic mulches are available in a variety of colors and thicknesses to support specific agronomic goals. Most generally, plastic mulch film covers the soil causing microclimate changes that increase yield and improve quality. Aside from economic returns, plastic mulch also provides excellent in-row weed control, protects the soil underneath from erosion, and reduces evaporation of irrigation water (Tarara, 2000; Wilhoit and Coolong, 2013). While implementation of plastic mulching systems can be expensive, increased profits from higher yield, improved quality, and growing season extensions offset the costs for many high value crops (Steinmetz et al., 2016; Tarara, 2000). In 2006, it was estimated that plastic mulch covered roughly 162,000 hectares in the United States (Miles et al., 2012).

Although plastic mulch provides agronomic advantages, the long-term environmental sustainability of plastic mulch has been called into question (Steinmetz et al., 2016). One area of concern is the waste produced by plastic mulch, as plastic mulch and plastic drip irrigation lines are not recyclable and are typically only used for a single cropping cycle (Kasirajan and Ngouajio, 2012). This problem has been addressed with the development of biodegradable plastic materials (Kasirajan and Ngouajio, 2012). However, major problems related to soil conservation remain. For example, the soil warming properties of plastic mulch that lead to increased crop yields and an extended growing season also accelerate the degradation of soil organic matter (Schonbeck, 1999; Steinmetz et al., 2016). In addition, many growers manage weeds between plastic mulch beds through cultivation or herbicides, leaving the soil bare and highly susceptible to erosion. With roughly 50-75% of a field covered with impermeable plastic,

sediment loss and associated agrochemical runoff in the between-row area can be greatly intensified during rain events (Arnold et al., 2004; Rice et al., 2004; Wan and El-Swaify, 1999; Zhang et al., 2013). Confronting these issues will be necessary to promote both environmentally and economically sustainable vegetable production systems in the future.

Covering bare soil between plastic mulch beds with plant residues or living mulches can mitigate erosion issues and improve the soil through organic matter inputs, while also providing between-row weed control. However, these systems also have associated challenges. For example, if not produced on-farm, plant residue mulches can be expensive or contaminated with weed seeds, exacerbating future weed challenges (Brown and Gallandt, 2018). On-farm mulch production could eliminate this risk and reduce potentially high transport costs, but this is not always feasible, particularly on small acreage (Law et al., 2006). Furthermore, as plant residue mulches decompose over the season, their ability to suppress weeds can become compromised, necessitating additional applications (Teasdale and Mohler, 2000).

Planting a cover crop between plastic mulch beds as living mulch is an attractive alternative to cultivation and dead plant residue mulches. Living mulches are cover crops planted during the growing season with or between cash crops that protect the soil with a thick canopy above ground and soil-stabilizing roots below ground (Hartwig and Ammon, 2002). Living mulches can effectively suppress weeds and protect the soil from erosion, all while allowing farmers to grow an income-generating cash crop alongside a soil-building cover crop (Hartwig and Ammon, 2002). Living mulch between plastic mulch beds have been shown to reduce the runoff of agricultural chemicals by more than 70% (Rice et al., 2004). Additionally, with spatial separation between the cash crop and a living mulch growing in the between-row area, challenges with competitive inhibition commonly observed in other living mulch systems may be

reduced. Although these advantages can alleviate many of the issues seen in other systems, the use of living mulches is still made challenging by other factors like variable weed control and additional seed and management costs (Altieri et al., 1985). While interest in the integration of living mulches in plastic mulch systems is growing, information regarding cover crop selection, cash crop selection, and best management practices are lacking.

Weeds can serve many of the same functions as a living mulch without associated seed costs (Blaix et al., 2018). Ambient weeds could act as a free living mulch, with mowing used to minimize weed seed production (Baker and Mohler, 2015). Gibson et al. (2011) found that in some cases mowing alone could reduce additions to the weed seedbank to levels equivalent to a no seed threshold. However, this result was not consistent across years, demonstrating the importance and difficulty in properly timing mowing events. Furthermore, mowing alone can select for low-growing weed species that escape mower blades, leading to a shift in the weed community over time (Butler, 2012; Gibson et al., 2011; Villalobos et al., 2016). Some weeds can also serve as alternative hosts for diseases and insects that can be detrimental to cash crop production (Altieri et al., 1985).

Conclusions and Research Objectives

When making management decisions, organic growers are faced with tough short- and long-term tradeoffs (Zwickle et al., 2016) related to weed control and soil management. As of yet, tradeoffs associated with between-row management strategies have not been widely studied. The aim of this research was not to identify a single “best” strategy, but to evaluate the tradeoffs associated with individual management practice and advance research in plastic mulch-living mulch systems. This information will equip farmers with the knowledge to make decisions best suited to their management goals.

The objectives of the current research were to 1) identify the influence of various between-row weed and soil management practices including living and dead mulches on systems-level tradeoffs related to production (Chapter I), 2) determine the impact of these strategies on soil microbial communities (Chapter II), and 3) screen a range of cover crops species representing three functional groups for their suitability as a living mulch in a mixed plasticulture-living mulch system (Chapter III).

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CHAPTER I: System Tradeoffs of Alternative Weed-Soil Management Strategies in Organic Plasticulture Vegetable Production

Abstract

Plastic mulch films used in fresh-market, organic vegetable production provide excellent in-row weed control. However, weeds between plastic mulch beds remain a challenge. Weed management strategies in the between-row area vary in their efficacy and effects on other aspects of the agroecosystem. Thus, identifying tradeoffs associated with various between-row weed management practices is necessary for organic growers to make informed management decisions. A two-year experiment was conducted in southwest Michigan to address between-row management practice tradeoffs related to weed management, labor requirements, crop production, and nitrogen and soil organic matter dynamics. Strategies evaluated included wheel-hoe cultivation, rye (*Secale cereale* L.) residue dead mulch, mowed weeds, and three living mulch treatments: Italian ryegrass (*Lolium multiflorum*) monoculture, rye monoculture, and a rye-Dutch white clover (*Trifolium repens*) mixture. The treatments were implemented between plastic mulch beds planted with green bell peppers (*Capsicum annuum* cv. Paladin) and yellow summer squash (*Cucurbita pepo* cv. Lioness). Our results demonstrate that cultivation and dead mulch provide greater in-season weed control, with associated reductions in the weed seedbank, compared to living mulches and mowed weeds. Additionally, plants growing between plastic mulch beds were shown to compete with bell pepper cash crops for in-row resources in a dry year. However, cultivating between plastic mulch beds took over twice as long as mowing weeds and living mulches in both years. Furthermore, plants growing between plastic mulch beds showed the potential to reduce nitrogen leaching compared to cultivated and dead mulch plots. Results from this study can be used by growers to make decisions most applicable to their farm, considering specific environmental and agronomic goals.

Introduction

Effective weed management in organic production relies on the integration of multiple tactics to ensure effective weed control (Baker and Mohler, 2015). The use of plastic mulch film is common in the production of many fresh market vegetables because it provides excellent in-row weed control in addition to other crop production benefits (Kasirajan and Ngouajio, 2012). However, despite these advantages, the impervious surface of plastic films can lead to more aggressive soil erosion between plastic mulch beds, as well as increase the risk of leaching and runoff of nutrients and agrochemicals compared to bare ground production (Arnold et al., 2004; Rice et al., 2004; Wan and El-Swaify, 1999).

While plastic mulch can effectively suppress the majority of in-row weeds, additional weed management strategies must be employed to control weeds between plastic mulch beds (Bonanno, 1996). Without the benefit of affordable synthetic herbicides, organic producers most often rely on cultivating these between-row areas. Some choose an alternative practice such as dead mulching, cover crop living mulches, or simply mowing ambient weeds when other approaches fail. These practices are known to vary in their efficacy and in important systems-level influences on the cropping system as a whole (Mirsky et al., 2010; Mohler, 1996). Potential advantages and disadvantages of common practices are summarized in Table 1.1. Informed grower decision-making regarding the use of these between-row weed-soil management strategies in organic plasticulture production requires a more detailed understanding of the tradeoffs associated with each practice (Baker and Mohler, 2015).

While cultivation can effectively reduce in-season weed pressure and suppress weed seed production, excessive cultivation can lead to soil degradation and leave bare ground highly susceptible to erosion, agrochemical runoff, and muddy harvesting conditions (Brennan and

Smith, 2018). Additionally, successful cultivation schedules can be labor intensive and easily hindered by labor shortages, weather, and soil conditions (Chen et al., 2017).

Dead mulches can mitigate some of these challenges by protecting the soil (Wilhoit and Coolong, 2013), though also lead to a new set of management problems. If not produced on-farm, dead mulches can be expensive and contaminated with weed seeds, making weed problems worse (Law et al., 2006; Schonbeck, 1999). Furthermore, heavy residues make weed escapes in dead mulches difficult to control with traditional cultivation tools, requiring specialized high residue cultivation equipment or hand-weeding (Brainard et al., 2013; Zinati et al., 2017).

Covering the soil instead with live vegetation can protect the soil with an aboveground canopy and soil-stabilizing roots, as well as provide important regulatory and supporting ecosystem services like nutrient cycling, alternative habitat for beneficial insects, and enhance on-farm biodiversity (Baraibar et al., 2018; Blaix et al., 2018; Blanco-Canqui et al., 2015). Because of these advantages, there is high grower interest in the integration of a living mulch within plasticulture systems. However, there is relatively little research in this area, and benefits need to be placed within the context of additional seed and management costs, variable living mulch establishment, unreliable weed suppression, and the potential to compete with a cash crop (Brainard et al., 2012).

Among these challenges, potential competition with the cash crop is especially important. While we would expect plastic mulch to serve as a spatial and physical barrier to competition, previous research has still shown evidence of competition (Table 1.2). For example, Reid and Klotzbach (2013) found that cereal rye growing between plastic mulch beds reduced onion and tomato yields by 21% relative to when the between-row area was maintained as bare ground. Similarly, Chen et al. (2017) found that when weeds grew between plastic beds, eggplant and

sweet corn yields were reduced by 6% and 40% respectively compared to when the between-row area was cultivated. In contrast, Nelson and Gleason (2018) saw equivalent yields in acorn squash grown on plastic mulch with living mulch or corn stover dead mulch between-row. Similarly, Warren et al. (2015), found that equivalent yields could be achieved in broccoli grown on plastic mulch with living mulch and cultivated between-row areas by increasing nitrogen fertilization. However, these results were not consistent across the two years of the study.

Mowing ambient weeds can be a free source of soil protecting vegetative cover (Hartwig and Ammon, 2002), and is a common practice that growers adopt when other management strategies fail. Potential benefits can be counterbalanced with possible cash crop interference, uneven soil coverage, and if not mowed regularly, the production of weed seeds that worsen future weed problems (Kazakou et al., 2016). However, even though this is common practice in organic systems, the costs and benefits of this strategy have not been evaluated in the literature.

The most effective weed management practices in organic plasticulture production will ultimately dependent on a grower's unique goals and cropping system context. Quantifying important system tradeoffs of management practices, as well as understanding impacts in both an in-season production and long-term soil management context, will be necessary for growers to make informed management decisions (Brown and Gallandt, 2018; DeDecker et al., 2014). The objective of the current research was to investigate the mechanisms and effects of alternative between-row weed and soil management strategies in organic plasticulture production of bell pepper and yellow squash—including quantifying system tradeoffs related to weed control, crop productivity, labor requirements, and soil nitrogen (N) and organic matter dynamics.

Table 1.1. Summary of tradeoffs associated with commonly used between-row weed-soil management practices.

System	Advantages	Disadvantages
Cultivation	<ul style="list-style-type: none"> • Eliminate potential competition • Very little or no weed seed rain 	<ul style="list-style-type: none"> • Enhanced soil erosion • Increased agrochemical runoff • Potential muddy harvesting conditions • Labor intensive
Dead Mulch	<ul style="list-style-type: none"> • Soil protection • Additions of organic matter • Low effort weed suppression 	<ul style="list-style-type: none"> • Expensive off-farm input • Contamination with weed or grain seed • Difficult to manage weed escapes
Mowed Weeds	<ul style="list-style-type: none"> • Soil protection • Free • Additions of organic matter • Provide ecosystem services • Enhance on-farm biodiversity 	<ul style="list-style-type: none"> • Exacerbate weed problems • Unmanageable plant community • Uneven soil coverage • Potential competition with cash crop • Reliance on mowing schedule
Living Mulch	<ul style="list-style-type: none"> • Soil protection • Additions of organic matter • Uniform plant community • Unique cover crop window • Provide ecosystem services • Enhance on-farm biodiversity 	<ul style="list-style-type: none"> • Seed cost • Additional crop management • Variable weed suppression • Potential competition with cash crop • Reliance on mowing schedule

Table 1.2. Summary of previous studies evaluating the influence of various vegetative between-row covers on cash crop yield in plastic mulch production.

Cash Crop	Vegetative Cover	Comparison ¹	Yield Response ²	Citation
Acorn squash	annual ryegrass/red clover ²	corn stover	equal	Nelson and Gleason, 2018
Broccoli	Italian ryegrass/white clover	cultivation	mixed	Warren et al., 2015
Eggplant	weeds	cultivation	reduced	Chen et al., 2017
Muskmelon	annual ryegrass/red clover	corn stover	reduced	Nelson and Gleason, 2018
Onion	rye	cultivation	reduced	Reid and Klotzbach, 2013
Onion	triticale	cultivation	reduced	Ivy et al., 2014
Onion	annual ryegrass	cultivation	reduced	Ivy et al., 2014
Onion	triticale/Dutch white clover	cultivation	reduced	Ivy et al., 2014
Pepper	Dutch white clover	cultivation	reduced	Law et al., 2006
Pepper	weeds	cultivation	reduced	Law et al., 2006
Sweet corn	weeds	cultivation	reduced	Chen et al., 2018
Tomato	rye	cultivation	reduced	Reid and Klotzbach, 2013
Tomato	red clover	cultivation	reduced	Butler, 2012

¹ Comparison column represents the between-row management strategy used as a comparison to vegetative cover

² Yield response is reported as differences in the vegetative cover treatment relative to the comparison treatment

³ Vegetative cover written as crop one/crop two implies that the living mulch was a mixture of the two crops

Materials and Methods

Site Description

In May 2017 and 2018 a field experiment comparing different between-row management strategies in organic plasticulture vegetable production was conducted at the Michigan State University (MSU) Southwest Michigan Research and Education Center (SWMREC) in Benton Harbor, Michigan (42°N, 86°W). The experimental treatments were located in the same field and plot boundaries in both years of the study in order to investigate the combined in-season and cumulative impacts of between-row weed management practices on soil quality parameters and weed management. The soil at the experimental site was a Spinks (Sandy, mixed, mesic Lamellic Hapludalfs) and Selfridge (Loamy, mixed, active, mesic Aquic Arenic Hapludalfs) loamy sand. Initial soil chemical characteristics were as follows: organic matter 1.6%; pH 5.9; CEC 4.0 cmol kg⁻¹ ; and P, K, and Mg levels of 48, 133, and 54 mg kg⁻¹ respectively. The area used for the experimental site was a transitioning organic field that had been maintained as a rye-hairy vetch fallow for five years prior to trial establishment.

Experimental Design

Between-row management strategies evaluated included cultivated bare ground, cereal rye residue dead mulch, mowed ambient weeds, and three cover crop living mulch species: rye (*Secale cereale* L.) monoculture, Italian ryegrass (*Lolium multiflorum*) monoculture, and a rye-Dutch white clover (*Trifolium repens*) mixture. The experiment was arranged in split-plot randomized complete block design with four replications. Between-row management was the main plot factor and cash crop was the sub-plot factor. Cash crops included green bell peppers (*Capsicum annuum* cv. Paladin) and yellow summer squash (*Cucurbita pepo* cv. Lioness). Bell peppers and summer squash were chosen to represent a relatively long- and short-season crop

commonly grown in plasticulture systems. While main plot (between-row management) treatments were maintained in the same locations in both years of the study, the location of sub-plots (cash crop treatments) within main plots were rotated between years. Main plots measured 6.1 m wide x 5.0 m row length, contained four raised plastic mulch beds (each 0.61 m wide and 0.15 m tall), and three between-row soil areas that measured approximately 1 m wide.

Field Management

Dates of key field operations are outlined in Table 1.3. Existing plant cover was mowed and incorporated with a rototiller in early May of both years. In mid-May, the field received 112 kg total N ha⁻¹ as organic fertilizer (Nature Safe “All-Season 10-2-8,” Irving, TX) derived from feather meal, meat and bone meal, blood meal, and sulfate of potash. Plastic mulch beds (0.15 m bed height and 0.61 m bed top width) were laid on 1.67 m centers within 1 wk of fertilizer application using 1.22 m wide, 1 mil thick embossed black plastic mulch (Trickl-Eez Irrigation Inc., St. Joseph, MI), over single drip irrigation lines with 0.30 m emitter spacing (149 L 30.5 m⁻¹ h⁻¹), using a combined plastic mulch layer and bed shaper (Reddick Equipment Company LLC, Williamston, NC).

Bell peppers (‘Paladin’) were sown in 98 cell flats in a heated greenhouse at the end of March and transplanted into the field at the end of May. Yellow summer squash was direct seeded in the field at pepper transplanting. Peppers were grown staked in off-set double rows with 0.30 m between-row spacing and 0.46 m in-row spacing. Squash were grown in single rows with 0.61 m in-row spacing. All beds were drip irrigated simultaneously on a regular schedule during the summer, with two 20 min irrigation intervals per day per standard grower practice in the area.

Between-row management strategies were established at the same time as cash crop planting in both years. In 2017, plastic mulch beds were laid 1 wk before implementing between-row management treatments and a stale seed cultivation was completed in all between-row areas immediately before living mulch seeding in order to reduce potential weed competition. However, wetter spring conditions in 2018 did not allow for the same stale seed bed window between bed formation and establishing between-row treatments.

Living mulch cover crops were broadcast sown by hand into between-row areas and lightly incorporated using a rake. Italian ryegrass, rye, and Dutch white clover-rye treatments were seeded at 35.9 kg ha⁻¹, 168.1 kg ha⁻¹, and 22.4 kg ha⁻¹ respectively in 2017. In 2018, the rye seeding rate was doubled to 336.3 kg ha⁻¹, in an attempt to address poor establishment in 2017. In both years, Dutch white clover was seeded with rye at half the monoculture rate (84 and 168 kg ha⁻¹ in 2017 and 2018, respectively) to act as a nurse crop. Overhead irrigation was applied to benefit living mulch establishment in 2017, but not in 2018 due to higher precipitation around the time of seeding in that year. Between-row living mulches and weeds were mowed 2-3 times each season (2017: July 5, July 18, and August 2; 2018: July 19 and August 7) using a walk behind push mower with the mower deck set to 10.16 cm. Since broadleaf summer annual weeds were typically the tallest plant in the between-row area, mowing events were determined by the height of weeds. Cultivated plots were maintained through hand cultivation using a wheel-hoe with a 20 cm blade (Glaser wheel hoe, Johnny's selected seeds, Fairfield, ME). Dead mulch consisted primarily of cereal rye obtained from an adjacent field that had been established the previous fall and flail mowed two days before mulching. Mulch was applied at a rate of approximately 18 MT ha⁻¹. After mulch was applied, a 0.25 m² subsample was weighed and returned to the field. Since the mulch was still green, a smaller subsample was taken, dried to a

constant weight, and used to obtain a fresh to dry weight correction factor ($\text{g dry g}^{-1} \text{ wet}$). In order to obtain the mulch application rate, the 0.25 m^2 subsample that was weighed in the field was then multiplied by this correction factor, and converted to $\text{MT ha}^{-1}\text{t}$.

Data Collection

A summary of data collected is outlined in Table 1.4.

Cover crop and weed biomass: Biomass samples in dead mulch, mowed weeds, and living mulch plots were taken from permanent 0.125 m^2 quadrats in each between-row area per plot prior to all mowing events and at experiment termination in both years. Biomass was collected above 10.16 cm from ground level prior to mowing events (to reflect height of mowing) and to the ground at experiment termination. Weed and cover crop biomass were separated and dried to a constant weight at 60°C before dry weight determination. Ambient weeds in cultivated plots in 2017 were negligible, and thus biomass was not collected in cultivated plots in that year. However, in 2018, wet conditions caused delayed cultivation and greater weed escapes. Before the last cultivation event weed above ground biomass was collected in two replications from three 0.125 m^2 quadrats.

Labor requirements: The time required to cultivate and mow the bare ground cultivated and living mulch/weedy treatments, respectively, was measured after workers had gained experience with both methods in 2017. As a result, time requirements were estimated based on two of three mowing events, and three of four cultivation events in 2017. In 2018, time requirements for all mowing and cultivation events were recorded. Labor times were converted to minutes 10-m^{-1} row for more meaningful comparisons between treatments.

Vegetable crop performance: Peppers were harvested every 7-10 days, for a total of five harvest events in 2017 and four in 2018. Summer squash was harvested every other day for a

total of 10 harvest events in each year. Squash and pepper were harvested from the beds in the middle of each plot, with buffer plants between plots. This method resulted in a total of eight summer squash and 20 pepper data plants per plot. Harvested squash fruit were categorized based on market standards as either marketable (length between 11-23 cm) or unmarketable (over 23 cm in length, deformed, scarred, damaged by insects or decay), counted, and weighed. Harvested pepper fruit were categorized into either marketable (U.S. Fancy, U.S. No. 1, and U.S. No. 2) or unmarketable (deformed, damaged by sunscald, disease, insects, or decay) based on USDA standards (Agricultural Marketing Service, 2005), counted, and weighed. Cumulative yield in each season was obtained by summing individual harvests, and total yields were adjusted to the number of surviving data plants in each plot for analysis on a per plant basis.

In-row soil nitrogen and moisture dynamics: In-row soil samples were collected under plastic mulch beds for each crop (pepper and squash) on a monthly basis starting at trial establishment and analyzed for inorganic N concentration (NO_3^- and NH_4^+). All soil samples consisted of 10 composited cores per bed taken to 20 cm depth (2.22 cm inside diameter) and stored at 4°C until dried at 38°C for at least 36 h prior to passing through a 2 mm sieve. 10 g dried and sieved soil was extracted with 50 ml 1M KCl, and extracts were analyzed for NO_3^- and NH_4^+ concentrations using a Lachat injection flow autoanalyzer (Lachat QuickChem, Hach Company, Loveland, CO).

In-row soil volumetric water content in the top 20 cm of soil was also monitored regularly using a FieldScout time-domain reflectometer probe (Spectrum Technologies Inc., Aurora, IL). Measurements were taken to avoid close proximity to irrigation lines. 10 measurements were taken in each subplot (cash crop) and averaged before analysis.

Between-row weed seedbank, soil nitrogen retention, and soil organic matter: Soil samples to evaluate the weed seedbank after the 2017 season were collected on April 19, 2019. Eight soil cores were taken to a depth of 20 cm (2.22 cm inside diameter), matching the fall tillage depth, per between-row area for a total of 24 cores per plot. The eight cores per between-row area were homogenized and stored at 4°C before transferring to the greenhouse for weed seed germination within a week of field collection. 400 ml of field soil was mixed with 400 ml potting mix before the mixture was placed on top of a 25.4 cm x 25.4 cm tray filled with 3 L of potting soil. Noseeum mesh separated the bottom potting soil from the field soil mixture. Subsurface irrigation was used to maintain moisture near or at field capacity. Weed seedlings were counted and removed at least once a week for three weeks. Subsequently, soils were air dried for two weeks, before rewetting and a second flush of germinated weed seedling were counted and removed.

One 2.54 cm diameter soil core per main plot was collected to 60 cm depth in September of each year to evaluate potentially leachable nitrogen remaining in the soil profile at the end of each season. Prior to sampling, each between-row area was examined to choose a location that was representative of the plot and treatment. Continuous cores were collected into plastic liners using a steel sampling tube driven into the ground by a portable gas-powered hammer (AMS Inc., American Fall, ID). Cores were transported from the field and stored at 4°C until being separated into 20 cm depth intervals, processed, and analyzed for inorganic N concentration as described above for regular season soil N measurements.

Soil organic matter (SOM) was evaluated at trial initiation in May 2017 and again at trial termination in August of 2018. Soil samples consisted of 12 composited cores per plot taken to 20 cm depth (2.22 cm inside diameter) and were stored at 4°C until dried at 38°C for at least 36 h

prior to passing through a 2 mm sieve. SOM was determined from a subsample of the sieved sample using the loss on ignition method.

Statistical Analysis

All data was analyzed separately by year and mean separations were conducted using Fisher's protected least significant difference test at $P < 0.05$. Total weed and cover crop biomass and cash crop yield data were analyzed using a mixed model ANOVA with the MIXED procedure in SAS (Version 9.4, SAS Institute, Cary, NC). Treatment was treated as a fixed factor and block (replication) as a random factor. When necessary, data were either log transformed or unequal variance models used in order to satisfy assumptions of normality and equal variance. Data presented are not transformed.

Weed seedbank data was analyzed using a mixed model ANOVA with the MIXED procedure in SAS. Treatment was treated as a fixed factor and block (replication) as a random factor. Data was log transformed to meet assumption of normality. Untransformed data are presented.

Total in-row soil inorganic N and soil moisture were analyzed using repeated measures mixed model ANOVA using the MIXED procedure in SAS. Treatment and sampling date were treated as fixed factors and block (replication) as a random factor. Assumptions of normality were checked before proceeding with analysis and heterogeneous variance was corrected with the appropriate variance-covariance structure. A first-order autoregressive variance-covariance structure was used given the roughly equally spaced timings of inorganic N and soil moisture measurements. Model selection was verified by fit statistics. Soil profile nitrogen was also analyzed using repeated measures mixed model ANOVA using the MIXED procedure in SAS. Treatment and depth were treated as fixed factors and block (replication) as a random factor. Fit

statistics were used to determine the most appropriate variance-covariance structure. An equal variance first-order autoregressive variance-covariance structure was used.

Table 1.3. Dates of key field operations.

Activity	2017	2018
CC mowed/incorporated	12/15 May	4/7 May
Field fertilized	22 May	22 May
Plastic laid	22 May	29 May
Living mulch planted	31 May	29 May
First mowing event	5 July	19 July
Second mowing event	18 July	7 Aug
Third mowing event	2 Aug	---
Squash harvest ¹	7 July-1 Aug	9 July-30 July
Pepper harvest ²	28 July-25 Aug	3 Aug-28 Aug

¹ Squash was harvested a total of 10 times in both years

² Pepper was harvested a total of five times in 2017 and four in 2018

Table 1.4. Data collected to evaluate potential tradeoffs among between-row management strategies.

Potential Tradeoff	Metric
Weed suppression	<ul style="list-style-type: none"> • Cover crop and weed biomass collection • Weed seedbank
Labor requirements	<ul style="list-style-type: none"> • Mowing time • Cultivation time
Vegetable crop performance	<ul style="list-style-type: none"> • Crop yield • Crop plant biomass
Potential competitive factors	<ul style="list-style-type: none"> • In-row nitrogen • In-row soil moisture • Cash crop leaf tissue analysis
Nitrogen retention	<ul style="list-style-type: none"> • Deep soil cores
Soil health	<ul style="list-style-type: none"> • Soil organic matter

Results

Weather

Average temperatures were similar in both years of the study and comparable to the 30-yr average for the location, with the exception of relatively higher temperatures in May 2018 (Table 1.5). Compared to seasonal precipitation averages, 2017 was a relatively dry year and 2018 a relatively wet year. Spring rainfall in 2018 was much heavier than in 2017, with over two and three times more rain in May and June, respectively. Within the week following living mulch sowing, the field received 1.5 mm of rain in 2017 compared to 44.2 mm in 2018. In 2017, overhead irrigation was used two weeks following living mulch sowing to encourage establishment. Overhead irrigation was not necessary in 2018, as heavy rain caused flooding in some areas of the field in late May and early June, and may have hindered cover crop establishment.

Table 1.5. Monthly temperature averages and precipitation totals during the 2017 and 2018 season and the reported 30 year averages.

	Average Temperature			Precipitation		
	2017	2018	30-yr ¹	2017	2018	30-yr
	-----°C-----			-----mm-----		
May	13.9	18.1	14.7	68.3	183.6	90.7
June	20.9	20.6	20.2	46.7	140.2	84.6
July	21.6	22.5	22.6	134.6	54.4	85.9
August	20.0	22.5	21.7	22.6	90.7	95.3
September	18.9	19.3	17.8	64.77	81.3	100.3

¹30-yr average temperature and precipitation reported by NOAA for Benton Harbor, Michigan from 1981-2010. Retrieved from <https://www.ncdc.noaa.gov/cdo-web/datatools/normals>; verified on February 15, 2019.

Cover Crop and Weed Biomass Production

Cumulative above-ground living mulch biomass production was greater in 2017 than in 2018, reflecting differences in establishment between the two years. Ryegrass produced significantly more biomass than rye or clover in both years (Figure 1.1). However, living mulch growth was generally modest, with the greatest cover crop biomass produced by ryegrass in 2017 at only 191 dry g m⁻² (Table 1.6). In both years, rye establishment was poor, and the development of leaf rust by mid-July nearly eliminated stands. Clover was slow to establish in 2017 and failed to establish in some plots in 2018.

As a result of poor cover crop living mulch establishment and competitiveness, total plant biomass production in all living mulch treatments was dominated by weeds. Greater overall biomass was produced in 2018 compared to 2017. No significant differences in cumulative biomass production were observed in 2017. However, in 2018, the clover-rye treatment had greater total biomass production relative to other living mulches and the weedy treatment (Figure 1.1). Dominant weed species in the field included crabgrass species (*Digitaria* spp.), common lambsquarters (*Chenopodium album* L.), ladythumb (*Persicaria maculosa*), oak-leaf goosefoot (*Chenopodium glaucum*), purslane (*Portulaca oleracea* L.), ragweed species (*Ambrosia* spp.), and wood sorrel species (*Oxalis* spp.). Perennial weeds including quackgrass (*Elymus repens*) and dock species (*Rumex* spp.) were common in localized areas of the field in 2017, but were much less abundant in 2018.

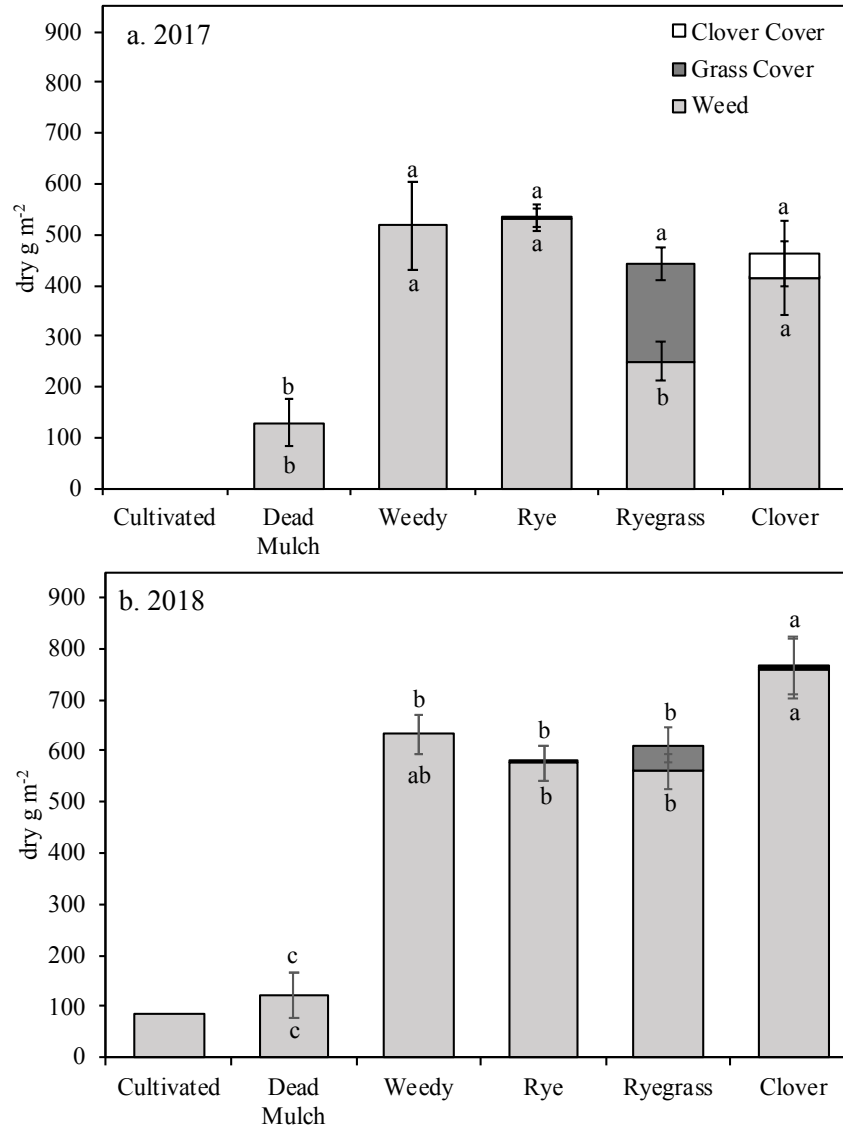


Figure 1.1. Mean cover and weed biomass production in a) 2017 and b) 2018. Error bars represent \pm standard error around cumulative weed biomass mean and cumulative total (weed biomass + living mulch biomass) biomass mean. Letters above bars represent significant differences related to cumulative total biomass production ($P < 0.05$). Letters within bars represent significant differences related to cumulative weed biomass ($P < 0.05$).

Table 1.6. Mean (standard error) of cumulative living mulch biomass production, percent reduction of in-season weed biomass, and germinable weed seedbank after the 2017 season. Means within columns followed by the same letter are not significantly different ($P < 0.05$).

	Biomass		Weed reduction ¹		Germinable weed seedbank ²	
	2017	2018	2017	2018	2018	
	-----g m ⁻² -----		-----%-----		seeds 500 ml ⁻¹ soil	
Cultivated	---	-	---	-	100	-
Weedy	---	-	---	-	87	-
Dead Mulch	---	-	---	-	75 (15)	a
Rye	6.57 (0.58)	c	0.43 (0.43)	c	9 (18)	c
Ryegrass	191.67 (28.93)	a	50.65 (19.37)	a	9 (3)	b
Clover ³	98.81 (10.29)	b	7.95 (2.51)	b	12 (8)	b
					0 (0)	b
					147 (14)	cd

¹Reduction of in-season weed biomass compared to the weedy control

²Soil to evaluate the weed seedbank was collected in early spring 2018, the seedbank following the 2018 has not been evaluated

³Biomass presented is cumulative clover and rye biomass

Weed Suppression

Cultivation and dead mulch reduced in-season weed biomass more than any of the living mulch treatments (Figure 1.1). Weed biomass production in the dead mulch treatment was similar in both years at 130 and 122 dry g m⁻² in 2017 and 2018 respectively. The cultivation schedule in 2017 resulted in these plots remaining weed-free during the season. Conversely in 2018, wet field conditions and delayed cultivation resulted in some weed biomass production in the cultivated treatment at the end of July. Since weed biomass in the cultivated treatment was collected in only two of four treatment replicates in 2018, this treatment was excluded from statistical analysis. However, cumulative weed biomass production in the cultivated treatment appeared to be substantially less than all treatments other than the dead mulch. Ryegrass reduced weed biomass by 51% compared to the weedy check in 2017 (Figure 1.1a), but had no detectable effect on weeds in 2018 (Figure 1.1b). In comparison, weed suppression by rye and clover was highly variable and neither significantly reduced in-season weed biomass compared to the weedy control in either year.

The 2017 pattern of in-season weed suppression was reflected in estimates of the germinable weed seedbank from between-row areas in spring 2018. The cultivated treatment had the smallest weed seedbank with 68 seeds 500 ml⁻¹ soil, while the weedy treatment was over three times greater (Table 1.6). The seedbank in dead mulch was not significantly different from cultivated plots. In the ryegrass treatment, the seedbank was less than half of the weedy check and was similar to the dead mulch treatment.

Labor Requirements

To maintain clear between-row areas for ease of harvesting, vegetative covers were mowed regularly. Since vegetation was dominated by weeds in weedy and living mulch plots, mowing events were determined by the height of weeds. Cultivation took 2 to 3 times longer than mowing living covers and weeds in both years (Table 1.7). However, additional management challenges in mowed plots are not reflected in the management time presented. Primarily, weeds along the edge of plastic mulch beds were difficult to mow with our method, and mowed plots often required additional hand removal of weeds that spread from the edge into the crop row. Mowing selected for prostrate growing weeds, particularly crabgrass, ladysthumb, and oak-leaf goosefoot. These weeds would spread along the edge of plastic mulch beds and, as the season progressed, into the crop row. In an attempt to mediate this challenge, weeds were hand cultivated along the edge of plastic early in the 2018 season. Hand cultivation along the edge of plastic was relatively undemanding, taking about 9.6 sec 10 m⁻¹. This strategy alleviated some management problems at the first mowing; however, effective mowing still proved challenging at the second event.

Table 1.7. Average labor time associated with differing management requirements.

	Date		Time	
	2017	2018	----min 10 m ⁻¹ ----	
Cultivation 1	6/20	6/14	2.80	3.23
Cultivation 2	7/6	7/3	2.86	2.42
Cultivation 3	7/18	8/7	2.56	7.71
Cultivation 4 ¹	8/2	---	---	---
Mowing 1 ²	7/5	7/19	---	1.48
Mowing 2	7/18	8/7	1.06	1.64
Mowing 3	8/2	---	2.73	1.56
Cultivation Avg.	---	---	2.74	4.45
Mowing Avg.	---	---	1.90	1.56

¹Time to cultivate at last cultivation in 2017 was not recorded

²Time to mow at the first event in 2017 was not recorded

Cash Crop Performance

Peppers. Pepper yield and pepper plant biomass were greater in the cultivated treatment compared to all other treatments in 2017 (Table 1.8; Table 1.10). Mulch treatments resulted in pepper yield losses of between 46 and 60% in 2017, but had no effect on yields in 2018.

Total N concentration in pepper leaves sampled at the last harvest was greater in the cultivated treatment relative to all other treatments in 2017 (Table 1.11). However, no significant differences in soil inorganic N levels in the crop row were resolved among treatments, and considerable plant available N (ranging from 28 to 57 mg N kg soil⁻¹ in weedy and rye treatments respectively) remained under pepper plastic mulch beds at the end of the season. Though not significantly different, in-row soil moisture was generally higher in the cultivated control compared to all other treatments during pepper fruit development in 2017 (Figure 1.2).

Squash. Squash yields and squash plant biomass were unaffected by between-row treatment in both years (Table 1.9; Table 1.10). Similarly, plant tissue N analysis at final harvest

did not reveal differences among treatments (Table 1.11). Additionally, soil moisture and inorganic N in squash rows were consistent across treatments and years (Figure 1.3).

Table 1.8. Mean (standard error) of pepper total and marketable yield in 2017 and 2018. Means within columns followed by the same letter are not significantly different ($P<0.05$).

	2017		2018	
	<u>Total¹</u>	<u>Marketable²</u>	<u>Total</u>	<u>Marketable</u>
	-----kg plant ⁻¹ -----			
Cultivated	1.42 (0.08) a	1.32 (0.11) a	0.97 (0.11)	0.87 (0.09)
Dead Mulch	0.88 (0.15) b	0.70 (0.11) b	0.99 (0.10)	0.85 (0.08)
Weedy	0.80 (0.05) b	0.58 (0.08) b	0.99 (0.09)	0.89 (0.08)
Rye	0.80 (0.05) b	0.61 (0.06) b	0.84 (0.10)	0.72 (0.10)
Ryegrass	0.75 (0.11) b	0.58 (0.09) b	0.92 (0.07)	0.79 (0.07)
Clover	0.68 (0.07) b	0.51 (0.01) b	0.98 (0.06)	0.86 (0.06)

¹Includes marketable and cull fruit
²Excludes damaged or misshapen fruit

Table 1.9. Mean (standard error) of squash total and marketable yield in 2017 and 2018.

	2017		2018	
	<u>Total¹</u>	<u>Marketable²</u>	<u>Total</u>	<u>Marketable</u>
	-----kg plant ⁻¹ -----			
Cultivated	2.4 (0.3)	1.8 (0.2)	2.2 (0.2)	1.8 (0.2)
Dead Mulch	2.5 (0.2)	2.0 (0.2)	2.2 (0.1)	1.9 (0.1)
Weedy	2.7 (0.1)	2.1 (0.1)	2.4 (0.1)	2.0 (0.2)
Rye	2.4 (0.2)	1.7 (0.2)	2.0 (0.1)	1.7 (0.1)
Ryegrass	2.2 (0.3)	1.8 (0.2)	1.9 (0.2)	1.7 (0.2)
Clover	2.0 (0.1)	1.6 (0.1)	2.0 (0.2)	1.7 (0.2)

¹Includes marketable and cull fruit
²Free of defects, measure under 22.86 cm in length, and tender skinned

Table 1.10. Mean (standard error) of pepper and squash dry plant biomass at the end of harvesting in 2017 and 2018. Means within columns followed by the same letter are not significantly different ($P<0.05$).

	Pepper			Squash	
	2017		2018	2017	2018
	-----g dry plant ⁻¹ -----				
Cultivated	61.5 (4.7)	a	64.9 (7.6)	237.6 (28.4)	302.1 (38.2)
Dead Mulch	37.5 (6.1)	b	62.8 (10.1)	243.5 (30.8)	314.5 (12.1)
Weedy	40.4 (3.5)	b	54.2 (2.7)	248.2 (16.7)	302.8 (22.6)
Rye	36.9 (3.7)	b	53.4 (3.5)	217.5 (11.1)	295.6 (14.8)
Ryegrass	45.6 (5.3)	b	49.0 (5.0)	234.4 (13.3)	279.5 (15.8)
Clover	45.1 (5.9)	b	56.3 (1.7)	226.9 (13.6)	281.9 (26.8)

Table 1.11. Mean (standard error) of percent nitrogen in leaf tissue at final harvest in 2017 and 2018. Means within columns followed by the same letter are not significantly different ($P<0.05$).

	Pepper			Squash	
	2017		2018	2017	2018 ¹
	-----% N-----				
Cultivated	3.36 (0.16)	a	2.72 (0.10)	3.77 (0.16)	1.72 (0.10)
Dead Mulch	3.04 (0.09)	b	2.81 (0.24)	3.87 (0.36)	1.69 (0.17)
Weedy	2.61 (0.10)	c	2.66 (0.10)	3.62 (0.11)	1.56 (0.10)
Rye	2.58 (0.05)	c	2.69 (0.15)	3.42 (0.22)	1.66 (0.05)
Ryegrass	2.48 (0.05)	c	2.72 (0.06)	3.25 (0.11)	1.63 (0.04)
Clover	2.52 (0.07)	c	2.81 (0.17)	3.06 (0.14)	1.56 (0.08)

¹Whole squash plants were analyzed in 2018

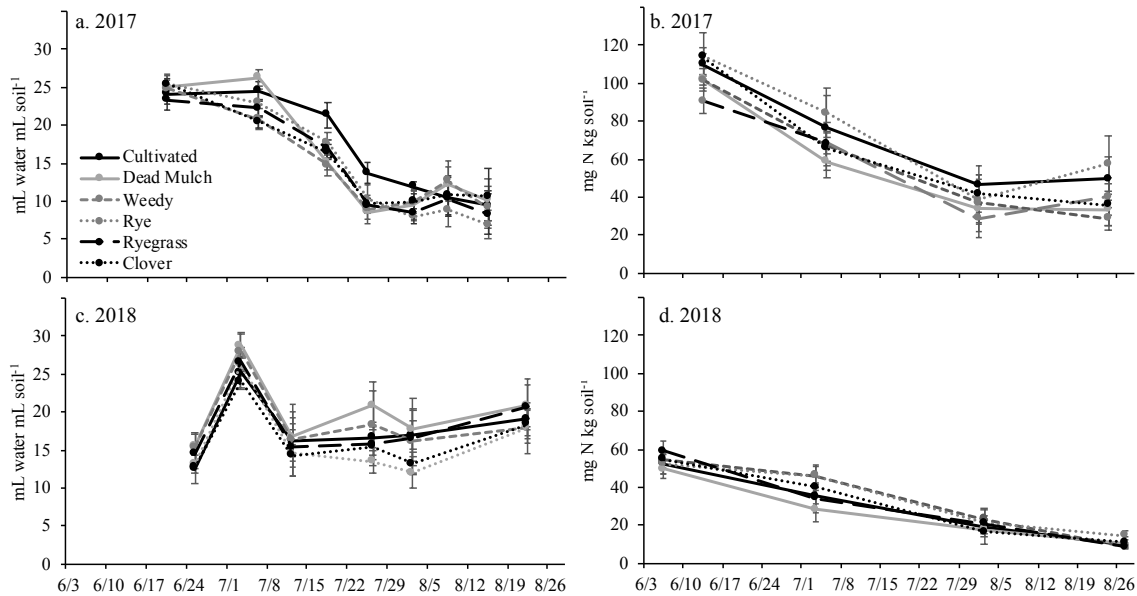


Figure 1.2. Mean of a) 2017 in-row soil moisture, b) 2017 in-row inorganic nitrogen, c) 2018 in-row soil moisture, and d) 2018 in-row inorganic nitrogen in the top 20 cm of pepper beds. Error bars represent \pm standard error.

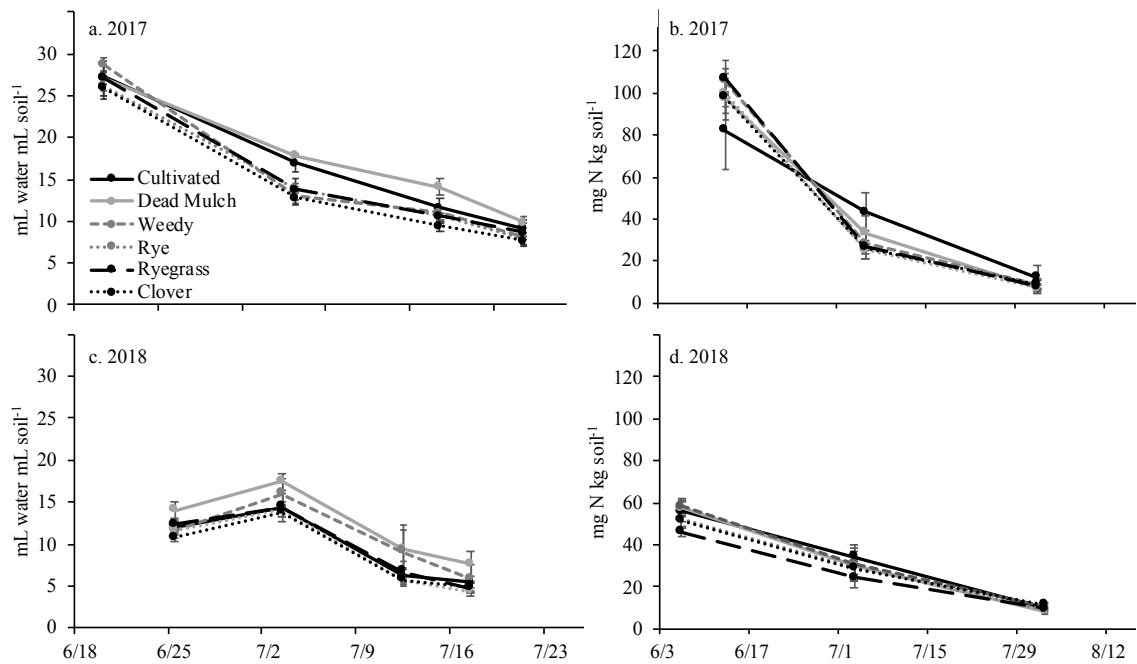


Figure 1.3. Mean of a) 2017 in-row soil moisture, b) 2017 in-row inorganic nitrogen, c) 2018 in-row soil moisture, and d) 2018 in-row inorganic nitrogen in the top 20 cm of squash beds. Error bars represent \pm standard error.

Between-Row Nitrogen Retention and Soil Organic Matter

Where plants were actively growing during the 2017 season, potentially leachable N in the soil profile was reduced (Figure 1.4). Differences in soil profile N were most pronounced in the top 20 cm, and decreased with increasing soil depth. In 2018, no significant differences in potentially leachable nitrogen were observed.

Shoot biomass inputs into mowed weeds and living mulch treatments averaged 490 g dry m⁻² in 2017 and 647 g m⁻². In comparison, dead mulch was applied at an average rate of 1,778 g m⁻² and had an additional input of 130 g m⁻² of weed shoot biomass in 2017 and 122 g m⁻² in 2018. Weed biomass production in the cultivated treatments was negligible in 2017 and averaged 85 g m⁻² in 2018. Despite these differences in organic matter inputs between treatments, SOM measured at trial establishment in 2017 and at trial termination in 2018 was not different among treatments. Across the field, SOM decreased an average of 0.2% in all between-row areas. Initial SOM measurements were as follows: cultivated 1.48±0.13; dead mulch 1.50±0.22; weedy 1.80±0.14; rye living mulch 1.48±0.10; ryegrass living mulch 1.43±0.17; clover-rye living mulch 1.55±0.06. SOM measurements at trial termination were as follows: cultivated 1.25±0.13; dead mulch 1.33±0.10; weedy 1.58±0.17; rye living mulch 1.35±0.13; ryegrass living mulch 1.33±0.13; clover-rye living mulch 1.28±0.17.

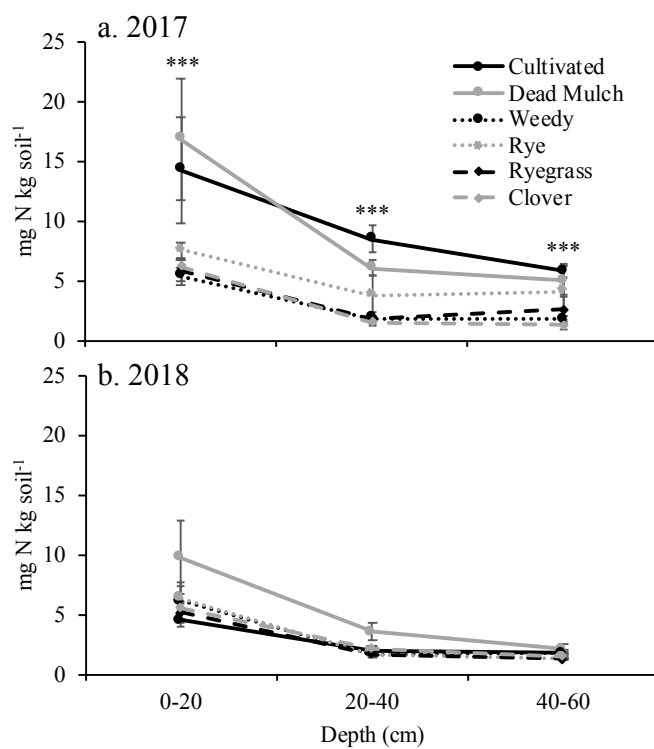


Figure 1.4. Mean of inorganic nitrogen at depth measured before fall tillage in a) 2017 and b) 2018. Error bars represent \pm standard error. Significance specified at *** $P < 0.001$.

Discussion

The objective of this research was to quantify inherent tradeoffs associated with various between-row management strategies in plasticulture vegetable production, with a focus on weed management, cash crop performance, soil nitrogen and organic matter dynamics, and labor requirements. Additionally, given grower interest in integrated plastic mulch-living mulch systems, and the relative lack of recommendations, we were seeking to identify some of the specific advantages and disadvantages of utilizing cover crop living mulches in this setting. As expected, between-row management strategies differed in their effects on the measured tradeoffs and are addressed in detail below.

Living mulch establishment was a challenge in both years. Though only three living mulch species were included in this trial, they were chosen to reflect a range of desirable living mulch characteristics. Rye was chosen because of its ability to establish rapidly, produce allelochemicals, and remain low growing in absence of vernalization (Ateh and Doll, 1996; Walters and Young, 2008). In contrast, Italian ryegrass is a warm-season, turf type grass that was chosen because of its ability to effectively cover the ground while remaining low growing (Masiunas et al., 1997; Sarrantonio and Gallandt, 2003). With the ability to fix atmospheric nitrogen, clover was chosen because of its presumed adaptability to the low fertility between-row area (Hartwig and Ammon, 2002; Verret et al., 2017). In this study, the quick germination and season-long vigor of Italian ryegrass was important for establishment in a field with heavy weed pressure. Conversely, rye and clover were easily outcompeted by ambient weeds in both years (Figure 1.1). With a relatively slow growth rate, clover was unable to establish competitive stands before aggressive annual weeds. Similar results of poor clover living mulch establishment have been observed previously (Law et al. 2006) and suggest that additional management would

be necessary in promoting clover as a living mulch species in an annual system. Low rye biomass accumulation may be attributed to the combination of summer stress, a low growth habit that was often below the biomass sampling height, and the development of leaf rust by mid-July that nearly eliminated stands. However, rye was planted outside of its climatic niche in this study, and may have performed better if planted in cooler temperatures. For example, Brainard and Bellinder (2004) found that rye emerged more rapidly and was better able to compete with Powell amaranth when grown under cool (15°C/10°C day/night) compared to warm (30°C/25°C day/night) initial temperatures.

The importance of living mulch establishment and biomass accumulation in weed suppression observed in this study has been previously demonstrated in several studies (e.g. Alonso-Ayuso, et al., 2018; Bybee-Finley et al., 2017). Italian ryegrass produced more biomass than either rye or clover in both years and was the only living mulch species to significantly decrease in-season weeds, reducing weed biomass by >50% compared to the weedy check in 2017 (Table 1.6). However, cultivation and dead mulch reduced in-season weed biomass more than the best living mulch treatment in both years. Additionally, the inconsistency of ryegrass performance between years reveals inherent risks in managing weeds with cover crops (Teasdale, 1996; Sarrantonio and Gallandt, 2003).

Our results confirm that uncontrolled weeds during the summer have the potential to contribute to long-term weed challenges via additions to the weed seedbank. The weed seedbank after the 2017 season followed a trend similar to weed biomass production in that year, with the cultivated treatment having the smallest seedbank and the weedy check having the largest (Table 1.6). Reductions in weed biomass by Italian ryegrass in 2017 resulted in a 55% reduction in the weed seedbank compared to the weedy check. Reductions in weed seed production and seed

viability in the presence of competition have been previously cited (Nurse and DiTommaso, 2005), and may explain these modest reductions in the germinable weed seedbank even in weak performing living mulch treatments. Alternatively, the weed seedbank could have been impacted by differences in weed seed predation or pathogens that affect seed mortality. However, even though declines in the weed seedbank were measured, weed biomass production in 2018 exceeded that in 2017 with no significant differences in weed biomass between the weedy check and living mulch treatments (Figure 1.1). Favorable warm and wet conditions in 2018 may explain greater weed biomass production in that year. Additionally, weeds may have compensated for reduced density with greater biomass accumulation per plant (Teasdale, 1996). These results suggest that if reducing weed pressure is a primary interest, the use of heavy residue mulches or cultivation should be used.

Though cultivation provided more effective weed control than living mulches, mowing living mulches and weeds generally took less time than cultivation. However, it should be noted that mowing times were only recorded after trialing various methods. A walk-behind mower was ultimately used because it did not damage plastic mulch beds and was narrow enough to fit in the between-row areas. Nevertheless, weeds with a spreading growth habit (i.e. crabgrass, ladysthumb, and oak-leaf goosefoot) often needed to be pulled from the edge of plastic and from the crop row before mowing. Even after this additional step, these weeds could escape the mower blade. The reported mowing time does not take into account this extra step in the mowing process or capture general frustrations in mowing a diverse plant community with dissimilar growth habits. In addition, these results may change if tractor-mounted mechanical cultivation equipment was used instead of manual cultivation. Furthermore, contradictory to the results in this study, Pfeiffer et al. (2015) found management time was greater in mowed living mulches

plots compared to hand-cultivation in broccoli, bell pepper, and snap bean production. These conflicting results may be attributed to differences in mowing thresholds, with Pfeiffer et al. (2015) mowing living cover plots four to six times per season.

A primary limitation of living mulch adoption by growers is the risk of cash crop interference (Hartwig and Ammon, 2002). While plastic mulch has the potential to serve as a spatial buffer and physical barrier to competition, previous studies investigating the potential of vegetative covers between plastic mulch beds have demonstrated variable effects on cash crop yields (Table 1.2), suggesting competition between cash crops and between-row plants is still a challenge in some cases. Bell peppers and summer squash were included in this study to represent a relatively long- and short-season crop with variable competitive abilities. The results from this study contribute to the existent body of literature that crop response to between-row management is crop and year dependent.

Pepper plant biomass and yields were reduced in all treatments relative to the cultivated control in 2017 (Table 1.8). Since weed biomass was far greater than cover crop biomass in living mulch treatments, this reduction was likely due to competition predominately from weeds. Differences in pepper leaf nitrogen status at the end of the season suggested nitrogen competition (Table 1.11), though in-row soil inorganic nitrogen was similar across treatments during the season, and nitrogen remaining in the soil at the end of the season ranged from 28 to 57 mg N kg soil⁻¹; levels that do not indicate nitrogen limitations (Figure 1.2). However, in-row soil moisture during pepper fruit development was consistently lower in all treatments compared to the cultivated control, suggesting water stress likely contributed to yield reductions (Figure 1.2). Differences in in-row soil moisture may have also impaired nitrogen transport and uptake,

explaining disparities in leaf nitrogen status and contributing to reduced plant biomass and yields.

Similar pepper yield reductions in the dead mulch and vegetative cover treatments are initially surprising given significant differences in plant biomass growing in the between-row among these treatments. However, weeds that were present in the dead mulch treatment were concentrated along the edge of beds where they are the most likely to compete for in-row resources.

Aside from potential competitive inhibition leading to yield differences among treatments, a cooler microclimate caused by between-row plant transpiration may have contributed to reduced yields in all treatments relative to the cultivated treatment. However, microclimate temperature was not monitored in this study. Another possible explanation of yield differences may be the induction of a shade-avoidance response. Even after eliminating potential N competition by increasing fertility rates, yields of broccoli grown on plastic mulch beds were reduced where living mulch was grown in the between-row compared to when between-row areas were left weed-free (Warren et al. 2015). Though it was not measured directly, the authors suggested that differences in crop yields resulted from a shade avoidance response.

Pepper yield and plant biomass were not significantly different across treatments in 2018. Similar pepper yields across treatments in 2018 may be attributed to heavier precipitation and steadier in-row soil moisture during the 2018 season across treatments. Additionally, peppers were subjected to higher temperatures at the time of transplanting in 2018 relative to 2017, causing girdling of the stem near the level of plastic mulch in 2018 that was not seen in 2017. This initial stress may have also contributed to more uniform yield results across treatments in 2018.

In comparison to variable pepper performance, squash plant biomass and yields were unaffected by treatment in both years. Additionally, regular monitoring of in-row soil moisture and N revealed similar levels across treatments in 2017 and 2018 (Figure 1.3). Likewise, squash leaf tissue N measured at the end of the season was consistent among treatments (Table 1.11). As a short-season crop with a rapid growth rate, squash may have had a greater competitive advantage over between-row vegetation. In addition, the squash canopy extended over plastic mulch beds, shading plants along bed edges that are most likely to compete for in-row resources.

Understanding variation in crop response to between-row management could help growers optimize management decisions. While the goal of some organic growers is to maintain the area between plastic mulch beds as bare ground, labor shortages or weather can disrupt cultivation schedules. As weeds grow beyond what is manageable by cultivation tools, mowing weeds is used as a last resort. By focusing resources on crops that appear to benefit most from cultivation, a grower can ensure the highest yields in all crops. For example, in this study, summer squash yield was unaffected by between-row management strategy in either year. Therefore, delayed or suspended cultivation in summer squash may be less detrimental than in sensitive crops like pepper. Additionally, strategies that could be employed to mitigate potential crop yield reductions include keeping the between-row area weed-free for some critical period unique to plastic mulch systems before planting living mulch, applying a dead mulch, or mowing ambient weeds. Research in inter-seeding living mulches in vegetable cropping systems has revealed great promise in delayed planting to mitigate yield penalties (Brainard and Bellinder, 2004; Ciaccia et al., 2015; Gibson et al., 2011). Though previous research has been done to evaluate the influence of in-row weeds on plasticulture vegetable production, (Bertucci et al., 2019; Buckelew et al., 2006; Chaudhari et al., 2016; Garvey et al., 2013; Norsworthy et al.,

2008) to the best of our knowledge, research identifying the critical weed-free period for plants growing between plastic mulch beds has not been done.

While living mulches and weeds can present challenges related to weed control and cash crop performance, their potential to provide other ecosystem services may still support their adoption. For example, the maintenance of vegetative covers during the season lead to average shoot residue contributions of 4900 kg ha⁻¹ and 6460 kg ha⁻¹ to between-row area in 2017 and 2018 respectively. Considering typical biomass production of a productive summer cover crop like sorghum-sudangrass in the north central United States of 4000-6000 kg ha⁻¹ (Clark, 2007), this represents a significant addition of organic material, even without considering root biomass contributions. However, residue additions were only representing 40% of the soil surface (the between-row area). This may help explain why residue inputs did not translate into detectable differences in SOM over the relatively short experimental period. Furthermore, changes in SOM are particularly difficult to detect, especially on sandy soils (Tiemann and Grandy, 2015).

In addition to the potential soil improvement benefits of vegetative cover, weeds and living mulches can also reduce agrochemical runoff and leaching. This can be particularly beneficial in plasticulture systems where agricultural runoff and erosion can be markedly higher with impermeable plastic concentrating precipitation in the between-row area (Rice et al., 2004). The use of vegetative covers between plastic mulch beds have repeatedly been shown to reduce soil and agrochemical runoff (Arnold et al., 2004; Nachimuthu et al. , 2017; Rice et al., 2007, 2004; Wan and El-Swaify, 1999). Rice et al. (2004) found an up to eight-fold decrease in runoff volumes from tomatoes grown on plastic mulch with rye living mulch grown between rows compared to when this area was maintained as bare ground.

Though surface runoff and erosion were not evaluated in this study, vegetative cover treatments did reduce potentially leachable N by 61% in the soil profile (0-60 cm) compared to cultivated and dead mulch treatments at the end of the 2017 season (Figure 1.4). However, these results were not replicated in 2018. Differences in rainfall between the two years may explain these conflicting results. In May and June of 2018, heavy rain caused flooding in some areas of the field. Anaerobic conditions encourage denitrification, and heavy rain can induce N leaching. Similar levels of N in the soil profile of cultivate, weedy, and living mulch plots could be explained by the loss of N from cultivated plots via denitrification and leaching and the assimilation of N in plant biomass in weedy and living mulch treatments. Higher levels of N in the top 20 cm of soil in the dead mulch treatment relative to the other treatments may be explained by anaerobic conditions inhibiting nitrification during particularly wet times, and thus preserving inorganic N in the less mobile ammonium (NH_4^+) form. This is supported by a more than 8 times greater NH_4^+ level in the dead mulch treatment relative to vegetative cover and cultivation in June 2018. Higher N levels in the dead mulch treatment in 2018 may also be explained by mineralization of the previous year's dead mulch residue. Though potentially leachable N results were not consistent across years in this study, the potential for reducing environmental contamination is an important consideration for organic growers who are typically more willing to forgo short-term yield advantages for environmental safety (DeDecker et al., 2014). Additionally, N retained in the vegetative biomass may become available to subsequent crops with decomposition of organic matter, thus improving nutrient cycling (Blanco-Canqui et al., 2015).

Conclusions

In conclusion, we found that cultivation and dead mulch between plastic mulch beds provided greater in-season weed control and reduced contributions to the weed seedbank more than the use of living mulch cover crops. With the exception of Italian ryegrass in one year, living mulch cover crops were largely ineffective at suppressing weeds under the conditions of this study, and vegetation in living mulch treatments was largely dominated by weed biomass at rates similar to the mowed ambient weeds treatment. Between-row management influenced vegetable crop performance, depending on the crop grown and conditions during the season. Summer squash appears particularly robust to competition from between-row vegetation, with yields unaffected by between-row management in either year. However, all treatments relative to the cultivated control reduced pepper yields in one year, and evidence of water competition from between-row vegetation may explain yield reduction. Pepper yields were not reduced relative to the cultivated control in the second year of the study under conditions of higher moisture. While living mulches between plastic mulch beds did not provide adequate weed control, reductions in nitrogen leaching and possible reductions in labor time could counterbalance these drawbacks. In addition, other ecosystem services not measured in this study, including biological pest control, increased farm biodiversity, and reduced soil erosion could be improved with the integration of vegetative covers between plastic mulch beds (Bond and Grundy, 2001; Teasdale, 1996). Future research should seek to investigate these other potential advantages, particularly as the demand for sustainable intensification necessitates the quantification of larger agroecosystem impacts beyond provisioning services (Garnett et al., 2013). For farmers interested in promoting these services, attention to cash crop selection, fertilizer and irrigation management, and living mulch management and species selection may help reduce negative impacts to productivity.

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CHAPTER II: Influence of Between-Row Weed and Soil Management on Soil Microbial Communities

Abstract

The use of black plastic mulch films in fresh-market vegetable production is common because it often leads to higher total and marketable yields; however, cultivation used to manage weeds between plastic mulch beds leaves the soil bare, and combined with the greater impervious surface of plastic mulch can greatly increase erosion potential in plasticulture production. The use of organic mulches between plastic mulch beds can protect the soil from erosion and contribute organic matter, with differences in organic mulch quantity and quality having distinct influences on the soil microbial community. A two-year experiment was conducted in southwest Michigan to identify the impact of different between-row mulch types on the soil microbial community in organic plasticulture vegetable production. Strategies for evaluation were chosen to represent commonly used management practices and included cultivation, rye residue dead mulch, and vegetative cover. To investigate the in-season and cumulative impact of between-row weed and soil management practices on soil microbial communities, the study was repeated in the same location in both years. No significant differences in microbial biomass carbon or the Shannon diversity index calculated from extracellular enzyme activity were observed in either year of this study. However, significantly greater total enzyme activity in dead mulch and vegetative cover treatments and significant differences between individual enzymes indicate the development of divergent soil microbial communities driven by between-row management strategy.

Introduction

Without the use of chemical fertilizers in organic farming, crop production is optimized through the enhancement of soil physical, chemical, and biological properties, with an emphasis on soil organic matter (SOM) management (Watson et al., 2002). However, vegetable cropping systems often rely on intensive management that can have negative impacts on soil health (Norris and Congreves, 2018). For example, the use of plastic mulch films in organic and conventional fresh market vegetable production is common because it often leads to higher total and marketable yields (Kasirajan and Ngouajio, 2012). However, the microclimate changes responsible for increased productivity (Tarara, 2000) can also lead to accelerated SOM degradation (Steinmetz et al., 2016). Additionally, cultivation used to manage weeds between plastic mulch beds, leaves the soil bare and highly susceptible to erosion (Rice et al., 2004). Thus, while plastic mulch films can result in short-term yield gains, associated soil degradation may negatively impact future productivity (Steinmetz et al., 2016). Given that the use of plastic mulches are common in organic vegetable cropping systems, addressing soil related challenges in plasticulture systems is necessary in advancing sustainable production.

Replacing cultivation in between-row areas with organic mulches has the potential to provide excellent between-row weed control (Wilhoit and Coolong, 2013), while alleviating soil degradation from erosion and excessive cultivation. Organic mulches can mediate erosion by reducing raindrop impact, increasing water infiltration, and decreasing runoff velocity (Smets et al., 2008). In addition to reducing erosion, organic mulches can improve soil health by contributing organic matter to support the soil microbial community. However, previous research has focused on mitigating erosion (Rice et al., 2004; Wan and El-Swaify, 1999; Zhang et al., 2013), without addressing the influence of between-row management strategies on the soil

biological properties. With a growing consensus that microbial-derived compounds are a crucial component of stable SOM (Bradford et al., 2013; Grandy and Neff, 2008; Lehmann and Kleber, 2015), managing for soil microbe abundance and activity can be a critical for SOM improvement.

Organic mulches include both dead and living mulches. Examples of dead mulches include plant residues, straw, compost, and wood chips. Growers may also use cover crops planted as living mulches, or commonly when other strategies fail, resort to mowing weeds between plastic mulch beds. While mowing ambient weeds is not always a planned weed management tactic, this strategy can be functionally similar to planting a cover crop living mulch, providing both dead shoot biomass and root exudates to feed the microbial community (Hartwig and Ammon, 2002).

Though dead mulches and vegetative covers protect the soil, and provide a carbon (C) source for soil microbes, differences in residue quality likely confer community structure and function differences (Bending et al., 2002; Wardle et al., 2004). Notably, vegetative covers as planted living mulches or mowed weeds add organic residues through continuous root exudates as they are growing and via shoot biomass after death, with roots alone having been shown to increase microbial biomass more than shoots (Buyer et al., 2010). Thus, we would expect that vegetative covers would be able to support a larger microbial population by supplying greater labile C inputs and more diverse C sources than either cultivation or dead mulching.

The objective of this study was to evaluate how between-row management in organic plasticulture vegetable production systems influence soil microbial communities. We hypothesized that the presence of either a dead rye mulch or living vegetative cover between plastic mulch beds would increase soil microbial biomass compared to bare soil achieved

through repeated cultivation. Furthermore, we hypothesized that the more diverse organic inputs from root exudates and shoots of living mulches would result in a more functionally diverse microbial community compared to dead mulch and bare soil treatments.

Materials and Methods

Site Description

In May 2017 and 2018 a field experiment comparing different between-row management strategies in organic plasticulture vegetable production was conducted at the Michigan State University (MSU) Southwest Michigan Research and Education Center (SWMREC) in Benton Harbor, Michigan (42°N, 86° W). To investigate the cumulative impact of between-row weed management practices on soil microbial communities, the trial was placed in the same field with treatments in the same areas in both years of the experiment. The soil at this site is a Spinks and Selfridge loamy sand, with initial soil chemical characteristics as follows: organic matter 1.6%; pH 5.9; CEC 4.0 cmol kg⁻¹; and P, K, and Mg levels of 48, 133, and 54 mg kg⁻¹ respectively. The area used for this experiment was a transitioning organic field that had been maintained as a rye-hairy vetch fallow for five years prior to trial establishment.

Experimental Design

Between-row management strategies evaluated included bare ground, dead mulch, mowed weeds, rye (*Secale cereale*) living mulch, Italian ryegrass (*Lolium multiflorum*) living mulch, and a rye-Dutch white clover (*Trifolium repens*) mixture living mulch. The experiment was arranged in a randomized complete block design with between-row management as the main plot factor. Main plots measured 6.1 m x 5.0 m, contained four plastic mulch beds planted with either bell pepper (*Capsicum annuum* cv. Paladin) or summer squash (*Cucurbita pepo* cv. Lioness), and three between-row areas measuring approximately 1 m wide.

Field Management

Winter cover crops were mowed and incorporated by rototiller in early May of both years. In mid-May, individual plots received 112 kg total N ha⁻¹ Nature Safe (Irving, TX) All-

Season Fertilizer 10-2-8 (N-P-K) derived from feather meal, meat and bone meal, blood meal, and sulfate of potash. Plastic mulch beds were laid on 1.67 m centers within a week of fertilizer application and raised to 0.15 m. 1.22 m 1 mil embossed black plastic mulch was used, with top of bed widths measuring 0.61 m and single drip lines with 0.30 m emitter spacing installed under plastic.

Between-row management strategies were established at the same time as cash crop planting in both years. In 2017, plastic mulch beds were installed a week before between-row management treatments were implemented to allow for stale seed bedding. However, wetter conditions in spring 2018 did not allow for a delay between plastic mulch bed formation and between-row treatment establishment.

Italian ryegrass, rye and Dutch white clover were seeded at 35.9 kg ha⁻¹, 168.1 kg ha⁻¹, and 22.4 kg ha⁻¹ respectively. In 2018 the rye seeding rate was doubled to 336.3 kg ha⁻¹, in response to poor establishment in 2017. In both years, Dutch white clover was seeded with a half rate of rye monoculture to act as a nurse crop, as clover is slow to establish. Living mulches were broadcast sown and lightly incorporated using a rake. Overhead irrigation was applied in 2017 to encourage living mulch establishment, however heavy precipitation in 2018 mitigated the need in that year. Between-row living mulches and weeds were mowed regularly using a walk behind push mower with the mower deck set to 10.16 cm. Cultivated plots were maintained through wheel-hoe cultivation. Rye residue dead mulch was applied at an estimated rate of 17,800 dry kg ha⁻¹. Mulch was obtained from an adjacent field in both years. In 2017, dead mulch was a 16:1 mixture of rye and hairy vetch, while in 2018 it was rye. In both years rye was cut at anthesis and before vetch flowering in 2017. Weed escapes in the dead mulch treatment were removed from plots.

Data Collection

Biomass samples in weedy and living mulch plots were taken in permanent 0.125 m² quadrats in each between-row area per plot before each mowing event and at experiment termination in both years. Samples taken before mowing were raised to 10.16 cm, to reflect the mowing height, and to the ground at experiment termination. Biomass were separated into cover crop and weed categories and dried to a constant weight at 60°C before dry weight determination.

Soil samples were taken between-row for microbial analysis at monthly intervals starting at trial establishment. Four samples were taken per between-row area in each plot, for a composite sample of 12 cores (20 cm depth by 2.22 cm inside diameter) that were homogenized in the field. Samples were placed in a cooler for temporary storage in the field and during transport to the lab where they were stored at 4°C until processing. Within 1 week of sampling, soil was processed through a 4mm sieve, and sieved samples were evaluated for microbial biomass C and N, gravimetric water content, and a subsample frozen for later use in extracellular enzyme assays. A subsample of the composite sample was also used for inorganic N determination.

Gravimetric water content was determined from a 10 g sieved subsample. Samples were dried at 100°C for at least three days before soil dry weight determination. Gravimetric water (θ) content was calculated by the formula:

$$\theta = \frac{(g \text{ wet soil} - g \text{ dry soil})}{g \text{ dry soil}}$$

Soil used for gravimetric water determination was also used to calculate a wet to dry soil correction factor that could be used to convert soil microbial biomass and extracellular enzyme assay data to a g⁻¹ dry soil basis.

A field moist sieved subsample was subjected to the chloroform-fumigation-extraction method adapted from Vance et al. (1987) to determine microbial biomass C. For each treatment replicate, 8 g of soil was weighted into two 50 ml centrifuge tubes. One sample was immediately extracted with 40 ml 0.5M K₂SO₄ while the other was subjected to fumigation with chloroform stabilized with non-polar hydrocarbons for 24 h. After 24 h, lids were removed and residual chloroform allowed to evaporate from fumigated samples for two hours in a fume hood before also being extracted with 0.5M K₂SO₄. Extracts were kept at -20°C until analysis using a Shimadzu total organic carbon analyzer with total N analyzer (TOC-V cpn, Shimadzu Corporation, Kyoto, Japan) in 2017 and using a Vario TOC Cube (Vario TOC select, Elementar Americas Inc, Ronkonkoma, NY) in 2018. Mass of extractable C in samples was calculated from the raw data using the equation

$$C = EC * \left(\frac{(FW - DW + EV)}{DW} \right)$$

Where C is the extractable carbon in a sample in $\mu\text{g g}^{-1}$ soil, EC is the extractable carbon in a sample in $\mu\text{g mL}^{-1}$ extractant, FW is the fresh weight of the sample; DW is the sample dry weight, and EV is the extractant volume.

Microbial biomass carbon was calculated from the equation

$$MBC = C_f - C_u$$

Where MBC is the microbial biomass carbon, C_f is the extractable carbon in the fumigated sample, and C_u is the extractable carbon in the unfumigated sample. Since an extraction coefficient was not calculated from the sample, and goal was to compare treatment means within study and not between studies, an extraction coefficient was not used in calculating microbial biomass carbon.

Extracellular enzyme assays (EEA) were performed on 1 g subsamples frozen at -80°C for three days and transferred to -20°C long-term storage. EEA performed were to assess the activities of the following eight enzymes: two cellulases (β -1,4-glucosidase (BG) and cellobiohydrolase (CBH)); a chitinase (β -1-2-N-acetylglucosaminidase (NAG)); a peptidase (leucine amino hydrolase (LAP)); urease; an enzyme responsible for releasing phosphorus from soil organic matter (phosphate-monoester phosphohydrolase (PHOS)); phenol oxidase; and peroxidase. Urease, phenol oxidase, and peroxidase were measured using colormetric assays, all other enzymes were measured fluorometrically with corresponding methylumbelliferone (MUB) or methyl coumarin (MC) labeled substrates added to soil slurries in 96-well microplates as described by Saiya-Cork et al. (2002). However, unlike Saiya-Cork et al. (2002), soil slurries were prepared with 125 mL DI H₂O instead of acetate buffer. BG and CBH were added together and analyzed as cellulase activity because trends in activity were similar. Likewise, phenol oxidase activity was low and was added to peroxidase activity to give total oxidase activity. However, when calculating the Shannon diversity index, all enzymes were kept separate.

To evaluate soil functional diversity, the Shannon diversity index was calculated from extracellular enzyme activity as has been described by previous authors (Bending et al., 2002; Zak et al., 1994). The equation used was

$$H = - \sum_{i=1}^R p_i \ln p_i$$

Where p_i is the ratio of activity of a particular enzyme to total extracellular enzyme activity.

Inorganic soil nitrogen (NO₃⁻ and NH₄⁺) analysis was performed on a subsample of the composite sample. The sample was dried at 38°C for at least 36 h. 10 g dry soil were extracted

with 50 ml 1M KCl, and extracts were analyzed for NO_3^- and NH_4^+ concentrations using a Lachat injection flow autoanalyzer (Lachat QuickChem, Hach Company, Loveland, CO).

Statistical Analysis

Data was analyzed separately by year and mean separations were conducted using Fisher's protected least significant difference test at $P < 0.05$. While data were collected in each treatment separately, similarities in plant composition and the lack of significant differences in microbial response between weedy and living mulch treatments encouraged combining these treatments into a single category referred to as vegetative cover, to evaluate meaningful differences between different organic material inputs (low input, dead mulch input, and living plant inputs). All data were analyzed using repeated measures mixed model ANOVA using the MIXED procedure in SAS. Treatment group and sampling date were treated as fixed factors and block (replication) as a random factor. When necessary, data was either square root of log transformed to meet assumptions of normality. Untransformed data are presented. Heterogeneous variance was corrected with the appropriate variance-covariance structure. Variance-covariance structures were selected based on fit statistic results. Slicing to evaluate the significance of treatment group differences within sampling dates was performed when the interaction between sampling date and treatment group was significant at $P < 0.05$.

Results

Weather

Average temperatures were similar in both years and comparable to the area 30-yr average, with the exception of relatively higher temperatures in May 2018 (Table 2.1). Compared to seasonal precipitation averages, 2017 was a relatively dry year and 2018 a relatively wet year. Spring rainfall in 2018 was much heavier than in 2017 with over two and three times more rain in May and June respectively. In 2018 heavy rain caused flooding in some areas of the field in late May and early June.

Table 2.1. Monthly temperature averages and precipitation totals during the 2017 and 2018 season and the reported 30 year averages.

	Average Temperature			Precipitation		
	2017	2018	30-yr ¹	2017	2018	30-yr
	-----°C-----			-----mm-----		
May	13.9	18.1	14.7	68.3	183.6	90.7
June	20.9	20.6	20.2	46.7	140.2	84.6
July	21.6	22.5	22.6	134.6	54.4	85.9
August	20.0	22.5	21.7	22.6	90.7	95.3
September	18.9	19.3	17.8	64.77	81.3	100.3

¹30-yr average temperature and precipitation reported by NOAA for Benton Harbor, Michigan from 1981-2010. Retrieved from <https://www.ncdc.noaa.gov/cdo-web/datatools/normals>; verified on February 15, 2019.

Shoot Biomass Inputs

Initial shoot residue additions in rye residue dead mulch plots were 1778 g m⁻² in both years. This far exceeds the cumulative shoot inputs over the season in vegetative cover plots in either year (Table 2.2). In 2017, the cultivation schedule resulted in minimal weed growth in cultivated plots. Conversely, wet conditions delayed cultivation in 2018, allowing for more weed escapes in the cultivated treatment in that year (Table 2.2). However, weed biomass

contributions were still lower in the cultivated treatment compared to either dead mulch and vegetative cover treatments.

Table 2.2. Mean (standard error) of cumulative living plant shoot biomass production in 2017 and 2018.

	2017	2018
	-----g m ² -----	
Cultivation	0 (0)	85 (-) ¹
Dead Mulch	130 (45)	122 (43)
Vegetation	490 (51)	647 (41)

¹Value represents the average production in two plots and does not have an associated standard error.

Soil Moisture and Between-Row Nitrogen

The wetter season in 2018 is reflected in greater average soil gravimetric water content in that year. Additionally, treatment differences in between-row soil moisture were more apparent in 2017 relative to 2018 (Figure 2.1). Soil moisture was consistently higher in dead mulch plots compared to the cultivated treatment and the vegetative cover treatment group in both years. In 2017, the drier of the two years, soil moisture was generally higher in dead mulch and cultivated treatments compared to vegetative covers. However, in 2018, soil moisture followed similar trends as was seen in dead mulch and the cultivated treatments.

Despite broadcast application of 112 kg total nitrogen (N) ha⁻¹, as organic fertilizer across the entire field prior to plastic mulch bed establishment in both years, soil inorganic N levels were relatively low (Figure 2.2). This was likely the result of the concentration of much of the applied N in-row during bed formation. In 2017, inorganic N levels were the highest in the cultivated treatment for the duration of the season. In 2018, inorganic N levels in the cultivated treatment and vegetative cover plots were similar throughout the season, and dead mulch had higher levels for most of the season.

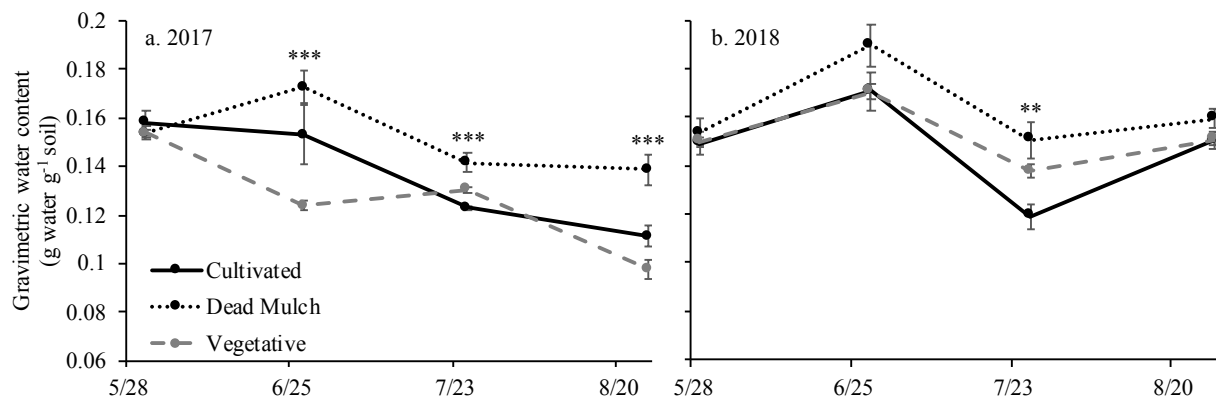


Figure 2.1. Mean of gravimetric water content in a) 2017 and b) 2018. Error bars represent \pm standard error. Significant specified at ** $P < 0.01$ and *** $P < 0.001$.

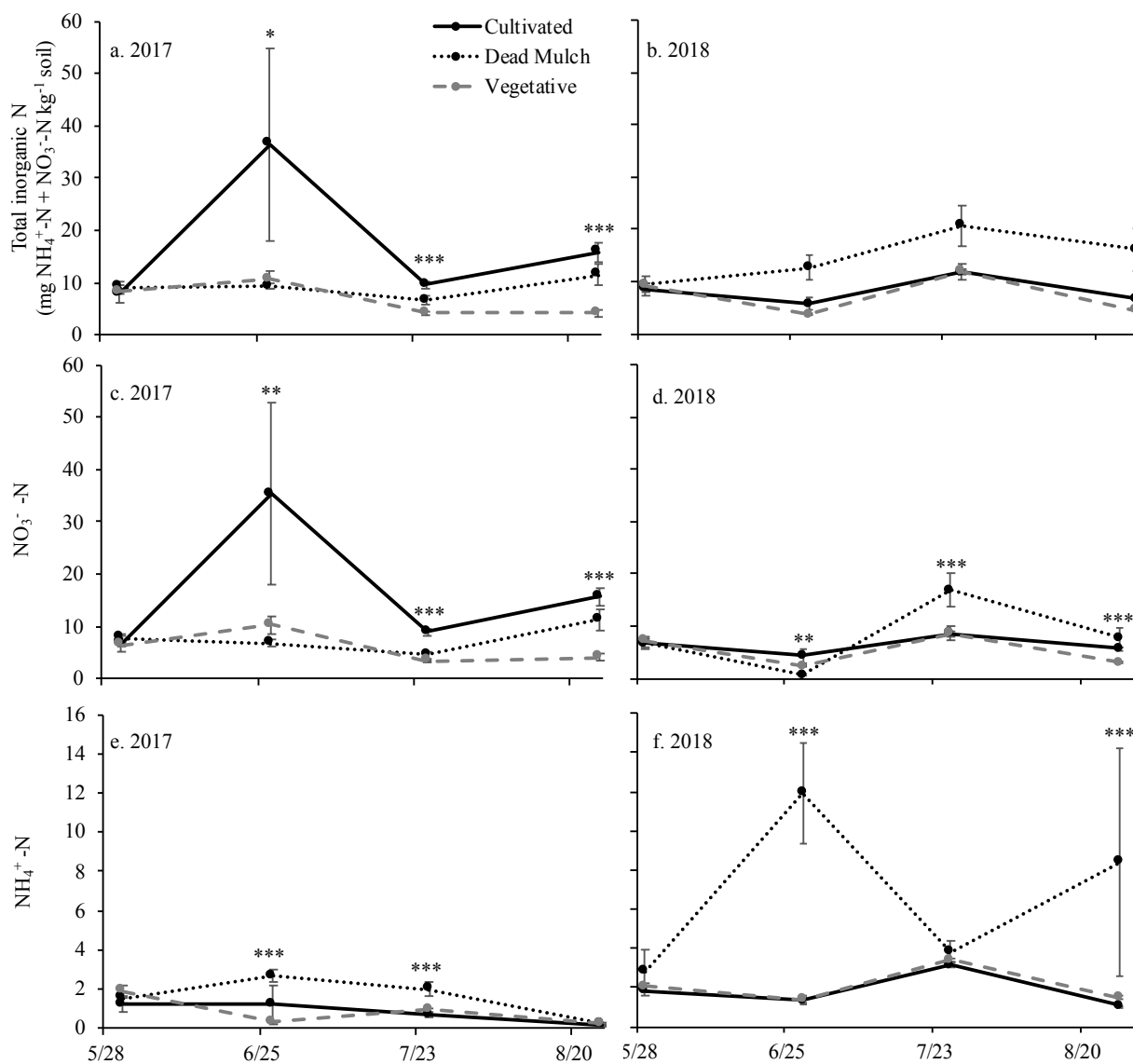


Figure 2.2. Mean of total soil inorganic N measured in the top 20 cm in a) 2017 and b) 2018; $\text{NO}_3^-\text{-N}$ in c) 2017 and d) 2018; and $\text{NH}_4^+\text{-N}$ in e) 2017 and f) 2018. Error bars represent \pm standard error. Significant specified at ** $P < 0.01$ and *** $P < 0.001$.

Microbial Biomass Carbon, Total Extracellular Enzyme Activity, and Shannon Diversity Index

Dissimilar patterns in microbial biomass C and soil gravimetric water content suggest that microbial biomass C was not driven by soil moisture. No significant differences were observed in microbial biomass C between cultivated, dead mulch, or vegetative cover plots in either year. However, microbial biomass C was on average highest at the end of the season in the dead mulch treatment in 2017 and in vegetative cover plots in 2018 (Figure 2.3).

Contrary to expectations, baseline soil sampling in May 2017 showed higher total extracellular enzyme activity in cultivated plots compared to the other areas of the field, despite this sampling date being before application of treatments. It is unclear why this was the case, but may be a result of the heterogeneous nature of the soil habitat. The greater extracellular enzyme activity in cultivated treatments at this first sampling date was driven by higher NAG, LAP, urease, PHOS and cellulase activity compared to dead mulch and vegetative cover plots. Higher enzyme activity documented in cultivated plots in May 2017 did not carry over to subsequent sampling dates. Since our between-row strategies were not established at this first sampling date, and because this unexpected trend in the cultivated treatment did not carry over to subsequent sampling dates, the first date was removed from analysis. By the end of the 2017 season, total extracellular enzyme activity was on average highest in dead mulch plots and similar in the cultivated treatment and vegetative cover plots. Total enzyme activity was similar in all treatments at the beginning of the 2018 season, but began separating in June. As the season progressed, the dead mulch treatment consistently had the greatest total enzyme activity, with vegetative cover as an intermediate, and cultivation having the lowest overall extracellular enzyme activity.

Greater overall enzyme activity in the cultivated treatment before treatment application drove significant differences in the Shannon diversity index (H) in May 2017. Aside from this initial sampling, significant differences were observed between treatments at the end of the 2017 season. However, this pattern did not carry over into 2018. No significant differences between treatments were detected for the entirety of the 2018 season. However, in 2018 H was on average greater in dead mulch and the vegetative cover treatment groups relative to the cultivated treatment (Figure 2.3).

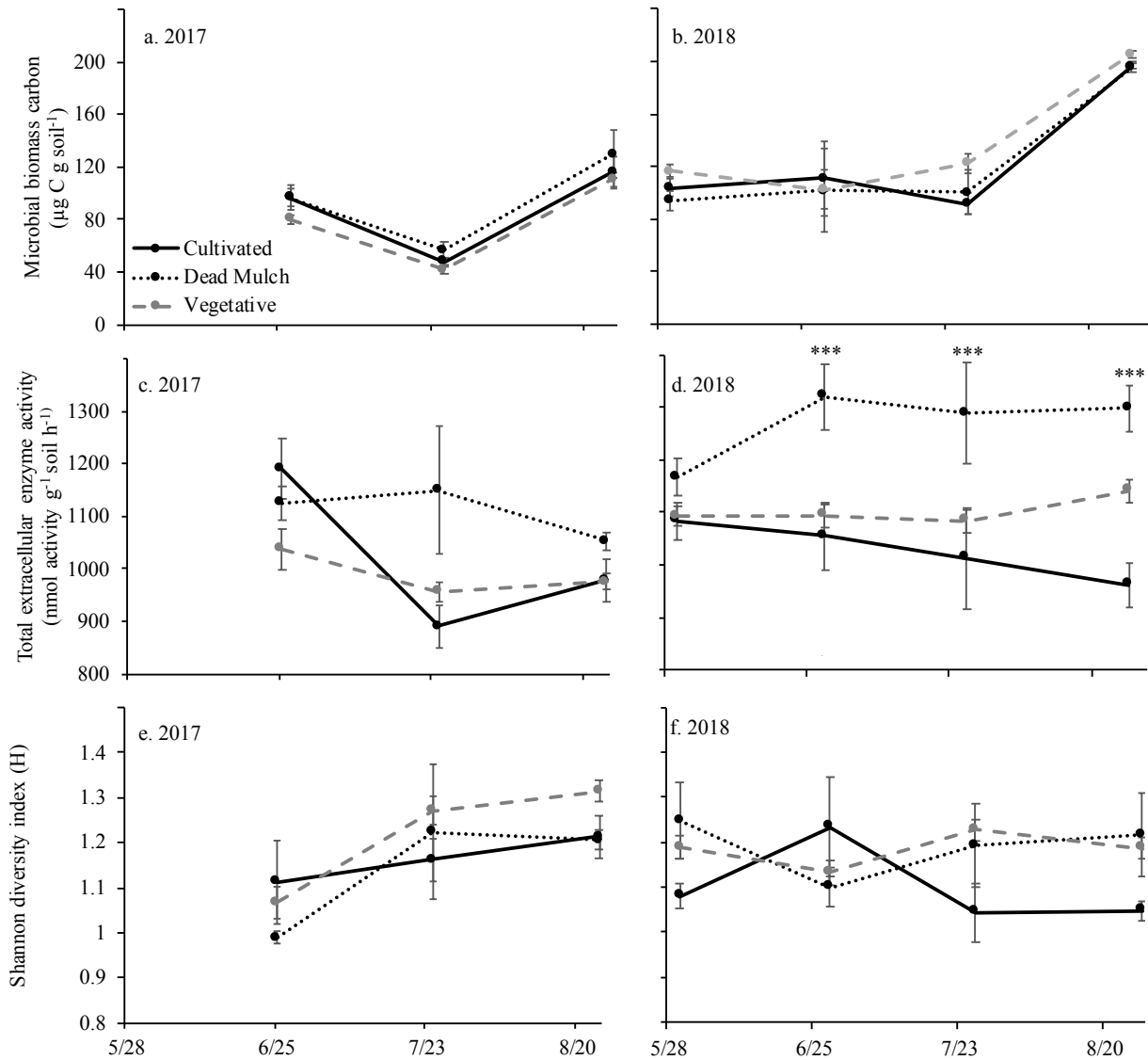


Figure 2.3. Mean of microbial biomass carbon in a) 2017 and b) 2018; total extracellular enzyme activity in c) 2017 and d) 2018; and Shannon diversity index in e) 2017 and f) 2018. Error bars represent \pm standard error. Significant specified at *** $P < 0.001$.

Individual Extracellular Enzyme Activity

The seasonal patterns of N-acquisition enzyme (NAG, LAP, urease) activity were comparable in 2017, though different by treatment. In cultivated plots, N-acquisition enzymes decreased through the July sampling date before increasing at the end of the season. In contrast, dead mulch and vegetative cover plots experienced an initial reduction in activity from May to June, then a steady increase from June through the end of the season. An exception was a July

spike in NAG activity in the dead mulch treatment. Aside from initial significant differences in PHOS activity between cultivated and the other treatments, no differences were observed in PHOS activity during 2017. Treatment influence on oxidase activity was marginally significant in 2017 ($P=0.0654$), with dead mulch having greater oxidase activity relative to the cultivated treatment or vegetative cover treatment group. Cellulase activity in the cultivated treatment continued decreasing as the 2017 season progressed, while cellulase activity in the dead mulch treatment and vegetative cover plots steadily increased during the season.

N-acquiring enzyme activity was dissimilar among enzymes and differed among treatments in 2018. NAG (chitinase) activity in dead mulch and vegetative cover plots steadily increased throughout the season. Conversely, NAG activity in the cultivated treatment increased slightly from May to June, then declined for the remainder of the season. LAP (peptidase) activity at the beginning of the season was greater in dead mulch and vegetative cover plots compared to the cultivated treatment and remained higher at subsequent sampling dates. However, in all treatments, LAP activity peaked in July before decreasing in August. 2018 urease activity was similar in dead mulch and cultivated treatments, declining as the season progressed. In comparison, urease activity in the vegetative cover treatment group was relatively stable throughout the season, ranging from 105 to 121 nmol activity g^{-1} soil h^{-1} . PHOS activity was similar across all treatments, with activity decreasing from May to June, increasing from June to July, then declining again at the end of the season. More pronounced differences between oxidase activity were observed in 2018 compared to 2017, though presented a similar pattern with higher rates in dead mulch plots relative to the cultivated treatment or vegetative cover plots. Cellulase activity followed a similar trend among treatments in 2018, with decreasing activity May through July before increasing in August (Figure 2.4).

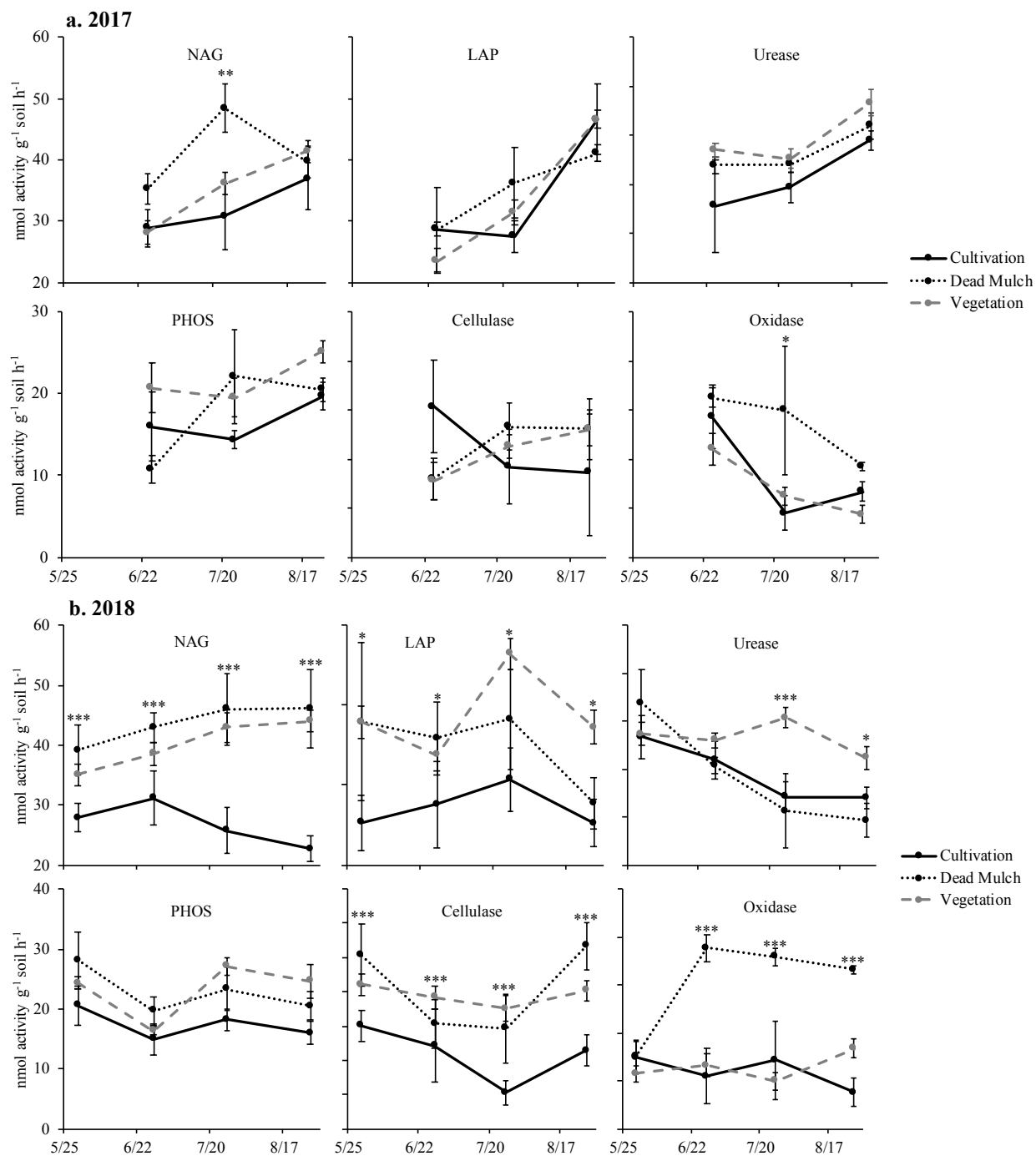


Figure 2.4. Mean of extracellular enzyme activity in a) 2017 and b) 2018. Error bars represent \pm standard error. Significance specified at $*P<0.05$; $**P<0.01$; and $***P<0.001$.

Discussion

The purpose of this study was to evaluate the effect of between-row management practices on soil microbial communities by examining the abundance (microbial biomass C) and functional diversity (Shannon diversity index calculated from extracellular enzyme activity) of soil microbes. While we had hypothesized that organic matter inputs from rye residue dead mulch and living covers would support a larger microbial community relative to bare ground cultivation, our results did not confirm this hypothesis. No significant differences in microbial biomass C were observed between treatments evaluated in either year of this study. This result was surprising, particularly given large organic matter inputs in dead mulch treatments. However, similar results have been previously observed in strip tillage vegetable production system where cover crop residues are left on the surface (Tillman et al., 2015). It is probable that differences were not detected in dead mulch treatments because the soil sampling depth was 20 cm and microbial community differences that may have been present nearer to the dead mulch were diluted by deeper soil layers. However, we had also expected to see an increase in microbial abundance in the vegetative cover treatment group. Previous studies evaluating soil microbial response to living plants demonstrated a positive correlation between living shoot and root biomass and soil microbial biomass (Buyer et al., 2010; Finney et al., 2017). However, in these other studies, phospholipid fatty acid profiles were used to evaluate microbial biomass, which may have provided greater precision for detecting differences. Though no statistically significant differences in microbial biomass were found, at the end of the 2018 season, vegetative cover plots had numerically the greatest measured microbial biomass C. This observation was in-line with our original hypothesis, and may indicate that in the relatively short

duration of this study, our experimental design had insufficient power to detect differences in microbial biomass.

Between-row management influence on the Shannon diversity index was not conclusive with few differences detected. Aside from the initial unanticipated differences between treatments observed at trial establishment, significant differences in Shannon diversity index were observed at the end of the 2017 season, when H was greatest in vegetative cover plots. At the end of the 2018 season, H was on average greater in dead mulch and vegetative cover plots, though no significant difference between treatments was observed that year. However, previous research has observed differences in enzyme activity diversity related to organic matter inputs (Bending et al., 2002).

At the end of the 2017 season and for the duration of the 2018 season, total enzyme activity was greatest in the dead mulch treatment. This pattern was driven by higher oxidase activity in dead mulch plots compared to the cultivated treatment and the vegetative cover treatment group. Additionally, in 2018, total enzyme activity in the vegetative cover treatment group was greater relative to the cultivated treatment. Since extracellular enzymes play a critical role in C, N, and P cycling (Xiao et al., 2018), greater overall extracellular enzyme activity found in dead mulch and vegetative cover plots may imply better nutrient cycling within these management strategies compared to cultivation.

Aside from differences in total enzyme activity, interesting differences in specific enzyme activities were demonstrated. Allison et al. (2011) posited that extracellular enzyme activity is driven by both nutrient limitations by the producer of an enzyme targeting a specific resource and by suitable substrate availability. In this study, greater oxidase activity in dead mulch plots likely reflects higher availability of more recalcitrant carbon compounds found in

mature rye tissues. Similarly, greater cellulase activity in dead mulch and vegetative cover plots compared to the cultivated treatment may reflect greater labile carbon availability in these plots from plant root exudates and green shoot biomass additions.

Generally, higher levels of N-acquiring enzyme activity were seen in dead mulch and vegetative cover plots compared to the cultivated treatment in 2017. This may have been caused by greater N limitations in dead mulch and vegetative cover plots relative to the cultivated control, reflected in inorganic soil N (Figure 2.2). Conversely, in 2018 soil inorganic N levels were similar in vegetative covers and the cultivated treatment, and generally higher in dead mulch plots. Therefore, disparities in N-acquiring enzymes between treatments are more likely explained by substrate availability differences than N limitations. This is further supported by dissimilar patterns of N-acquiring enzymes in 2018, suggesting treatment influence on the type of N being utilized by soil microbial communities. For example, higher levels of chitinase activity (NAG) in vegetative cover and dead mulch plots in 2018 may suggest greater fungal hyphae in those treatments given chitin is a main component of fungal cell walls.

Conclusions

Contrary to expectations, no significant differences in microbial abundance or enzyme functional diversity were detected between the treatments evaluated in this study. However, general patterns were in line with expectations. Lack of statistical significance in these trends may be attributed to the relatively short duration of this experiment, insufficient statistical power to detect small differences, and perhaps the coarse textured soil at the experimental field where changes in the microbial communities can be harder to detect than on heavier soils (Tiemann and Grandy, 2015). Although no significant differences were seen in microbial biomass or the Shannon diversity index, differences in individual enzyme activity suggest possible shifts in microbial composition driven by management practices.

This research confirms that between-row management strategies influence soil microbial communities, though perhaps not as drastically as originally hypothesized. Future research should aim to investigate the effect of the evaluated between-row management practices on a longer timescale.

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LITERATURE CITED

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CHAPTER III: Performance of Nine Cover Crops as Between-row Living Mulch in Plasticulture Production

Abstract

The use of plastic mulch films is standard in many fresh market vegetable production systems. While this practice can enhance cash crop yields, the impervious surface of plastic mulch can also increase agrochemical runoff and soil erosion in the between-row area. Keeping the soil covered with a cover crop living mulch may help address this challenge; however, information regarding living mulch species selection in plasticulture vegetable production is lacking. To help optimize choice of cover crop species between plastic mulch beds, a two year screen evaluating biomass production, in-season weed control, and the potential to compete with a cash crop for in-row resources was implemented. Cover crops included Italian ryegrass (*Lolium multiflorum*), teff (*Eragrostis tef*), sudangrass (*Sorghum x drummondii*), barley (*Hordeum vulgare*), rye (*Secale cereale* L.), wheat (*Triticum*), Dutch white clover (*Trifolium repens*), New Zealand white clover (*Trifolium repens*), and yellow blossom sweet clover (*Melilotus officinalis*). All living mulches significantly reduced in-season weed biomass relative to the weedy check in one year of this study. A significant negative correlation between weed and living mulch biomass demonstrated the importance of biomass production in weed suppression. Teff produced the most biomass and provided the greatest weed suppression. However, despite physical separation, all living mulch reduced available nitrogen and soil moisture in neighboring plastic mulch beds. For growers interested in integrating a living mulch into plasticulture production, caution should be taken to minimize risk to cash crop yields while optimizing other desired benefits including weed suppression, soil health, erosion control, and cleaner harvesting conditions.

Introduction

The use of plastic mulch film is standard practice in many fresh market vegetable production systems (Lamont, 2005; Tarara, 2000). Key benefits of plastic mulch include temperature moderation, increased moisture retention, reduced nitrogen leaching, effective weed suppression in the crop row, reduced soil splash, and lower incidence of some diseases. These benefits account for the higher total and marketable vegetable yields often observed in plastic mulch systems (Kasirajan and Ngouajio, 2012; Tarara, 2000).

Although plastic mulch offers many advantages, it can also lead to increased soil degradation (Steinmetz et al., 2016). This is particularly true when cultivation is used to manage weeds between plastic mulch beds, leaving the soil bare. In addition to excessive cultivation disrupting physical and biological soil properties, soil erosion can be markedly greater in plastic mulch systems because the impervious surface of the plastic induces aggressive runoff after rain events (Steinmetz et al., 2016; Wan and El-Swaify, 1999). Furthermore, bare ground between plastic mulch beds increases the risk of pesticide and nutrient leaching, contaminating local water resources (Rice et al., 2004).

Planting a cover crop as a living mulch between plastic mulch beds is an attractive weed management alternative to cultivation that could help alleviate the aforementioned soil degradation challenges (Depalo et al., 2017; Paine and Harrison, 1993; Rice et al., 2004). In addition to suppressing weeds, living mulches can protect the soil with an above-ground canopy and roots below that stabilize the soil. However, properly selecting a living mulch species is critical in ensuring the delivery of anticipated benefits.

Desirable living mulch traits have been identified by multiple authors and include 1) rapid germination and establishment to exclude weeds, 2) a low and easily managed growth

habit, 3) thorough soil coverage, 4) resistance to drought and low nutrient environments, and 4) allelopathy (Adamczewska-Sowińska et al., 2009; Brennan and Smith, 2018; den Hollander et al., 2007; Hartwig and Ammon, 2002; Leary and DeFrank, 2000). While finding a cover crop that possesses all of these traits is unlikely, various cover crop functional groups have a suit of these desirable characteristics. For example, clovers have a relatively low growth habit, produce a ground covering canopy, and have the ability to fix atmospheric nitrogen, making them easily adaptable to low-fertility environments and less likely to compete with a cash crop for nitrogen than grasses (Hartwig and Ammon, 2002). Alternatively, planted in the appropriate climatic niche, warm-season grasses can accumulate large amounts of biomass and provide exceptional weed control (Wedryk and Cardina, 2012). Finally, cool-season, small-grain grasses establish quickly, remain low growing in the absence of vernalization, and produce allelopathic chemicals (Brainard and Bellinder, 2004; Lowry and Smith, 2018; Nelson et al., 2011).

Previous studies have found that living mulches from these three broad functional groups can provide excellent weed control. For example, subterranean clover living mulch has been shown to provide up to 99% weed control in sweet corn production (Ilnicki and Enache, 1992). Infante and Morse (1996) showed that white clover over-seeded into broccoli two days after transplanting could control weeds equivalent to the application of a preemergent herbicide. Chase and Mbuya (2008) saw a 56% reduction in weed density when broccoli was planted with annual ryegrass living mulch. Rye living mulch in zucchini squash production resulted in 80% control of redroot pigweed and 82% control of smooth crabgrass (Walters and Young, 2008). However, weed control by a cover crop is not always guaranteed (Baraibar et al., 2018), with the efficacy of weed control by a living mulch depending on a range of factors including living

mulch species, dominate weed species, and environmental conditions that can influence competitive dynamics between living mulch and weeds (Radicetti et al., 2018)

In addition to variable weed control, living mulch adoption by vegetable growers is limited because living mulches that are competitive enough to suppress weeds, typically will also suppress a cash crop (Hartwig and Ammon, 2002; Lowry and Smith, 2018). Therefore, management strategies that mediate competition between a living mulch and cash crop need to be considered. These may include supplemental use of nutrients or irrigation to minimize competition for these critical below-ground resources; delayed planting of low growing cover crop species to minimize competition for light; and complementary weed management practices including cultivation or herbicides (Brainard and Bellinder, 2004). In addition, plastic mulch systems are ideally suited for the integration of a living mulch because plastic mulch can provide both a spatial and physical barrier to competition. However, previous studies have demonstrated that cash crop yield reductions remain a challenge in some cases, though mechanisms have rarely been investigated (Law et al., 2006; Warren et al., 2015).

Though there is considerable grower interest in mixed plastic mulch-living mulch systems, recommendations are currently lacking. In particular, there is little available information regarding which cover crop species function best in this specific niche and how potential competition between a living mulch and cash crop could be avoided. The objective of this research was to evaluate the potential performance of cover crop species from distinct functional groups as living mulches in organic plasticulture production systems with respect to biomass production, in-season weed suppression, and their potential to compete with a cash crop for in-row water and nitrogen resources.

Materials and Methods

Site Description

In May 2017 and 2018 a cover crop screen evaluating cover crops for use as living mulch between plastic mulch bed was established at the Michigan State University (MSU) Horticulture Teaching and Research Center in Holt, MI (42°67' N, 84°48' W). The study was conducted in adjacent, organically-managed fields characterized by Spinks loamy sand (sandy, mixed, mesic Lamellic Hapludalf). Initial soil chemical characteristics in 2017 were as follows: 1.7% organic matter; pH 6.5; CEC 3.7 cmol kg⁻¹; and P, K, and Mg levels of 81, 60, and 64 mg kg⁻¹ respectively. In 2018 initial soil chemical characteristics were as follows: 1.7% organic matter, pH 7.4; CEC 5.0 cmol kg⁻¹; and P, K, and Mg levels of 153, 127, and 98 mg kg⁻¹ respectively. The field had previously been planted with beans and peas in 2016 and with cabbage in 2017.

Experimental Design

Three species from three distinct functional groups, totaling nine cover crops, were evaluated for their potential as a living mulch grown between plastic mulch beds (Table 3.1). Cool-season grasses included rye, barley, and wheat. Italian ryegrass, teff, and sudangrass represented warm-season grasses. Dutch white clover (DW), New Zealand white clover (NZ), and yellow blossom sweet clover represented legumes. All clover seed was pretreated with rhizobium inoculum (Prevail, Verdesian Life Sciences, Cary, NC) and seeded with a half rate of cereal rye monoculture to act as a nurse crop. Living mulches were compared to a cultivated (hand-hoed) control in both years, and to a cultivated control and weedy check in 2018.

Treatments were arranged in a randomized complete block design with four replications. Individual plots were 5.5 m long in 2017 and 3.4 m long in 2018, and consisted of three plastic mulch beds and two between-row areas. Between-row areas measured approximately 1 m wide.

Table 3.1. Seeding rate, seed cost, and per hectare cost based on living mulch seed price included in this study.

Common Name	Scientific Name	Cultivar	2017	2018	Seed cost ¹	
			Seeding rate		---	---
			-----kg ha ⁻¹ -----		---\$ kg ⁻¹ ---	---\$ ha ⁻¹ ---
Italian Ryegrass	<i>Lolium multiflorum</i>	Tetila	35.9	35.9	3.30	118.47
Teff	<i>Eragrostis tef</i>	Dessie	11.2	11.2	9.24	103.49
Sudangrass	<i>Sorghum x drummondii</i>	Piper	56.1	-	6.01	336.94
Barley	<i>Hordeum vulgare</i>	Thoroughbred	168.1	336.3	0.92	308.28
Rye	<i>Secale cereal</i>	VNS ²	168.1	336.3	0.81	273.75
Wheat	<i>Triticum</i>	Emerson	168.1	336.3	0.99	332.94
White Clover	<i>Trifolium repens</i>	Dutch-VNS	22.4 (84.1) ³	22.4 (168.1)	11.44	393.13
White Clover	<i>Trifolium repens</i>	New Zealand-Crusade	22.4 (84.1)	22.4 (168.1)	8.36	324.14
Yellow Clover	<i>Melilotus officinalis</i>	Sweet blossom-VNS	22.4 (84.1)	22.4 (168.1)	3.74	220.65

¹\$ ha⁻¹ calculated using the 2018 higher seeding rates of barley, rye, and wheat. For clovers, \$ ha⁻¹ accounts for half rate of 2018 rye monoculture

²Variety not stated

³Clovers seeded with a half rate of rye monoculture; number in parentheses represents rye seeding rate

Field Management

In late May of both years, Nature Safe (Irving, TX) All-Season Fertilizer 10-2-8 (N-P-K) was broadcast applied at 56 kg total N ha⁻¹ and incorporated with a rototiller. Plastic mulch was laid within a day of field fertilization. Plastic mulch beds were raised to 0.15 m and laid on 1.67 m centers. 1.22 m 1 mil embossed plastic mulch was used. Top of bed widths measured 0.61 m and single drip lines with 0.30 m emitter spacing were installed under plastic.

Living mulch treatments were broadcast sown and lightly incorporated with a rake on May 30 of both years. Seeding rates (Table 3.1) were selected based on the highest recommended rates (Clark, 2007; Lindquist, 2014). Since clovers are slow to establish, clover species were planted with a half rate of rye monoculture to act as an early season nurse crop (Chase and Mbuya, 2008). Poor establishment of cool season grasses (cereal rye, winter barley, and winter wheat) in 2017 warranted increasing the seeding rate in 2018. In 2017, 4 mm of overhead irrigation was applied after five days of no rain to encourage living mulch germination and another 4.8 mm of water was applied a week later. Overhead irrigation was not necessary in

2018, with ~19 mm of rainfall the evening after planting and a total of 33.5 mm of rain the week following living mulch sowing. In-row irrigation was applied weekly, though no cash crop was growing, to observe differences in in-row soil moisture throughout the growing season.

A walk behind push mower with the mower deck set to 10.16 cm was used to manage living mulches and weeds. Mowing times were determined by the height of the tallest plants in the between-row area, which were typically broadleaf weeds, resulting in all living mulches being mowed at the same time. Cultivated control plots were maintained through hand hoeing every 2-3 weeks.

Data Collection

Total weed and cover crop living mulch biomass was assessed using a permanently placed 0.125 m² quadrat in each between-row area per plot. Biomass samples were taken by clipping shoots at 10.16 cm before the first two mowing events and to the ground at experiment termination. Weed and cover crop biomass were separated and dried to a constant weight at 60°C before dry weight determination.

Weed and cover crop density counts were taken three weeks after living mulch sowing in 2017 and two weeks after sowing in 2018. In 2017, density counts were taken in two 0.25 m² quadrats per plot (one per between-row) and separated into grass living mulch, lambsquarters, pigweed, ladythumb, Solanaceae species, other broadleaves, and other grass weeds. Due to difficulty in counting, clover living mulch was counted in four randomly selected 0.0064 m⁻² squares within the quadrats. In 2018 density counts were taken in two 0.125 m² quadrats per plot and were separated in to grass living mulch, clover living mulch, lambsquarters, pigweed, purslane, other broadleaf weeds, and other grass weeds. Teff projected density was 5,694 seeds m⁻², making accurate counts difficult to perform, so teff density counts were not taken.

In-row and between-row soil samples were taken to a 20-cm depth at month intervals starting at trial establishment. In-row soil samples were composites of 10 cores per plot taken from the center plastic mulch bed. For between-row samples, 12 cores per plot (six cores from each between-row area) were composited. Soil samples were stored at 4° C until dried at 38° C for at least 36 h. To assess inorganic N levels, 10 g dry soil was extracted with 50 ml 1M KCl, and extracts analyzed for NO₃⁻ and NH₄⁺ concentrations using a Lachat injection flow autoanalyzer (Lachat QuickChem, Hach Company, Loveland, CO). In-row soil moisture in the top 20 cm of soil was monitored regularly using a FieldScout time-domain reflectometer probe (Spectrum Technologies Inc., Aurora, IL). 10 measurements in the center plastic mulch bed were taken per plot and averaged before analysis.

Statistical Analysis

Given the differences in the treatments evaluated between years, the two years of data collected were analyzed separately. All mean separations were conducted using Fisher's protected least significant difference at $P < 0.05$. Total weed and cover crop biomass was analyzed using a mixed model ANOVA with the MIXED procedure in SAS (Version 9.4, SAS Institute, Cary, NC). Treatment was treated as a fixed factor and block (replication) as a random factor. When necessary, data were either log transformed or unequal variance model used in order to meet assumptions of normality and equal variance. Data presented are not transformed. A linear regression analysis was performed using the REG procedure in SAS to evaluate the relationship between weed and cover crop biomass. Data were combined across years to test this relationship.

Total in-row soil inorganic N and soil moisture were analyzed using repeated measures mixed model ANOVA using the MIXED procedure in SAS. Treatment and date were treated as fixed factors and block (replication) as a random factor. Assumptions of normality were checked

before proceeding with analysis and heterogeneous variance was corrected with the appropriate variance-covariance structure. A first-order autoregressive variance-covariance structure was used given the equally spaced timings of inorganic N and soil moisture measurements. Model fit was verified using fit statistics.

Results

Weather

Average temperatures during May-August were similar in both years and comparable to seasonal averages (Table 3.2). However, temperatures were slightly higher in May relative to the previous year and 30-yr average. Similarly, May precipitation in 2018 was greater than in 2017 and above expected levels. Within a week of sowing living mulches in 2018, the field received a total of 33.5 mm of rain. In contrast, 2017 was drier during cover crop establishment, with only 5.6 mm of precipitation and supplemental overhead irrigation within a week of living mulch planting (Table 3.2). Although precipitation in June and July of 2017 far exceeded that in 2018, and total season precipitation was similar in both years.

Table 3.2. Monthly average temperatures and total precipitation during the 2017 and 2018 season and the 30 year averages.

	Average Temperature			Precipitation ¹		
	<u>2017</u>	<u>2018</u>	<u>30-yr²</u>	<u>2017</u>	<u>2018</u>	<u>30-yr</u>
	-----°C-----			-----mm-----		
May	13.5	17.6	14.7	65.8	121.8	84.6
June	19.9	20.0	20.0	92.5	37.2	88.9
July	21.6	21.9	22.1	67.3	26.2	82.8
August	19.3	21.8	21.3	34.8	112.5	83.8

¹Overhead irrigation was used in 2017, precipitation totals refer to total water input for specified month.

²30-yr average temperature and precipitation reported by NOAA for East Lansing, Michigan from 1981-2010. Retrieved from <https://www.ncdc.noaa.gov/cdo-web/datatools/normals>; verified on March 12, 2019

Cover Crop Establishment and Biomass Production

Early season density counts demonstrated higher density for all cool-seasons grasses in 2018 compared to 2017 (Table 3.3). The same was true for ryegrass and yellow clover. However, both Dutch white clover and New Zealand white clover had greater density in 2017.

Warm-season grasses on average produced more living mulch biomass than cool-season grasses or clovers in both years, with teff producing the most of all species evaluated (Table 4.4). Sudangrass produced similar levels of biomass as teff, but its tall growth habit, even after mowing, made it difficult to control and walk through, and therefore was not included in 2018. Of the warm-season grasses, Italian ryegrass produced on average less biomass than Sudangrass in 2017, and significantly less than teff in both years.

Without a vernalization period, cool-season grasses maintained a low growth habit during the season. In July of both years, leaf rust infected all cool-season grasses, weakening stands. As a result, cool-season grasses produced on average the least amount of biomass of the functional groups. Particularly low biomass production in 2017 necessitate increasing the seeding rate from 168 kg ha⁻¹ in 2017 to 336 kg ha⁻¹ in 2018. While this increased seeding rate at least in part resulted in greater biomass production, cool-season grasses still produced less biomass on average than warm-season grasses and clovers in 2018. However, barley produced significantly more biomass than rye or wheat in both years, and in 2018 was the second greatest biomass producer behind teff (Table 3.4).

There were no significant differences between biomass production among clover-rye bicultures in either year (Table 3.4). However, in 2017, the Dutch white clover-rye mixture treatment produced on average the most biomass in the group followed by New Zealand white

clover-rye, followed by yellow clover-rye. This pattern was reversed in 2018 with yellow clover producing the most biomass and Dutch white clover the least (Table 3.4).

Though differences in living mulch biomass production were apparent, total cumulative biomass production was similar across all vegetative cover treatments in 2017. In 2018 teff, ryegrass, and the weedy check produced similar amounts of total biomass, while all other treatments produced significantly less.

Table 3.3. Mean (standard error) of living mulch and weed density counts.

	Living Mulch		Weed	
	2017	2018	2017	2018
	-----plants m ⁻² -----			
Cultivated	-	-	304 (58)	1430 (225)
Weedy	-	-	-	1299 (143)
Ryegrass	362 (98)	1006 (117)	359 (74)	951 (167)
Teff	-	-	277 (54)	887 (157)
Sudangrass	301 (72)	-	218 (27)	-
Barley	347 (64)	1423 (265)	330 (67)	829 (129)
Rye	238 (33)	554 (75)	370 (98)	886 (212)
Wheat	278 (33)	1274 (315)	296 (28)	743 (118)
DW	2485 (415)	1678 (82)	222 (19)	564 (72)
NZ	2368 (240)	1410 (124)	238 (82)	983 (154)
Yellow	872 (148)	963 (103)	197 (46)	736 (156)

Table 3.4. Mean (standard error) of cumulative living mulch biomass production. Means within columns followed by the same letter are not significantly different ($P < 0.05$).

	2017		2018	
	-----g m ⁻² -----			
Ryegrass	97.0 (14.4)	bc	202.1 (32.1)	bc
Teff	262.6 (76.4)	a	559.6 (60.8)	a
Sudangrass	165.4 (7.2)	ab	---	---
Barley	35.9 (12.3)	e	210.9 (29.3)	b
Rye	8.5 (2.5)	f	114.3 (13.2)	cd
Wheat	0.7 (0.4)	g	52.9 (17.9)	d
Yellow ¹	50.7 (5.9)	de	206.7 (22.4)	b
DW	88.5 (8.9)	cd	125.8 (27.4)	bcd
NZ	69.6 (17.6)	cd	164.3 (16.5)	bc

¹Clover biomass values are presented as combined production of clover and rye.

Weed Suppression

Dominant weed species in the field in 2017 included mustards (*Brassica* spp.), lambsquarters (*Chenopodium album* L.), crabgrass species (*Digitaria* spp.), ladysthumb (*Persicaria maculosa*), and Pennsylvania smartweed (*Polygonum pensylvanicum*). In 2018, dominant weeds were lambsquarters (*Chenopodium album* L.), pigweed species (*Amaranthus* spp.), crabgrass species (*Digitaria* spp.), ladysthumb (*Persicaria maculosa*), and Pennsylvania smartweed (*Polygonum pensylvanicum*). Regrowth of lambsquarters and pigweed was limited after mowing, but the low, spreading growth habit of crabgrass, ladysthumb, and Pennsylvania smartweed allowed these weeds to escape the mower blade and become progressively problematic as the season progressed.

Weed density was high in both fields, ranging from 197 weeds m⁻² in 2017 to 1430 weeds m⁻² in 2018 (Table 3.3). Early season density counts revealed higher weed density in 2018 relative 2017. However, overall in-season weed biomass was lower in 2018 compared to 2017. Better cover crop establishment in 2018 may explain differences in in-season weed biomass,

with all cover crops screened reducing weed biomass compared to the weedy control in 2018 (Figure 3.1).

A significant negative correlation between living mulch and weed biomass suggest that weed suppression was driven by living mulch biomass accumulation (Figure 3.2). In both years warm-season grasses reduced in-season weed biomass more than the other functional groups, followed by clover-rye bicultures, then cool-season grasses. Teff on average reduced in-season weed biomass more than any other living mulch. Of the cool-season grasses, barley reduced in-season weed biomass more than rye or wheat. Among clover-rye treatments, no significant differences were observed in terms of in-season weed suppression in either year (Figure 3.1).

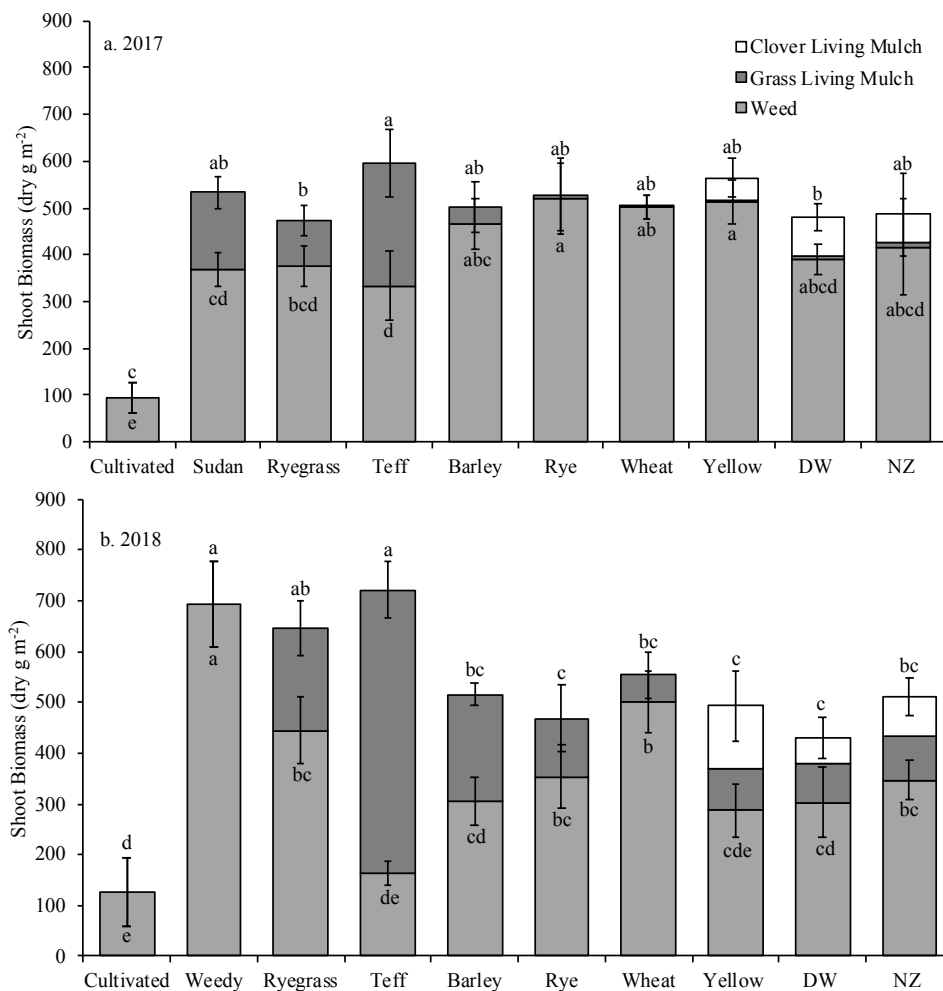


Figure 3.1. Mean cover and weed biomass production in a) 2017 and b) 2018. Error bars represent ± 1 standard around cumulative weed biomass mean and total cumulative biomass mean. Letters above bars represent differences in cumulative biomass production ($P < 0.05$). Letters within bars represent differences in cumulative weed biomass ($P < 0.05$).

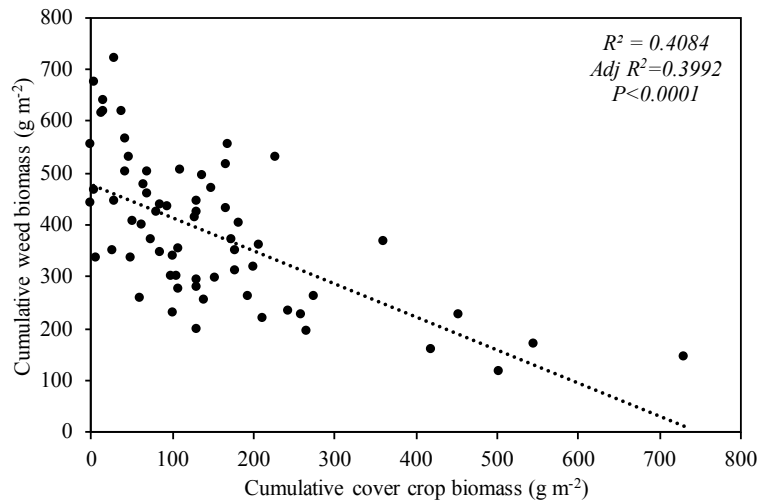


Figure 3.2. Correlation between cumulative weed biomass and cumulative cover crop biomass. Data points were combined over both years.

Potential Competition

All cover crops and weeds showed the potential to compete with a cash crop for in-row soil inorganic nitrogen and water relative to the cultivated control (Figure 3.3, figure 3.4). Living mulch and weed utilization of in-row inorganic nitrogen and soil moisture increased as the growing season progressed, with differences between the cultivated control and vegetative covers becoming pronounced by mid-July of both years.

All living mulches followed a similar in-row soil moisture pattern, with higher volumetric water content in the cultivated treatment relative to all other treatments with the exception of the first measurement (Figure 3.3; Figure 3.4).

In addition to utilization of in-row soil water, all living mulches reduced in-row available nitrogen compared to the cultivated treatment. By August, in-row soil inorganic N was decreased by 76% and 52% where living mulch or weeds were growing between plastic compared to the cultivated treatment in 2017 and 2018 respectively (Figure 3.3; Figure 3.4). However, significant differences among treatments were not detected.

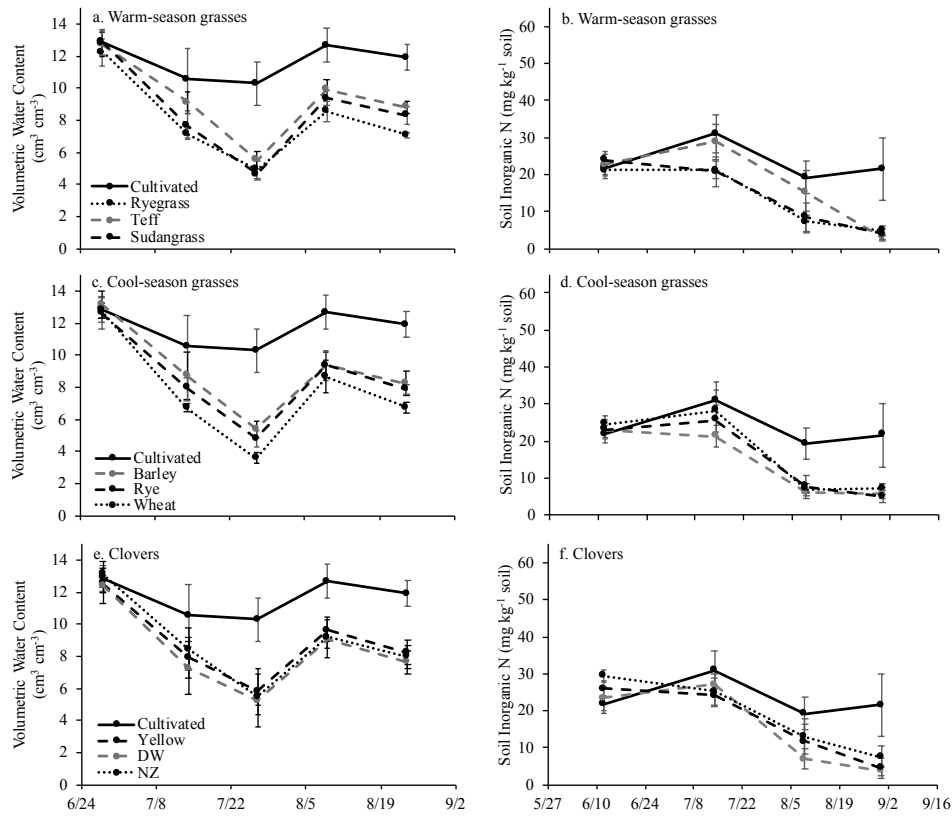


Figure 3.3. 2017 volumetric water content of (a) warm-season grasses, (c) cool-season grasses, and (e) clovers and in-row soil inorganic nitrogen in (b) warm-season grasses, (d) cool-season grasses, and (f) clovers. The treatment by sampling date interaction was not significant with regards to in-row soil inorganic nitrogen, however treatment was marginally significant ($p=0.0883$).

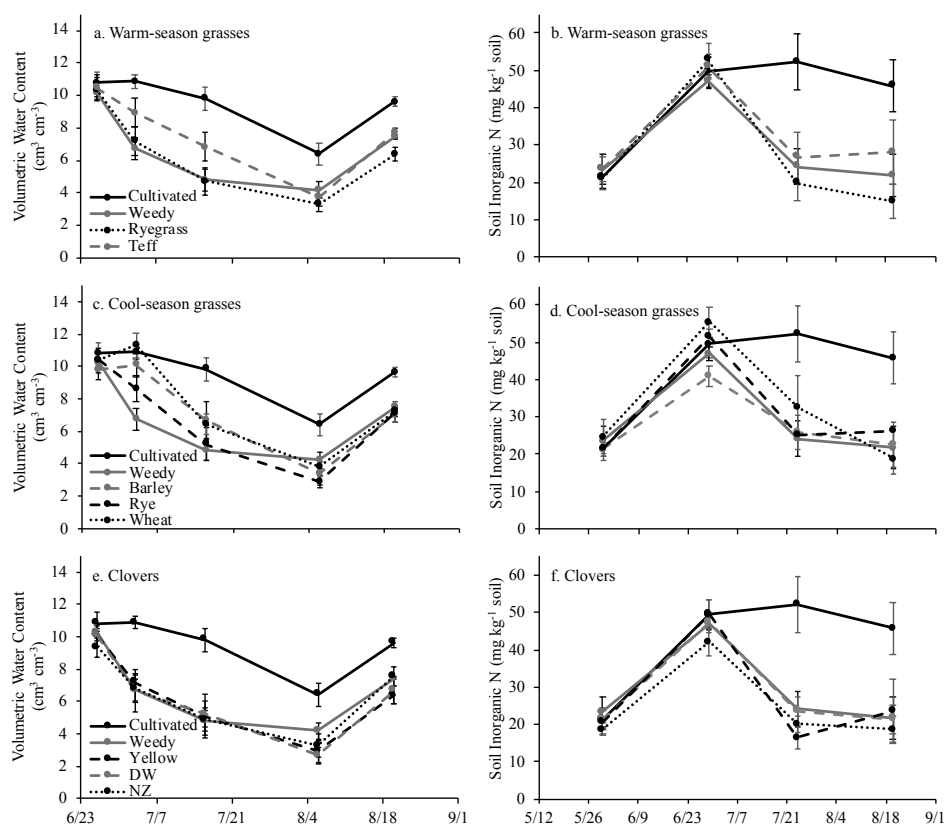


Figure 3.4. 2018 volumetric water content of a) warm-season grasses, c) cool-season grasses, and e) clovers and in-row soil inorganic nitrogen in b) warm-season grasses, d) cool-season grasses, and f) clovers.

Discussion

The purpose of this study was to evaluate the performance of different living mulch species for use between plastic mulch beds. Of particular interest was variation among living mulches in terms of biomass production, weed suppression, and in-row resource utilization. Two general themes emerged from this research. The first was that a living mulch can reduce weed pressure, with weed suppression being driven by living mulch biomass production. Second, despite spatial separation and the physical barrier of the plastic mulch, vegetative cover between plastic mulch beds demonstrate the potential to compete with a cash crop. A more detailed examination of the nuances within these two themes and future research directions are examined below.

The role of sufficient precipitation or irrigation in living mulch establishment was demonstrated in this study, with higher precipitation in 2018 relative to 2017 leading to better establishment. Differences in establishment rates between years is reflected in higher densities of cool-season grasses, ryegrass, and yellow clover in 2018 compared to 2017 (Table 3.3). Though this pattern was not observed in Dutch white clover or New Zealand white clover, this difference may have been caused by density counts taken 11 days earlier in 2017. Clovers grow slowly, making this timing gap in data collection potentially noteworthy.

Better establishment rates in 2018 may have led to greater living mulch biomass production in that year relative to 2017. Greater living mulch biomass production in 2018 may also be explained by higher May temperatures in that year promoting weed seed germination prior to tillage at trial establishment, killing these young seedlings and giving living mulches a competitive edge over the weed community. Low precipitation in June and July in 2018 may

have also hindered mid-season weed seed germination, alleviating some competition between weeds and living mulches, again promoting living mulch growth.

A significant negative correlation between cover crop biomass and weed biomass was found, demonstrating the importance of living mulch biomass production in weed suppression (Figure 3.2). Previous research has also demonstrated the influence of cover crop biomass on weed suppression, and suggested that biomass production is often an important factor driving differences in weed suppressive ability between cover crops (Alonso-Ayuso et al., 2018). Weed control through hand-hoe cultivation resulted in the least weed biomass production in both years. However, both teff and yellow clover provided weed control that was statistically similar to the cultivated treatment in 2018, with a 77% and 59% reduction in weed biomass relative to the weedy control by teff and yellow clover respectively.

Even though living mulches were able to reduce in-season weed pressure, mowing in vegetative cover plots caused a shift in the weed community to species that are more difficult to control particularly in plastic mulch systems. Before mowing, the field was dominated by broadleaf summer annuals (common lambsquarters, mustards, and *Amaranthus* species). However, crabgrass, ladysthumb, and Pennsylvania smartweed have a low, horizontal growth habit allowing them to escape the mower blade, and become more dominate as the season progressed. This community shift is in line with previous findings by Butler et al. (2013), showing that repeated clipping of barnyardgrass, giant ragweed, and common lambsquarters reduced plant dry weight by as much as 82%. However, clipping did not significantly reduce large crabgrass dry weight because of its prostrate growth habit. Prostrate growing weeds were particularly problematic in this system because they would grow along the edge of plastic mulch

beds, eventually growing along bed tops. As the season progressed, mowing became more cumbersome as these weeds need to be pulled from the in-row area.

In addition to frustrations with vegetative cover control, we found that despite spatial separation and the physical barrier of plastic mulch, plants growing between plastic mulch beds have the potential to compete for in-row resources. The degree of in-row resource utilization was uniform across living mulch treatments in 2017 and across living mulch treatments and the weedy control in 2018. These findings are in line with previous studies that have found yield reductions in vegetable crops when living mulch was planted between plastic mulch beds (Warren et al., 2015; Reid and Klotzbach, 2013). In these cases, yield reduction mechanisms were not studied directly though, Warren et al. (2015) included nitrogen rate as a factor in a study evaluating the effects of a Italian ryegrass-white clover living mulch mixture on broccoli yields. In the first year of the study, yield reductions were only present at low nitrogen fertility rates, suggesting nitrogen competition between living mulch and the cash crop. In the second year however, yield reductions were present in all living mulch treatments compared to cultivation regardless of nitrogen rate. The authors proposed competition for resources other than nitrogen or the induction of a shade-avoidance response resulting from the reflected light quality off of neighboring living mulch caused yield reductions.

To minimize yield reductions caused by living mulches, the suppression of living mulch and associated weeds through mowing, the use of selective herbicides, spatial separation of living mulch and cash crops, or delayed planting of living mulches have been suggested (Masiunas, 1998). In this study, we found that spatial separation and mowing were not enough to mitigate potential competition. Similar observations were made by Biazzo and Masiunas (2000) where mowing red clover, perennial ryegrass, and canola living mulches mitigated some pepper

yield reductions when compared to un-mowed plots, but yields were still reduced when compared to a conventionally tilled control. The use of mowing alone to mediate competition between a cash crop and living mulch has previously been questioned (Teasdale, 1996).

Conversely, the delayed planting of living mulches has shown great promise to alleviate yield reductions of cash crops. Tomato yields were maintained by delaying between-row buckwheat sowing until after the critical weed-free period (Gibson et al., 2011). Cauliflower yield reductions caused by competition with burr medic living mulch were curbed by waiting three weeks after transplanting to sow living mulch (Ciaccia et al., 2017). Delayed sowing of rye living mulch by 10 days after transplanting mitigated yield reductions in broccoli (Brainard and Bellinder, 2004). Indeed, the delayed utilization of in-row resources by living mulches and weeds in this study suggest that competitive inhibition could be reduced or eliminated if living mulch was planted later, and between-row areas kept weed-free for some critical period unique to this specific management system. Future research aimed at determining the critical weed-free period between plastic mulch beds of important warm-season vegetable crops commonly grown in plasticulture would be fundamental to optimizing an integrated plastic mulch-living mulch system.

While living mulch between plastic mulch beds has the potential to reduce cash crop yields, other ecosystem services provided by a living mulch could be worth the tradeoff. For example, living mulch can reduce soil and agricultural chemical runoff (Masiunas, 1998), provide above- and below-ground habitat for beneficials (Depalo et al., 2017; Frank and Liburd, 2005) and maintain cleaner harvesting conditions. Further investigation and economic quantification of these potential agroecological tradeoffs will enable growers to make decisions specific to their unique management goals.

In addition to more in-depth evaluations of ecosystem services, exploring other living mulches species would be an insightful future research direction. While previous living mulch screens have been done (Chase and Mbuya, 2008; Nicholson and Wein, 1983), to the best of our knowledge this is the first screen evaluating species within a plasticulture production system. Though living mulch species were selected to represent a range of desirable traits, the list of species included was certainly not exhaustive. Other cover crop species or functional groups could potentially fit well into this niche. For example, Brennan and Smith (2018) evaluated the use of mustard living mulch sown between raised plastic mulch strawberry beds in organic production. The authors suggested that mustard would be an ideal living mulch in an organic system because it is easily killed via mechanical means. Buckwheat is also easily controlled via mowing or cultivation, and has been shown to provide excellent control of summer annual weeds (Gibson et al., 2011). Future research including species beyond grasses and clovers could help elucidate other desirable living mulch characteristics specific to this particular management system.

In terms of cover crop mixtures, only clover-rye mixtures were used in this study, though other mixtures might perform better in this niche. While barley was able to suppress weeds early in the season, a combination of rust and a low growth habit made it less competitive later. Teff did not establish as rapidly as barley, but was able to consistently produce biomass and suppress weeds through the middle and end of the growing season. Clovers did not start to put on significant amounts of biomass until August and persisted even after the first frost. A cover crop mixture with a representative from each functional group screened here has the potential to integrate the best qualities of each while minimizing individual weaknesses. However, caution should be taken when designing cover crop mixtures as research confirming the cumulative weed

suppressive ability of cover crop mixtures is sparse and indeed weed suppression is typically performed by the most weed suppressive species in the mixture (Lowry and Smith, 2018).

Conclusions

In summary, living mulches can reduce in-season weed pressure in plastic mulch systems, with all living mulches evaluated significantly reducing in-season weed biomass compared to the weedy check in 2018. Teff produced the most biomass in both years, reduced in-season weed biomass more than any other living mulch screened, and even comparable to cultivation in terms of weed suppression in 2018. Additionally, based on seed cost, it was the most inexpensive species screened on a per hectare basis (Table 3.1). Though living mulches could suppress weeds, and despite spatial separation and regular mowing, plants growing between plastic mulch beds were shown to be potentially competitive for in-row resources. Addressing other strategies for competition mitigation, such as delayed planting of living mulches relative to the cash crop, hold promise for optimizing both cash crop yields and weed control. Additionally, living mulches may provide other desirable ecosystem services that offset possible yield penalties. Future research assessing such tradeoffs would aid in grower decision making to optimize this type of system for specific agronomic and ecological goals. Furthermore, evaluation of other cover crop species and cover crop combinations could reveal other relevant traits and synergies suited for use in plasticulture production.

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