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# IMPACTS OF COVER CROP MIXTURES ON PRODUCTIVITY OF CROPPING SYSTEMS

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Natural and Applied Science, Agriculture

By

Brionna Lee West

December 2018

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## IMPACTS OF COVER CROP MIXTURES ON PRODUCTIVITY OF CROPPING

## **SYSTEMS**

Agriculture

Missouri State University, December 2018

Master of Natural and Applied Science

Brionna Lee West

## **ABSTRACT**

A field study and a meta-analysis were conducted to compare the impact of cover crops on carbon and nitrogen dynamics and cash crop yield. The field study included six treatments: winter cereal rye, winter cereal rye-Austrian winter pea at two seeding rates, cereal rye-Austrian winter pea-radish at two seeding rates, and a no cover control. Meta-analysis data sets were compiled from studies published between 1994 and 2017. Treatments were grouped into the following classifications: monoculture, binary mix, polyculture, or control. In both the field study and meta-analysis, cover crops did not affect cash crop yield. However, plant biomass and plant biomass N in binary mixtures containing legumes were greater in both the field study and meta-analysis. Binary mixtures proved as beneficial as polycultures potentially reducing grower's seed costs and management decisions. The meta-analysis allowed data to be analyzed across all regions, environments, and crops, and highlighted a lack of cover crop mixture data emphasizing the need for additional future research

**KEYWORDS**: cover crop, cover crop mixtures, cover crop biomass, cover crop biomass N, cover crop C/N ratio, cash crop yield, meta-analysis

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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

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## LITERATURE REVIEW

## Introduction

Soil health is imperative for future agricultural productivity and environmental quality (Reeves, 1997). Cover crops are one method used to preserve or improve soil health. Cover crops are any living ground cover simultaneously planted with or after a cash crop and are frequently terminated before growing the following cash crop (Hartwig and Ammon, 2002). Cover crops influence soil health through reduced soil erosion and increased soil organic matter (SOM; Blanco-Canqui et al., 2013; Hartwig and Ammon, 2002). Soil organic matter has profound effects on physical, chemical, and biological soil properties, making it a central factor in soil health and quality (Magdoff and Weil, 2004).

Green manure is from cover crops plowed into the soil. Green manure enriches the soil by allowing the nutrients in the cover crops to be released to the succeeding cash crop. The use of green manure can increase economic viability while reducing environmental impacts, but the management, species, and interactions with the environments can make these approaches difficult (Cherr et al., 2006).

In addition to increasing soil organic matter, cover crops impact soil physical properties, water use, soil temperature, yield, N, and C. They also impact disease, weed, and insect populations. The effects vary across experiments but are generally favorable.

## **Cover Crop Use**

Cover crops have long been used in crop production. The increase in yields of crops planted after legumes is recognized in Chinese writings 2000 years ago. Documents from early

434 to 355 B.C. recognized the value of green manure and specifically discussed the use of legumes for soil improvement (Smith et al., 1987). Thomas Jefferson recommended the use of the legumes clover (*Trifolium* spp) or hairy vetch (*Vicia villosa* L.) as winter cover crops for N supply during the colonial period in Virginia. In Alabama, winter cover crops including hairy vetch, crimson clover (*Trifolium incarnatum* L.), and cereal rye (*Secale cereale* L.) were systematically evaluated as N sources for cotton (*Gossypium hirsuturn* L.) as early as 1898 (Meisinger et al., 1991).

More recently, a 2005 survey of 3500 farmers in Indiana, Iowa, Illinois, and Minnesota questioned respondents about their knowledge and attitudes about cover crops and use of cover crops at any time in the previous five years (Singer et al., 2007). Farmers in the survey primarily raised wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), or soybeans (*Glycine max* L.). Eighteen percent of respondents used cover crops in their lifetime, 11% in the past five years, and 8% planted them in the fall of 2005. Of the respondents who used cover crops, 86% utilized other conservation practices such as conservation tillage, waterways, and different N applications times. Ninety-six percent of respondents agreed that cover crops are primarily effective at reducing soil erosion, and 74% said it increased SOM (Singer et al., 2007).

The 2012 United States Department of Agriculture (USDA) census reported that operators of 133,124 farms planted cover crops on 10.3 million acres; this does not include land in USDA's Conservation Reserve Program (USDA 2012). A 2016 cover crop survey collected data from 2,020 respondents from 48 states, excluding Nevada and New Hampshire (SARE, 2016). Thirty six percent of respondents were from Iowa, Minnesota, Illinois, Indiana, and Ohio. Of the 2,020 farmers to complete the survey 81% used cover crops. Compared to the survey in 2005, there was an increase in cover crop use of 63% over 11 years (SARE, 2016).

The top three benefits of cover crops listed by farmers were increased overall soil health (86%); reduced soil erosion (83%); and increased SOM (82%). When asked to assess the impact of cover crops on overall farm profitability, 34% of respondents reported, on average, an increase of profitability. Additionally, 26% said profitability neither increased nor decreased, while 6% reported a decrease.

Respondents were asked what cover crop species were planted. Cereal rye was planted by 82%, radish (*Raphanus raphanistrum* L.) by 95%, and winter wheat accounted for 65% (SARE, 2016). The selected cover crop species have desirable characteristics such as quick establishment, adequate biomass, easy overwintering, and quick termination (Creamer et al., 1997).

## **Effects of Cover Crops**

Soil Physical Properties. Agricultural practices and the type of plant residues returned to the soil influence SOM properties (Ding et al., 2006). Soil organic matter affects soil properties such as nutrient availability, soil structure, and water holding capacity. Cover crops can increase SOM lost through tillage and improve physical conditions. Soil compaction can impede plant development by restricting water and nutrient uptake, as well as plant root growth. Cover crops like radish and other brassica species can reduce compaction through deep taproot penetration (Chen and Weil, 2010). The repetitive break down of exposed topsoil attributes to reductions in water availability and soil fertility (Pimentel et al., 1995). Wind erosion in the Great Plains results in soil losses from 5 to 18 Mg ha<sup>-1</sup>yr<sup>-1</sup>, a serious environmental issue (Hansen et al., 2012). Growing cover crops to protect the soil during the fall and spring after cash crops are harvested can reduce the risks associated with wind erosion.

Long-term tillage can result in weaker soil structures because it breaks down soil aggregates and encourages the decomposition of SOM (Chen and Weil, 2010). Cover crops provide aggregate protection by reducing soil detachment from the impacts of rain drops (Dabney et al., 2001). A study replacing fallow ground with cover crops showed improved soil aggregation and reduced overall erosion (Blanco-Canqui et al., 2013). Gómez et al. (2009) reported most sediment losses on bare ground occurred in a few intense events causing reduced infiltration, rainfall, and nutrients to be used by a later crop. In contrast, a treatment using cover crops resulted in significant reductions in runoff, sediment, organic matter, and nutrient loss. Zhu et al. (1989) reported chickweed (*Stellaria media* L.), blue grass (*Poa pratensis* L.), and downy brome (*Bromus tectorum* L.) uncontrolled winter weeds reduced runoff by 44, 53, and 45 % respectively compared to no cover.

Water Use. The amount of soil water used by cover crops is a primary concern for many farmers because it can result in reduced water availability for the cash crop. Additional biomass produced by cover crops allow for greater transpiration, increased infiltration, and decreased runoff (Dabney, 1998). If infiltration and precipitation are adequate, cover crops regularly have a positive or neutral effect on the following cash crop. In water-limited environments, cover crops can potentially reduce water available to subsequent cash crops (Unger and Vigil, 1998).

The growing season of a cover crop, method and time of termination, and planting time of the following crop influence the success of cover crops. Vaughan and Evanylo, (1998) reported termination by mowing may potentially conserve soil moisture at a similar level to chemical desiccation methods. Compared to disking, cover crops terminated with herbicides in a no-till system had an increased soil moisture percentage up to 2.4 % to a depth of 61 cm (Daniel

et al., 1999b). Burgess et al. (2014) found a positive or neutral effect on grain yield, regardless of the reduced soil water availability following cover crops.

Soil Temperature. Cover crops rarely have an impact on soil temperature when incorporated by tillage; however, living cover crops can alter soil temperatures significantly (Dabney et al., 2001). During the winter, cover crop residues can increase soil temperatures, while keeping soil cooler in the spring (Kahimba et al., 2008). Teasdale and Mohler (1993) reported cover crop residues reduced maximum and daily soil temperatures and reduced soil water losses in drought conditions. Similarly, Blanco-Canqui et al. (2011) found soil temperatures under winter wheat, sorghum, and hairy vetch to be consistently lower in the spring.

Nitrogen. Nitrogen is one of the most essential nutrients for crops but can easily be lost to the environment (Van Delden, 2001). Losses occur by NO<sub>3</sub><sup>-</sup> leaching, NH<sub>4</sub><sup>+</sup> volatilization, and N<sub>2</sub>O transport to the atmosphere. Residual N is a potential source for water pollution, and producers can save on production costs if the N is used by the following crop instead of washed off by water (Tosti et al., 2012; Stivers-Young, 1998). Cover crops may reduce N losses by accumulating N in biomass, reducing soil NO<sub>3</sub><sup>-</sup> concentration, and reducing the drainage volume (Meisinger et al., 1991; Parkin et al., 2006; Tribouillois et al., 2016). Part of the N contained in winter cover crop residues can become mineralized when incorporated into the soil, making it available to the following crop (Stivers-Young, 1998).

Daniel et al. (1999a) noted that cereal rye showed similar N assimilation to legumes such as vetch or clover in two out of three years studied. Nitrogen assimilated by these cover crops should reduce leaching and runoff losses when compared to fields without cover crops (Daniel et al., 1999a; Clark et al., 1994). Ranells and Wagger (1996) suggested that a binary mixture using

cereal rye with crimson clover or hairy vetch had a lower potential for N immobilization compared to a cereal rye monoculture.

When evaluating soil NO<sub>3</sub><sup>-</sup> Lawson et al. (2015) noted that soil NO<sub>3</sub><sup>-</sup> was greatest after a vetch monoculture followed by mixtures and a cereal rye monoculture. Similarly, Tosti et al. (2014) found pure vetch to provide large amounts of N, but it had similar N leaching risks as bare soil. Kramberger et al. (2013) recommended using cover crop mixtures over pure leguminous stands for areas with frequent rains to reduce the risk of nitrate leaching.

Brassica species can scavenge residual soil N, but Gieske et al. (2016) found they had no effect on N accumulation in a corn cash crop. The N accumulated can be lost to the environment through leaching and runoff preventing availability to the subsequent cash crop. In order to provide the greatest net benefit, cover crop effects on N cycling and their impact on the environment need to be studied further.

Carbon. Rising atmospheric CO<sub>2</sub> levels have created interest in the potential of carbon sequestration as soil organic carbon (SOC). Globally, soils contain 1500 Gt of SOC, about double the C in the atmosphere (Schlesinger and Andrews, 2000). An estimated 30 to 50% of SOC has been lost in the United States due to cultivation (Kucharik et al., 2001). Continuous cropping results in a decline of SOC (Reeves, 1997). The introduction of cover crops as a green manure in conventional till systems could potentially maintain C input to compensate for soil mineralization rates (Mazzoncini et al., 2011).

Regardless of tillage practice, cover crop residue management and limited soil disturbance can have a significant impact on the amount of C that is stored in the soil. Metay et al. (2007) reported no-till systems implementing cover crops increased SOC in the upper 10 cm of soil by 0.35 Mg C ha<sup>-1</sup> year<sup>-1</sup> compared to conventional and disk-tilled plots. Similarly, cover

crops and N fertilization increased residue amounts of C in the soil from above and below ground biomass by 120 to 130 kg N ha<sup>-1</sup> of SOC compared to no cover or fertilization regardless of tillage system (Sainju et al., 2006). Carbon put into the soil from residue is directly related to SOC content; therefore, residue removal compared to residue returned to the soil will alter total SOC. An increase in C sequestration improves overall soil quality and productivity (Sainju et al., 2006).

**Yield.** Cover crops can potentially benefit yields of cash crops such as corn and soybean. Factors affecting the economics of cover crops include cash crop grown, cover crop used, method of establishment and termination, cost of fertilizer, fuel, and cash value applied to the environment and soil protection benefits (Clark, 2007).

Of the many potential benefits of cover crops, increased yield of marketable crops is the most direct benefit derived from cover crops (Snapp, 2005). Decisions to reduce production costs can potentially result in yield loss, in turn negating any other cost savings and decreasing profitability (DeVuyst et al., 2006). Cover crops can improve yield stability, reduce fertilizer costs through N mineralization, use less herbicide through weed suppression, and reduce need for fumigation and pesticides (Snapp et al., 2005).

Sainju et al. (2005b) reported greater cotton yields following weeds or cereal rye cover crop compared to a legume cover crop or cereal rye/vetch mixture. Sorghum yields responded oppositely and were significantly greater with a cereal rye/vetch mixture or a legume cover crop compared to cereal rye and weeds. Carrera et al. (2004) reported that sweet corn yields were 43% greater in vetch and 30% greater in cereal rye-hairy vetch mixture than bare control.

Multiple sites were used to evaluate cover crop treatments consisting of cereal rye, oat (*Avena Sativa* L.), oilseed radish, and oilseed radish mixed with cereal rye compared to a control

of weeds or no cover and no differences in yield were discovered (O'reilly et al., 2011; De Bruin et al., 2005). The overall effects of cover crops on yield are extremely variable depending on the type of cover crop and cropping system implemented.

Insects. Entomological studies of annual cash crops planted into winter cover crops such as cereal rye, wheat, various clovers, and clover/grass mixtures have shown both reductions and increases in pest pressure compared to those planted into fallow ground (Miklasiewicz and Hammond, 2001; Tillman et al., 2004; Koch et al., 2012). Tillman et al. (2004) evaluated the influence of five different cover crop treatments on insect populations in cotton. Economic threshold levels were greater in cotton without a cover crop. Results showed that corn ear worm, *Heliocoverpa zea* (Boddie) population was not increased as a result of cover crop treatments. It has been shown that the density of natural enemy populations enhances in cash crops when intercropped with winter cover crops.

Koch et al. (2012) reported soybean aphid, *Aphis glycines* (Matsumara), and potato leaf hopper, *Empoasca fabae* (Harris) densities were lower in soybeans with cereal rye mowed between rows compared to beans without cereal rye. Variable densities of bean leaf beetle, *Cerotoma trifurcata* (Forster) were observed with lower populations observed in treatments with cereal rye compared to those without. Additionally, winter cereal rye cover crops demonstrated the potential to reduce potato leaf hopper, soybean aphid, and bean leaf beetle populations (Koch et al., 2012). Conversely, cereal rye had the opposite effect in a two-year, commercial farm cereal rye cover crop study in corn rotated with soybeans (Dunbar et al., 2016). Cereal rye cover crops increased true armyworm, *Pseudaletia unipuncta* (Haworth) populations and corn defoliation.

Cover crops grown in concurrence with a cash crop and are not killed are called living mulches. Cover crops and living mulches are often used to increase diversity and abundance of insect populations (Costello and Altieri, 1995; Schmidt et al., 2007; Hartwig and Ammon, 2002). Prasifka et al. (2006) reported living mulches of alfalfa and clover may not be sufficient to suppress pests but have biological control and agronomic benefits to consider. Schmit et al. (2007) reported a living mulch increased natural enemies to soybean aphids resulting in a delay in aphid establishment and lower pest populations. Hooks and Johnson, (2004) reported living mulches were promising in reducing lepidopteran pests by increasing natural predator activity and maintaining crop quality and yield. A large variance across studies suggest the need for additional research on the ability of cover crops and living mulches to reduce populations of damaging insects.

**Disease.** Cover crops may suppress soil-borne diseases when used as biofumigants (Matthiessen and Kirkegaard, 2006). Biofumigation is a biological control method in which green manure releases toxic compounds during the intercrop period. Research indicates the use of green manure on potatoes (*Solanum tuberosum* L.) to reduce soil borne disease such as common scab, *Streptomyces scabiei* (Thaxter) and verticillium wilt, *Verticillium dahliae* (Kleb; Larkin et al., 2011; Ochiai et al., 2007; Sakuma et al., 2011). Motisi et al. (2009) concluded that growing mustard (*Brassica juncea* L.) significantly lowered disease incidence compared to a no cover crop control. When incorporating crushed residues, disease incidence and severity of root rot, *Rhizoctonia solani* (Kuhn) decreased further. Both management phases provided insight into cover crop ability to mitigate disease (Motisi et al., 2009).

Larkin et al. (2010) studied seven different cash crop rotations and assessed them with and without the use of a winter cereal rye cover crop. Canola (*Brassica napus* L.) Rapeseed

(*Brassica napus* L.) reduced soil borne diseases more frequently than green beans, barley/red clover mix, soybean, and sweetcorn with reductions ranging from 18 to 38%; The additional use of a cereal rye cover crop reduced black scarf caused by *R. solani* an average of 12.5% and common scab caused by *S. scabiei* an average of 7.2% across all rotations. Hartz et al. (2005) reported the opposite with mustard cover crops having no consistent effect on soil borne disease suppression. However, grower management of mustard resides likely affected the biofumigant properties. Bensen et al. (2009) reported mustard cover crops did not reduce disease incidence over a long-term experiment.

Weeds. Cover crops can have various influences on weeds as a living plant, or as plant residue after the crop terminates. Studies report vigorous living cover crops can suppress weeds through reduced weed seedling emergence, direct competition, and allelopathy (Brennan and Smith, 2005; Peachey et al., 2004).

Crop residues remaining on the surface can alter weed seed germination by influencing soil temperature, moisture content, light availability, and allelopathy (Creamer et al., 1996). Living cover crops have the ability to absorb red light, resulting in a reduced red:far-red ratio, which inhibits phytochrome-mediated seed germination (Teasdale and Daughtry, 1993). When evaluating cereal rye seeding rates, Boyd et al. (2009) found planting cereal rye at a higher seeding rate can improve biomass production, resulting in improved early-to midseason weed suppression from direct competition.

Allelopathy is a direct or indirect harmful effect by one plant on another through the production of chemical compounds released into the environment (Rice, 2013). Cereal rye cover crops have been proven to be allelopathic. Field studies were conducted to evaluate whether cereal rye cover crops could be leached of allelochemicals, and in turn be used to improve weed

suppression. Results proved allelochemicals leached from cereal rye shoot residues can cause weed suppression (Creamer et al., 1996; Barnes and Putnum, 1986).

Dhima et al. (2006) reported inhibitory substances were present in winter cereals and they all reduced weed growth and germination in corn. Similarly results from Yenish et al. (1996) reported early emergence of giant foxtail (*Setaria faberi* Herrm.), velvetleaf (*Abutilon theophrasti* Medik.), smooth pigweed (*Amaranthus hyridus* L.), and common lambsquarter (*Chenopodium album* L.) was reduced using cover crops in corn. Using cereal rye as a mulch generally controlled 90 % or more of weed populations in soybean (Liebl et al., 1992). In contrast, Moore et al. (1994) reported that small grain cereals did not affect weed seedling emergence compared to bare soil in soybeans.

Monocultures of cereal rye, barley, hairy vetch, and crimson clover were planted along with polycultures of crimson clover, barley, cereal rye and hairy vetch to suppress eastern black nightshade (*Solanum ptychanthum* Dunal) and yellow foxtail (*Setaria pumila* Poir; Creamer et al., 1996). Compared to the control, all five leached cover crop shoot residues reduced eastern black nightshade emergence indicating a distinct physical suppression component. Emergence was inhibited by approximately 98% in barley, leached cereal rye, and the clover-barley- cereal rye polyculture. The results indicate that cover crop weed suppression is species-specific and growing various mixtures does not necessarily eliminate a broad range of weeds. Use of cover crops as weed-suppressing mulches was demonstrated through the eastern black nightshade suppression independent of allelochemical mechanisms (Creamer et al., 1996).

Cover crop residue management optimization for weed control was analyzed using cereal rye and oilseed rape (Kruidhof et al., 2009). Winter cereal rye residue was most effective as surface mulch. When residues were incorporated into the soil, inhibitory effects were weaker,

and the time course of inhibition was difficult to predict. Oilseed rape was less effective when used as mulch compared to incorporated residue. Results suggested weed species response to residues depends on weed emergence time (Kruidhof et al., 2009).

Mixtures. Researchers and growers are becoming increasingly interested in the potential benefits a diverse-species cover cropping system may have (Lin, 2011). A mixture of cover crop species can help achieve multiple goals simultaneously (Tosti et al., 2014). Cover crop mixtures can positively impact winter survival, ground cover, biomass production and N cycling, weed and pest control, duration of active growing period, forage options, tolerance to adverse conditions, root architecture, C/N, and response to variable soil traits (Clark, 2007; Smith et al., 2014). Using multispecies mixtures may cause an increase in overall resilience, production, and resource efficiency; but management objectives should be the main consideration when selecting any cover crops (Wortman et al., 2012b). Maximized benefits will result from the proper choice and management of cover crops whether using a mixture or monoculture. Soil, water availability, farming system, cropping sequence, and cultural practices should be considered to ensure success for the grower (Ingels et al., 1994).

Growing multiple species together that have different temporal and spatial nutrient demands can potentially recover N in fertilizer and legume-based cropping systems (Crews and Peoples, 2005). Variance in growing conditions can cause cover crop monocultures to have the same risks associated with cash crop monocultures (Lin, 2011). Cover crop mixtures may be less desirable to growers due to increased cost for seed, difficulty establishing and managing complex mixtures, different termination requirements, different seed sizes, and different growth rates (Creamer et al., 1997; Clark, 2007; Wortman et al., 2012a; Blanco-Canqui et al., 2015).

According to a 2016 SARE survey, cover crop mixtures were planted on more than 161,000 acres which was similar to the amount of monoculture cereal rye planted. Of 999 respondents who used cover crop mixtures, respondents planted 79 acres of binary mixtures per respondent or around 64,549 acres, 35,755 acres of polycultures, and 61,214 acres of a mixture containing four species or more (SARE, 2016).

Cereal rye is commonly included in mixtures because it overwinters well, establishes quickly, has adequate biomass, and kills easily. Studies have shown cereal rye produces more biomass than a cereal rye/hairy vetch mixture (Daniel et al., 1999a; Lawson et al., 2015; Clark et al., 1994). When establishing a cereal rye- hairy vetch mixture, Lawson et al. (2015) found no benefit to increase hairy vetch seeding past 50% of the seed mixture weight since cereal rye dominated the species mixture regardless of the seeding ratio through quick establishment and large amounts of biomass; the vetch increased weed suppression and added N to the mix. Of thirteen polyculture mixtures of cover crops evaluated, cereal rye was competitive with other species and comprised 80% of above-ground biomass in five out of eight mixtures it was in (Creamer et al., 1997). Hairy vetch, barley, and crimson clover were also competitive, potentially making them good candidates when selecting cover crop species (Creamer et al., 1997).

Field experiments were conducted to study the effects of cereal rye, hairy vetch, and cereal rye- hairy vetch mixtures on biomass and density of winter weed communities (Hayden et al., 2012). All cover crop treatments reduced weed biomass with weed suppression compared to the control ranging from 71 to 91% for vetch to 95 to 98% for cereal rye. The cereal rye and cereal rye-hairy vetch mixtures suppressed the density of mustard weed species the most compared to vetch alone. The authors concluded cereal rye would most likely be the most inexpensive and effective winter cover crop for weed control, but if mixed with vetch it could

potentially restore N in the soil. Teasdale and Abdul-Baki (1998) compared a vetch monoculture to a cereal rye-hairy vetch cover crop mixture and found the increased biomass from the mixture improved overall weed suppression compared to the vetch monoculture.

Overall cover crop effects on agroecosystems are still controversial. Smith et al. (2014) reported cover crop mixtures did not enhance the overall agroecosystem services in summer cover crops (Smith et al., 2014). Blanco-Canqui et al. (2015) reported that cover crops can improve ecosystem services and enhance the multi-functionality of agroecosystems without drastically interfering with current management practices. Lin (2011) noted the practical and theoretical arguments for and against the use of diverse cover crop mixtures. There is conflicting research on the use of cover crop mixtures. As cover crop mixtures become more popular, further research is needed to evaluate their overall impact on agroecosystems.

## Ch. 1: IMPACTS OF COVER CROP MIXTURES ON EDAMAME IN SOUTHWEST MISSOURI: A FIELD STUDY

## Introduction

Cover crops are grown to enhance agroecosystems without drastic alteration of management practices (Blanco-Canqui et al., 2015; Dozier et al., 2017). Cover crops reduce erosion; (Blanco-Canqui et al., 2011), lower NO<sub>3</sub><sup>-</sup> losses in runoff and leachate; (Villamil et al., 2006; Kaspar et al., 2012; Clark et al., 1994; Daniel et al., 1999), reduce insect populations; (Miklasiewicz and Hammond, 2001; Tillman et al., 2004; Koch et al., 2012), suppress soil-borne diseases; (Matthiessen and Kirkegaard, 2006; Larkin et al., 2010), and suppress weeds (Brennan and Smith, 2005; Peachey et al., 2004). Additional ecological stability and increased crop resilience can occur with species diversity among plant communities (Wortman et al., 2012).

Cover crop mixtures can increase production and resource efficiency by combining multiple functional traits (Wortman et al., 2012; Smith et al., 2014). Mixtures of cover crops can increase tolerance of adverse conditions and positively impact biomass production and winter survival (Clark, 2007). Cover crop mixtures allow growers to achieve multiple goals such as soil erosion prevention, and the trapping of N to reduce leaching (Tosti et al., 2014). If mixtures are planted late or include an aggressive species such as cereal rye, species diversity can be compromised; to avoid grass dominance seeding rates of grasses should be lowered while legume seeds should comprise a larger portion of the mixture (Murrell et al., 2017). Creamer et al. (1997) studied multiple cover crop mixtures using various species and reported cereal rye dominated spring biomass in diverse mixtures.

Cover crops have the ability to restore N back into the soil that may otherwise turn into runoff and cause pollution (Tosti et al., 2012; Stivers-Young, 1998). Cover crops are able to accumulate N in biomass and different species result in different C/N ratios (Meisinger et al., 1991; Parkin et al., 2006; Tribouillois et al., 2016). Cereal rye was reported to have a greater C/N ratio than any other cover crop treatments including legume monocultures, and binary mixes (Sainju et al., 2005a, 2005b; Sainju et al., 2007a; Clark et al., 1994; Clark et al., 1997; Kuo and Jellem, 2002). Odhiambio and Bomke (2001) and Tosti et al. (2014) reported grass and small grain monocultures had the greatest C/N ratios while legume monocultures had the lowest ratio. Binary mixture C/N ratios are often intermediate to legume and grass monocultures. For example; Sainju et al. (2005b) reported a 57:1 ratio for cereal rye with a 12:1 ratio for hairy vetch, and a 32:1 ratio for a cereal rye-hairy vetch biculture. Net immobilization is more likely to occur in the soil when the C/N ratio is above 25:1 (Allison, 1966).

Edamame is a unique soybean that is harvested when beans are still immature, in the R6 stage. The cash crop grew in popularity during the 1970's when interest in organic agriculture peaked. Demand is on a slow increase and it is a nutritious addition to many diets. Edamame is used in salads, snacks, and can be served steamed and salted in restaurants (Konovsky et al., 1994). Taiwan exports 5,000 MT of edamame to the United States (Shanmugasundaram, 2004). The use of edamame could increase diversity of current Midwestern cropping system and reduce the need for imports.

Cover crop effect on cash crop yield are variable, depending on the cover crop species and management practices (Snapp, 2005). Snapp (2005) reported one of the most direct benefits derived from cover crops is increased yield in cash crops. Sainju et al. (2005a) reported greater cotton yields in cereal rye and weeds over two growing seasons compared to a legume cover

crop or mixture, but sorghum grain yields were greater following hairy vetch and a cereal rye-hairy vetch biculture. Additional studies reported increases in cash crop yields following the implementation of cover crops (Carrera et al., 2004; Clark et al., 1994; Kuo and Jellum, 2002; Vaughan and Evanylo, 1998). No difference in cash crop yield was discovered when evaluating multiple cover crop treatments over multiple sites (O'reilly et al., 2011; De Bruin et al., 2005).

There are practical arguments for and against the use of cover crop mixtures, and there is a need for additional research to understand their impact on agroecosystem functions (Lin, 2011). Therefore, the objective of this study was to examine the impact of cover crop mixtures on cover crop biomass, cover crop C/N ratios, and edamame yield. Specifically, this field study evaluated percentage cover crop canopy cover and biomass, weed biomass, cover crop and soil C and N, edamame stand, pod count, and pod yield.

## **Materials and Methods**

An experiment evaluating cover crop mixture effects on cover crop biomass, cover crop biomass N, cover crop C/N ratio, edamame yield, canopy cover, weed biomass, edamame stand, pod count, and pod yield was established at the State Fruit Experiment Station in Mountain Grove, Missouri, USA (37.1542° N, 92.2618° W). This location received 1135 mm of precipitation from August 2016-August 2017, and mean monthly temperatures ranged from 2°C (January) to 22°C (July) (Missouri Mesonet, Weather Station Network, Mountain Grove, MO). Soil at the location was Viraton silt loam (fine-loamy, siliceous, active, mesic Oxyaquic Fragiudalfs) with 2-8% slope and a pH of 5.6. Dates of crop management practices and data collection are listed in Table 1.

The experimental design was a randomized complete block with four replications. Pumpkin with no cover crop was grown prior to the experiment. Plots measured 9.1 by 4.6 m. Soil samples from the top 5 cm of soil were collected prior to cover crop planting and analyzed for total soil carbon and nitrogen by the MU Soil and Plant Testing Laboratory by combustion method using a variomax CN analyzer. Six treatments included winter cereal rye, cereal rye-Austrian winter pea (*Pisum sativum* L.ssp, *sativum* var. *arvense*) at two seeding rates, cereal rye-Austrian winter pea-radish at two seeding rates, and a no cover control (Table 2). Cover crop seeding rates were consistent with recommendations from the University of Missouri. Legume seed was mixed with water and Verdesian dry premium peat inoculant (Wannamaker seed Inc, Saluda, SC) prior to planting. Plots were disked on 23 Aug. 2016 to prepare for planting. Glyphosate (Buccaneer Plus) was applied at 2.5 kg a.i ha<sup>-1</sup>. Plots were established on 30 Sept. 2016 by broadcast seeding. A cultipacker was used to firm soil around seeds. An overhead irrigation sprinkler system applied 13 mm of water directly after cover crop planting.

Total green area canopy cover percentage was estimated using the Canopeo application (Patrignani and Ochsner, 2015) on both dates. Cover crop biomass was clipped at the soil surface and collected from a 0.1 m<sup>2</sup> quadrat randomly placed in each plot during Nov 2016, and three quadrats were collected 13 April. 2017. Cover crop and weed species were sorted, dried, and weighed. Samples were ground to pass a 2-mm screen using a Wiley Mill (Arthur H. Thomas Company). Cover crop biomass C and N were determined from duplicate samples from each plot by the MU Soil and Plant Testing Laboratory. Samples were analyzed by combustion method using a VarioMax CN analyzer.

Cover crops were terminated by disking on 19 April. 2017 and plots were disked twice more prior to edamame planting. Edamame [*Glycine max* 'Midori Giant' (Wannamaker Seeds

Inc.)] was hand planted on 26 May. 2017 in furrows 5-cm deep in six 76 cm rows 7.6 cm apart to give a seeding rate of approximately 185,000 seeds ha<sup>-1</sup>. Inoculant (N-Dure, INTX Microbials LLC., Kentland, IN, Wannamaker Seeds Inc., Saluda, NC) mixed with water was used on the edamame seed before planting. Fertilizer was applied at 20 kg K<sub>2</sub>O ha<sup>-1</sup> and 31 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> one day prior to planting, in accordance with soil test recommendations. Fertilization and pest management recommendations from the University of Arkansas were used to guide crop management decisions. The insecticide zeta-cypermethrin (Mustang® Maxx 0.8EC, FMC Corporation, Philadelphia, PA, USA) was sprayed at a rate of 0.02 kg a.i. ha<sup>-1</sup> using broadcast application method on 28 June. 2017 to manage an outbreak of Japanese beetles, *Popillia* japonica (Newman). Spring plots were extremely weedy from a previously established weed seed bank consisting of large amounts of common lambsquarters (*Chenopodium album L.*), Pennsylvania smartweed (*Polygonum pensylvanicum* L.), redroot pigweed (*Amaranthus* retroflexus L.), smooth pigweed, and carpetweed (Mollugo verticillate L.). Plots were tilled to terminate cover crops and control spring weed populations. The herbicide fomesafen (Flexstar®, Syngenta U.S., Greensboro, NC) was sprayed at a rate of 0.3 kg a.i. ha<sup>-1</sup> using a broadcast application method to manage summer weed populations in all plots on 28 June 2017 and 10 July 2017.

Edamame was harvested by hand on 9 Aug. 2017 when plants reached the R6 stage of development (fully expanded seeds). Plants were harvested from the middle 3 m of the two center rows. Four edamame plants were randomly selected from those harvested in each plot and pods were sorted by one, two, or three-seed then counted and weighed.

Data from the study were analyzed as a randomized complete block design with a oneway treatment structure using analysis of variance (PROC MIXED, SAS v 9.4). Cover crop mixture was a fixed effect and replication was included in the model as a random factor. LS means were separated using Tukey's pair-wise comparisons ( $\alpha$  <0.05).

## **Results and Discussion**

Estimates of canopy cover were similar among cover crop treatments and greater than the no cover control during fall, likely due to the fact cereal rye was in every mixture excluding the control. No differences in spring canopy cover were observed due to the large amount of weed species established in the control plots (Table 3).

Spring weed biomass was significantly less in all treatments compared to the control (Table 3). Large amounts of redroot pigweed, smooth pigweed, Pennsylvania smartweed, common lambsquarters, and carpetweed were present in control plots. Previous research suggests weed suppression may have been due to direct competition from cover crops (Brennan and Smith, 2005; Creamer et al., 1996; Peachey et al., 2004). Summer weed weight was not different (Table 3) likely due to disking and the delay between termination of the cover crops and edamame planting.

Cereal rye established quickly compared to winter pea and radish and contributed most of the biomass in the fall measurements (Table 4). Similarly, Creamer et al. (1997) and Lawson et al. (2015) reported cereal rye produced large amounts of biomass and was dominant in their mixtures. Fall cereal rye biomass was heavier than the control, but less than the radish in the polyculture with low recommended cereal rye rates. Fall pea weight was greater in the rye-pea binary mixture. Fall radish weight was more than any other treatments when planted with a low cereal rye recommendation rate (Table 4).

By spring, winter pea and radish were well established and there were no differences in total cover crop dry biomass among treatments (Table 4). Spring cereal rye rates were only greater than the control because all species were established in the other treatments. Spring pea weight was greater in the binary mixtures and polycultures using low cereal rye seeding rates. Spring radish weight was similar with both high and low recommended cereal rye rates. Appelgate et al. (2017) and Finney et al. (2016) reported similar findings of cover crop mixtures not producing more biomass than the most productive cover crop monocultures.

Cover crop biomass C was greater in all mixtures compared to biomass C accumulated by weeds in the no cover control (Table 5). Cover crop biomass N was different in the four polyculture treatments compared to the cereal rye monoculture and the no cover crop control (Table 5). The control and cereal rye monoculture were likely lower in biomass N because they did not have legumes to provide N fixation or brassicas to scavenge additional N. Varying N concentrations were also associated with different seeding ratios among grasses, legumes, and brassicas.

The soil C and N content was not different among treatments (Table 5). It is possible that no differences were found due to only one year of data. Previous research suggests that higher concentrations of SOC and soil total nitrogen were in the top layers of soil in no-tillage systems compared to conventional (Mazzoncini et al., 2011; alvarez et al., 1995; Mahboubi et al., 1993). This study implemented conventional tillage methods, which may have contributed to small differences among data.

Plant stand count was measured from three meter of row in each plot. Edamame height and stand count did not differ between treatments during the summer or at the time of harvest (Table 6). No differences were found among pod counts in single, double, and triple beans or

total pod count after harvest (Table 7). Single and double-bean pod weights did not differ from each other with the exception of triple-pod weight (Table 8). The difference in triple pod weight among all treatments is possibly attributed to the condition of the pods.

Pods with three beans in some plots were very healthy and large. Other plots had pods with three beans that had a large amount of insect damage from Japanese beetles. The beetles attacked weeds, cover crops, and edamame pods regardless of the treatment. Szendrei and Isaacs, (2006) reported that Japanese beetle behavior is affected by cover crop species and beetles were sparse in bare ground control plots compared to those with Alsike clover (*Trifolium hybridum* L.), buckwheat (*Fagopyrum esculentum* Moench), and perennial ryegrass (*Lolium perenne* L.). If pods distinctly had three beans they were classified as such even if a seed was damaged. Plots with more insect damage had smaller weights because pods and beans were damaged. The overall pod weight was not affected by the difference in the three-pod weight.

In conclusion, there were limited differences among all cover crop mixtures and treatments. Fall canopy cover was dominated by a quickly established cereal rye monoculture, but no differences occurred during the spring once other species had time to establish. Spring weed weights were excessive in control plots because there was no competition from cover crops. Cereal rye monoculture contained slightly less biomass N compared to the binary mixture and polycultures. Pea and radish in treatments with lower cereal rye seeding rates produced more biomass than pea and radish in treatments with recommended rye seeding rates. Three-bean pod weight difference was likely caused by insect damage altering results. No other significant differences were found between treatments.

Table 1. Cover crop planting and data collection dates.

Activity	Date
Initial soil sampling	1 Aug. 2016
Disking	26 Aug. 2016
Herbicide Spray	9 Sept. 2016
Cover crop planting	30 Sept. 2016
Biomass Collection 1	16 Nov. 2016
Biomass Collection 2	13 Apr. 2017
Cover crop termination	19 Apr. 2017
Disking	10 May. 2017
Disking	25 May. 2017
Soil sampling	25 May. 2017
Fertilizer application	25 May. 2017
Edamame planting	26 May. 2017
Herbicide Spray	28. June 2017
Insecticide Spray	28 June. 2017
Edamame Stand Count	28 June. 2017
Weed Biomass Collection	28 June. 2017
Herbicide spray	10 July. 2017
Edamame Stand Count	9 Aug. 2017
Edamame harvest	9 Aug. 2017

**Table 2.** Cover crop treatments and seeding rate for plots.

•	Winter	Austrian	Radish
Treatment†	Rye	Winter Pea	
		kg ha <sup>-1</sup>	
Rye	45	-	-
Rye-pea- high	23	14	-
Rye-pea- low	15	22	-
Rye-pea-radish -high	15	9	1
Rye-pea-radish -low	10	13	3
Control	0	0	0

†Rye= Cereal rye; Pea=Austrian winter pea; Radish= Ground builder Radish; High= High cereal rye seeding rate; Low=Low cereal rye seeding rate

**Table 3**. Least square means for cover crop canopy cover during spring and fall along with spring and summer dry weed biomass  $(g/0.1m^2)$ . Treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

	Canopy cover		Weed biomass	
Treatment†	Fall 2016 Spring 2017		Spring 2017	Summer 2017
	% Canopy cover		$g/0.1m^2$	
Rye	$31.4_{a}$	61.3	$-9.3_{a}$	14.7
Rye-pea- high	$22.3_a$	61.9	$-8.0_{a}$	16.0
Rye-pea- low	$18.2_{a}$	60.3	-1.5a	16.3
Rye-pea-radish -high	$23.0_a$	65.9	-5.7 <sub>a</sub> 18.2	
Rye-pea-radish -low	$20.8_{a}$	66.3	-0.6 <sub>a</sub> 18.1	
Control	$0.2_{b}$	62.5	$98.9_{b}$	16.6
Pr>F	< 0.001	0.702	<0.001 0.758	

†Cereal rye= 45 kg ha<sup>-1</sup>; Rye-pea – high = cereal rye at 23 kg ha<sup>-1</sup>, Austrian winter pea at 14 kg ha<sup>-1</sup>; Rye-pea – low = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 22 kg ha<sup>-1</sup>; Rye-pea-radish – high = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 9 kg ha<sup>-1</sup>, Ground Builder radish at 1 kg ha<sup>-1</sup>; Rye-pea-radish – low = rye at 10 kg ha<sup>-1</sup>, Austrian winter pea at 13 kg ha<sup>-1</sup>, Ground Builder radish at 3 kg ha<sup>-1</sup>; Control=No cover crop, weedy

**Table 4**. Least square means for spring and fall cover crop dry weights. Treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

		Fall 2016		S	Spring 2017	
Treatment†	Rye	Pea	Radish	Rye	Pea	Radish
·			g/0.	1m <sup>2</sup>		
Rye	$22.9_a$	$0_{\rm a}$	$2.3_a$	$369.9_{a}$	13.6a	$2.4_a$
Rye-pea- high	$10.6_{ac}$	$3.9_{ab}$	$5.0_{a}$	$340.3_{a}$	$32.7_{ab}$	$2.4_a$
Rye-pea- low	$10.3_{ac}$	$6.4_{\rm b}$	$2.3_a$	$342.4_{a}$	$36.1_{b}$	$2.4_a$
Rye-pea-radish -high	$12.9_{ad}$	$3.9_{ab}$	$6.5_a$	$369.5_{a}$	$27.1_{ab}$	$10.8_{b}$
Rye-pea-radish -low	$7.3_{\rm bcd}$	1.8a	$17.6_{b}$	$344.5_a$	35.7 <sub>b</sub>	$9.9_{ab}$
Control	$0_{\mathrm{bc}}$	$2.3_a$	$2.3_a$	$-10.1_{b}$	$14.0_a$	$2.4_a$
Pr>F	0.001	0.001	< 0.006	< 0.001	0.008	0.003

†Cereal rye= 45 kg ha<sup>-1</sup>; Rye-pea – high = cereal rye at 23 kg ha<sup>-1</sup>, Austrian winter pea at 14 kg ha<sup>-1</sup>; Rye-pea – low = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 22 kg ha<sup>-1</sup>; Rye-pea-radish – high = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 9 kg ha<sup>-1</sup>, Ground Builder radish at 1 kg ha<sup>-1</sup>; Rye-pea-radish – low = rye at 10 kg ha<sup>-1</sup>, Austrian winter pea at 13 kg ha<sup>-1</sup>, Ground Builder radish at 3 kg ha<sup>-1</sup>; Control=No cover crop, weedy

**Table 5**. Least square means of N and C from six soil samples per plot and three 0.1-m<sup>2</sup> quadrats per plot of cover crop biomass collected 25 May 2017. Treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

Treatment†	Soil C	Soil N	Biomass C	Biomass N		
	%		%		kg	ha <sup>-1</sup>
Rye	2.2	0.2	$42.5_{a}$	$1.7_a$		
Rye-pea- high	2.1	0.2	$42.6_a$	$2.2_{b}$		
Rye-pea- low	2.1	0.2	$42.1_{a}$	$2.2_{\mathrm{bc}}$		
Rye-pea-radish -high	2.2	0.2	$42.7_{a}$	$2.5_{\rm c}$		
Rye-pea-radish -low	2.3	0.2	$41.8_a$	$2.6_{\rm c}$		
Control	2.1	0.2	39.6 <sub>b</sub>	$1.9_{ab}$		
Pr>F	0.727	0.945	0.001	< 0.001		

†Cereal rye= 45 kg ha<sup>-1</sup>; Rye-pea – high = cereal rye at 23 kg ha<sup>-1</sup>, Austrian winter pea at 14 kg ha<sup>-1</sup>; Rye-pea – low = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 22 kg ha<sup>-1</sup>; Rye-pea-radish – high = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 9 kg ha<sup>-1</sup>, Ground Builder radish at 1 kg ha<sup>-1</sup>; Rye-pea-radish – low = rye at 10 kg ha<sup>-1</sup>, Austrian winter pea at 13 kg ha<sup>-1</sup>, Ground Builder radish at 3 kg ha<sup>-1</sup>; Control=No cover crop, weedy

**Table 6**. Least square means for edamame stands and height during summer months and harvest. Treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

	Summer 2017		Harvest 2017	
Treatment†	Edamame	Edamame	Edamame	Edamame
	Stand	Height	Stand	Height
		c	m	
Rye	22.6	28.0	24.3	53.1
Rye-pea- high	21.3	28.0	22.9	54.8
Rye-pea- low	21.1	29.4	23.1	56.3
Rye-pea-radish -high	20.3	29.3	22.0	52.2
Rye-pea-radish -low	23.4	30.7	23.0	55.2
Control	21.9	29.8	21.8	51.9
Pr>F	0.959	0.594	0.982	0.754

†Cereal rye= 45 kg ha<sup>-1</sup>; Rye-pea – high = cereal rye at 23 kg ha<sup>-1</sup>, Austrian winter pea at 14 kg ha<sup>-1</sup>; Rye-pea – low = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 22 kg ha<sup>-1</sup>; Rye-pea-radish – high = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 9 kg ha<sup>-1</sup>, Ground Builder radish at 1 kg ha<sup>-1</sup>; Rye-pea-radish – low = rye at 10 kg ha<sup>-1</sup>, Austrian winter pea at 13 kg ha<sup>-1</sup>, Ground Builder radish at 3 kg ha<sup>-1</sup>, Control=No cover crop, weedy; Edamame=Midori Giant

**Table 7**. Least square means of total edamame pods. Treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

	One-bean	Two-bean	Three-bean	Total
Treatment†	Pods	Pods	Pods	Pods
		# (	of pods	
Rye	3.9	17.9	12.8	33.1
Rye-pea- high	4.3	20.3	15.0	38.3
Rye-pea- low	3.3	20.7	12.4	34.9
Rye-pea-radish -high	3.4	14.7	8.8	25.5
Rye-pea-radish -low	2.1	11.0	9.4	21.0
Control	4.2	15.3	10.3	28.1
Pr>F	0.275	0.212	0.071	0.102

†Cereal rye= 45 kg ha<sup>-1</sup>; Rye-pea – high = cereal rye at 23 kg ha<sup>-1</sup>, Austrian winter pea at 14 kg ha<sup>-1</sup>; Rye-pea – low = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 22 kg ha<sup>-1</sup>; Rye-pea-radish – high = cereal rye at 15 kg ha<sup>-1</sup>, Austrian winter pea at 9 kg ha<sup>-1</sup>, Ground Builder radish at 1 kg ha<sup>-1</sup>; Rye-pea-radish – low = rye at 10 kg ha<sup>-1</sup>, Austrian winter pea at 13 kg ha<sup>-1</sup>, Ground Builder radish at 3 kg ha<sup>-1</sup>, Control=No cover crop, weedy; Edamame=Midori Giant

**Table 8**. Least square means for edamame pod weights based off pod size. Treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

Treatment†	Single Pod	Double Pod	Triple Pod	Total Pod
	Weight	Weight	Weight	Weight
		(	(g)	
Rye	2.8	21.8	26.7 <sub>ab</sub>	49.8
Rye-pea- high	2.7	30.2	$29.6_a$	61.2
Rye-pea- low	2.1	27.4	$27.2_{ab}$	55.3
Rye-pea-radish -high	1.8	18.8	$17.7_{b}$	36.9
Rye-pea-radish -low	1.0	13.9	$19.5_{ab}$	33.1
Control	3.9	16.7	$19.9_{ab}$	39.0
Pr>F	0.354	0.349	0.021	0.109

†Cereal rye= 45 kg ha -¹; Rye-pea – high = cereal rye at 23 kg ha⁻¹, Austrian winter pea at 14 kg ha⁻¹; Rye-pea – low = cereal rye at 15 kg ha⁻¹, Austrian winter pea at 22 kg ha⁻¹; Rye-pea-radish – high = cereal rye at 15 kg ha⁻¹, Austrian winter pea at 9 kg ha⁻¹, Ground Builder radish at 1 kg ha⁻¹; Rye-pea-radish – low = rye at 10 kg ha⁻¹, Austrian winter pea at 13 kg ha⁻¹, Ground Builder radish at 3 kg ha⁻¹; Control=No cover crop, weedy; Edamame=Midori Giant

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# Ch. 2: META-ANALYSIS OF COVER CROP AND CASH CROP RESPONSE TO COVER CROP MIXTURES

# Introduction

Cover crop mixtures can increase spatial and temporal plant diversity leading to enhanced ecosystem services in agronomic systems (Finney et al., 2016). Interest in the potential benefits of a diverse-species cover cropping system has increased among researchers and growers (Lin, 2011). Research on managing and optimizing cover crop mixtures has recently expanded because of rising interest in cover crop mixtures using more than two species (Storkey et al., 2015; Finney et al., 2016; Wortman et al., 2012b).

When evaluating the potential benefits of cover crops mixtures, Blanco-Canqui et al. (2015) reported cover crops mixtures have the ability to enhance the multi-functionality of agroecosystems and improve ecosystems services. Wortman et al. (2012b) noted increased overall resilience, production, and resource efficiency when using multi-species mixtures. Tosti et al. (2014) pointed out cover crop mixtures' ability to simultaneously achieve multiple agronomic goals.

Many studies evaluate cover crop mixture biomass and biomass N. Lawson et al. (2015) reported cover crop mixtures had greater N concentrations and lower C/N ratios compared to a monoculture, but mixtures had similar or less biomass than monocultures. Sainju et al. (2007a) reported hairy vetch and a cereal rye-hairy vetch mixture had greater N content and a lower C/N ratio than cereal rye or weeds alone, leading to greater soil N and cash crop N uptake. Finney et al. (2016) did not find any cover crop mixtures that produced more biomass than the most productive monocultures. Tosti et al. (2014) recommended the adoption of cover crop mixtures to optimize agroecosystem efficiency after discovering pure vetch alone supplied large amounts of N but resulted in large amounts of nitrate loss. Kramberger et al. (2013) had similar

recommendations after noting that leguminous cover crops increase the risk of nitrate leaching in areas with high rainfall.

Many factors contribute to overall crop yields. Vast differences among cover crop selection, management style, and cash crop species contribute to the varying results among studies requiring additional research. When analyzing cash crop yield, Leavitt et al. (2011) reported greater tomato, zucchini, and bell pepper yield following a no cover crop control compared to cover crop monocultures or bicultures. Appelgate et al. (2017) found no advantages of cover crop mixtures compared to a monoculture on weed suppression and yield. O'Reilly et al. (2011) reported no difference in sweet corn yield among cover crop monocultures and binary mixtures. Sainju et al. (2005b) reported hairy vetch and a hairy vetch- cereal rye biculture increased cotton and sorghum yields similarly. Tosti et al. (2012) reported maize and tomato yields among all cover crop treatments evaluated. Vaughn and Evanylo (1998) found hairy vetch increased corn yields more than cereal rye or a cereal rye-hairy vetch mixture. Parr et al. (2011) reported 2009 corn yields were greatest following late-terminated hairy vetch cover crop treatments, but in 2010 greatest grain yields followed a cereal rye -hairy vetch cover crop mixture. Most cover crop treatments yielded less than the highest yielding N-fertilized control treatment. Further research is needed to discover the various impacts of cover crops on cash crops yield.

A meta-analysis is a research tool that combines independent studies and quantifies the variability among treatment effects (Hedges and Olkin, 1985). A meta-analysis is important because it is a way to analyze data on a large scale across all environments, regions, and crops, given that many studies only draw conclusions from a local scale (Fisher, 2015). Meta-analysis statistical methods can help interpret and summarize multiple studies as an index of effect size

(Gurevitch and Hedges, 1999). The objective of this meta-analysis was to evaluate the impact of cover crop mixtures on agronomic and environmental benefits in agricultural production systems. A meta-analysis was used to address the following question: How do winter cover crop monocultures, bicultures, and polycultures of three or more species compare to each other and to no-cover-crop controls when evaluating cover crop biomass, cover crop biomass N, cover crop C/N ratio, and cash crop yield.

## **Materials and Methods**

A meta-analysis of published studies was conducted to evaluate the impacts of cover crop mixtures on productivity in cropping systems. Studies were found by searching the Agricola (USDA National Agriculture Library, Beltsville, MD) and Google Scholar (Google, Mountain View, CA) electronic databases. Keywords used in these searches were 'cover crop mixtures' alone and with 'biomass', 'biomass N', 'C/N ratio', and 'yield.' Citations from papers initially identified were also used to gather additional studies. Studies included in the analysis were published between 1990 and 2017; included a cover crop monoculture or control and at least one polyculture; and reported one or more of the following measurements: cover crop biomass, cover crop biomass N, C/N, or cash crop yield. A total of 29 published articles with 185 different site-years met the criteria. Due to lack of studies, soil NO<sub>3</sub>- and cash crop profit were excluded from the statistical analysis but are listed (Table 9).

Cover crop treatments were classified as monoculture, biculture, polyculture, or control.

Controls included either weedy treatments or bare ground. Monocultures consisted of grass,
legumes, or brassicas. Bicultures consisted of legume-grass (24 studies) or brassica-grass (4

studies). Polycultures consisted of a mixture of at least three different species with a large majority containing cereal rye, hairy vetch, and some type of clover or additional legume.

Data were analyzed using a mixed model ANOVA (Proc Mixed of SAS, SAS Institute Inc., Cary, NC) that included classification of cover crop mixture as a fixed effect and study as a random effect. Least squared means were weighted using the inverse of the squared SEM (standard error of mean) (St-Pierre, 2001; Lancaster et al., 2014) and means were separated by Tukey's pairwise comparison at  $\alpha$ =0.05. When P-value and LSD were reported, a function written in R was used to estimate SD using number of treatments, LSD value, and LSD P-value. Due to lack of reported SEMs in some studies, a SD was imputed by algebraic manipulation from the number of experimental units if the study reported an LSD (least significant differences) or P-value with no SEM (Gadberry et al., 2015). This was done using the qt() function in R to determine critical t value (http://www.r-project.org; M.S Gadberry, personal communication) in order to minimize the exclusion of already limited studies. If the P-value was ≤0.01 then 0.03 was applied, and for "nonsignificant" reported P-values 0.20 was assigned. The implementation of 0.03 was based off the idea that  $P \le 0.05$  is commonly reported and sets the threshold for the rejection or acceptance of a null hypothesis (Gadberry et al., 2015). To evade a smaller probability level among meta-results the .20 α probability level was implemented to avoid estimating a smaller SD as it was similar to other studies used. Papers using multisite and multiyear means had the SD calculated from the pooled means (Gadberry et al., 2015).

## **Results and Discussion**

**Biomass.** Cover crop monocultures, bicultures, and polycultures produced greater biomass than the control (Table 10). Planting any cover crop proved to provide more biomass

than weeds or a control plot because you are adding additional species to an agroecosystem. Seven out of 23 biomass studies reported a cereal rye-legume biculture produced larger quantities of biomass compared to monocultures or a control (Clark et al., 1997; Clark et al., 1994; Kuo and Jellum 2002; Sainju et al., 2005a, 2005b; and Sainju et al., 2007a, 2007b). Odhiambo and Bomke (2001) reported similar findings where a cereal rye-legume biculture had larger amounts of biomass in three out of four experiments; winter wheat produced greater biomass only when planted early.

A cereal rye monoculture produced more biomass than bicultures during certain years but not consistently in three of the reported studies (Vaughan and Evanylo, 1998; Ranells and Wagger, 1996; Leavitt et al., 2011). Monocultures consisting of cereal rye, crimson clover, and wheat had greater amounts of biomass than any of the bicultures in three reported studies (Parr et al., 2011; Daniel et al., 1999; Lawson et al., 2015). It is likely that overall results among cover crop monocultures, bicultures, and polycultures were not different because of the variance among individual studies.

**Biomass N.** The only difference in biomass N was between monocultures and bicultures. Mixtures had biomass N contents between 40 and 310 kg ha<sup>-1</sup>, leading to different results among monocultures and bicultures. A majority of the studies used monocultures and bicultures, resulting in small weighting factors for the control and polycultures, giving them less statistical importance.

There is a vast difference in legume and grass N content. Some studies report similar N content for grasses and legumes. Legumes fix N and commonly have a greater N content than grasses. Nitrogen fixation by legumes occurs by N-fixing rhizobia bacteria (*Rhizobiaceae*) that live in nodules on plant roots and convert unusable N<sub>2</sub> into biologically useful NH<sub>3</sub> (Flynn and

Idowu, 2015). The biculture had the benefit of grasses scavenging N and leguminous species fixing N making it significantly different from the monocultures.

Appelgate et al. (2017) found cereal rye and cereal-rye-associated cover crop mixtures accumulated the most above ground biomass N. The large amount of biomass produced by cereal rye likely allows it to accumulate more N per hectare than other cover crop treatments. Daniel et al. (1999) reported that cereal rye had comparable N assimilation to legumes such as clover and hairy vetch, or a cereal rye-hairy vetch mixture, suggesting that a cereal rye monoculture potentially has the ability to remove more N from the soil than a legume or mixture. Clark et al. (1994) reported the N content in cereal rye and cereal rye-hairy vetch mixtures were similar, and both were greater than a hairy vetch monoculture.

Clark et al. (1997); Sainju et al. (2007a); and Kuo and Jellum, (2002) reported conflicting results, but concluded that hairy vetch and a cereal rye-hairy vetch mixture usually had a greater N content than cereal rye alone. Sainju et al. (2005b) reported N content was greater in hairy vetch and a cereal rye-hairy vetch mixture compared to cereal rye and weeds. Teasdale and Abdul-Baki, (1998) reported a cereal rye monoculture to have a lower N content than hairy vetch or mixtures. Tosti et al. (2014) reported hairy vetch monoculture N to be consistently greater than barley monoculture, which accumulated very low to intermediate levels of N. When comparing barley monoculture to a barley-hairy vetch mixture intermediate amounts of N were assimilated. Based on the studies above, there is a large variance in N content among cover crop treatments. Cereal rye and hairy vetch monocultures varied from 19 to 165 kg ha<sup>-1</sup>.

C/N. Cereal rye had a greater C/N ratio than any other cover crop in nine of the 19 studies (Sainju et al., 2005a, 2005b; Sainju et al., 2007a; Clark et al., 1994; Clark et al., 1997; Kuo and Jellem, 2002; Lawson et al., 2015; Teasdale and Abdul-Baki, 1998; Vaughan and

Evanylo, 1998). Kuo and Jellem, (2002) reported that cereal rye had a greater C/N ratio than a cereal rye-hairy vetch mixture, demonstrating vetch's ability to fix atmospheric N. Clark et al. (1994) reported that regardless of kill date, all species except pure cereal rye were able to maintain a C/N ratio of <25:1 therefore reducing net immobilization, and a vetch monoculture had the lowest ratios.

Similar to findings from Odhiambio and Bomke (2001); Teasdale and Abdul-Baki (1998); and Tosti et al. (2014) reported grass and small grain monocultures had the greatest C/N ratios while legume monocultures had the lowest ratio. Appelgate et al. (2017) reported all cover crops and mixtures in their study had C/N ratios that were less than 14:1, including cereal rye, which conflicts with previous studies that had small grain ratios as great as 57:1. They attribute a majority of this to a lack of growth and development from limited postharvest heat unit accumulation.

No significant differences were found in the meta-analysis between C/N in different classifications. The major differences in ratios were between species of monocultures such as legumes and grasses, but for the purpose of this study they were classified together as monocultures. The vast difference in ratios among monocultures averaged out similarly to those of a mixture, or a control with weeds.

**Yield.** Clark et al. (1994) reported corn yields following no cover crop were significantly less than corn yield following all cover crop mixtures. Pure vetch and mixtures using high vetch seeding rates had the greatest yield. Kuo and Jellum, (2002) reported similar results where monoculture vetch produced the highest corn yields, followed closely by bicultures, with cereal rye producing the least. Vaughan and Evanylo, (1998) also found greater corn yields following hairy vetch compared to a biculture or cereal rye alone. Tosti et al. (2012) reported vetch had the

greatest yield on corn while a barley-hairy vetch mixture had the greatest tomato yield, but the effects on yield were similar from all cover crop treatments.

Sainju et al. (2005b) found sorghum yields to be greater with vetch and a biculture compared to cereal rye alone. The cotton yield in this study was excluded from our meta-analysis due to the small lint yield. It was not comparable to any other study and inadequately represented statistical models. Teasdale and Abdul-Baki, (1998) reported less marketable yield in tomato using cover crops without herbicide. Cover crop mixtures were noted for reducing weed populations but did not provide sufficient control to prevent yield loss due to weed interference. Due to the lack of studies, polyculture effect on yield cannot be validated. The overall model suggests cover crops do not affect yield. O'Reilly et al. (2011) reported no difference among cover crop treatments in marketable sweet corn yield.

In conclusion, a biculture can produce more biomass compared to a monoculture or control. The lack in biomass from polycultures can be attributed to competitive species such as cereal rye and other grasses outcompeting different species in the mix. Cover crops will produce more biomass than weedy or control plots contributing to the conservation of soil.

Binary mixes and monocultures produced biomass with different amounts of N. There was a large variance among legumes and grasses in monocultures. Polycultures assimilated biomass N similarly to monocultures. Cover crops utilize soil NO<sub>3</sub><sup>-</sup> to reduce runoff and leaching during winter periods. Cover crop biomass N results should lead to the increase of future research on NO<sub>3</sub><sup>-</sup> soil data to improve environmental conditions.

There were no differences in C/N ratios among classifications. Large differences between C/N of legumes and grass monocultures likely contributed to these results. The large spread in data likely averaged out overall results.

No differences were found in cover crop effect on yield over 11 studies. Cash crop yield is important to growers and our results imply that cover crops will not negatively impact overall cash crop yield, regardless of the species and number of cover crops used.

There is a deficiency in cover crop mixture research using three or more species. Current studies have primarily focused on binary mixtures. A meta-analysis should be conducted in the future after more research is published to validate our current findings and improve overall accuracy across multiple studies. A lack in studies prevented proper analyses of cover crop effect on soil nitrates and cash crop profits. Additional future research on cover crop mixtures, benefits, effect on cash crop yield, soil nitrates, and cash crop profit could motivate growers to expand the use of cover crop farming practices.

**Table 9**. Summary of studies used in the meta-analysis of biomass, biomass N, C/N ratio, and cash crop yield

cash crop yield.		1						
Reference	†Loc.	†Crop	†Bio	†Bio N	†C/N	NO <sub>3</sub> -	Yield	Profit
Appelgate et al. (2017)	Iowa	soybean	X	X	X	X		l
Clark et al. (1994)	Maryland	corn	X	X	X		X	<u> </u>
Clark et al. (1997)	Ohio	corn	X	X	X			
Crème et al. (2016)	France	corn			X			
Ćupina et al. (2011)	Serbia	sudan grass			X		X	
Daniel et al. (1999)	Virginia	cotton	X	X				I
Finney et al. (2016)	Pennsylvania	corn	X					Ι
Hartz et al. (2005)	California	tomato	X					Ι
Kuo and Jellum (2002)	Washington	corn	X	X	X	X	X	<u> </u>
Kramberger et al. (2013)	Slovenia	wheat		X	X			
Kramberger et al. (2014)	Slovenia	wheat			X			
Lawson et al. (2015)	Washington	sweet corn	X		X	X		<u> </u>
Leavitt et al. 2011	Minnesota	tom,zuc,bp	X				X	l
Odhiambo and Bomke	British	pea, potato	X	X	X			
(2001)	Colombia				1			<u> </u>
O'Reilly et al. (2011)	Canada	sweet corn		<u> </u>			X	X
Parr et al. (2011)	Slovenia	wheat		<u> </u>	X			<u> </u>
Ranells and Wagger	North	corn	X	X	1 [	[ ]	1	ļ
(1996)	Carolina						<u> </u>	
Sainju et al. (2005a)	Maryland	corn	X	X	X			<u> </u>
Sainju et al. (2005b)	Maryland	tomato	X	X	X		X	<u> </u>
Sainju et al.(2007a)	Georgia	cott,sorgh	X	X	X	X		
Sainju et al. (2007b)	Maryland	cott,peanut	X		X			l
Schipanski and Drinkwater, (2012)	New York	cereal grain	X	X				
Teasdale and Abdul-Baki (1998)	Maryland	tomato	X	X	X		X	
Thompson, (2013)	British Columbia	og, fescue	X					
Tosti et al.(2012)	Georgia	cott,sorgh	X	X	X		X	
Tosti et al. (2014)	Italy	sunflower	X	X	X			
Tribouillois et al.(2015)	France	faba bean, barley			X			
Vaughan and Evanylo (1998)	Italy	corn,tom	X	X			X	
Wayman et al. (2014)	Washingon	cc only	X		 			
AT T C 1								

<sup>†</sup>Loc=Location of study

<sup>†</sup>Crop=Cash crop used in rotation; tom=tomato; zuc=zucchini; bp=bell pepper; cott=cotton; sorgh=sorghum; og=orchard grass; cc only= only cover crops grown, no cash crop

<sup>†</sup>Bio= Cover crop biomass data

<sup>†</sup>Bio N= Cover crop biomass N data

<sup>†</sup>C/N=Cover crop C/N ratio data

 $\textbf{Table 10}. \ Least \ square \ means \ of \ cover \ crop \ classifications \ for \ biomass, \ biomass \ N, \ C/N \ ratio, \ and \ yield. \ Treatments \ that \ are \ not \ followed \ by \ the \ same \ letter \ are \ significantly \ different \ (p<0.05,$ 

using Tukey's pairwise comparisons).

Classification	Cover Crop	Cover Crop	C/N Ratio	Cash Crop
	Biomass	Biomass N		Yield
	Mg/ha <sup>-1</sup>	kg/ha <sup>-1</sup>		Mg/ha <sup>-1</sup>
Monoculture	$4.7_a$	$68.1_{bc}$	22.1	30.6
Binary	$4.9_{\rm a}$	$72.9_{a}$	19.9	30.7
Polyculture	$4.8_a$	$69.3_{ac}$	18.8	34.5
Control	$3.6_{b}$	$33.3_{ac}$	21.8	31.0
Pr>F	0.001	0.007	0.358	0.170
Number of papers	23	16	19	11

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#### **SUMMARY**

The primary objective of this research was to evaluate the impacts of cover crop mixtures on multiple cropping systems. The one-year field experiment provided a comparison to the multiple studies evaluated in the meta-analysis. The field study objective focused on the agronomic impacts of cover crop mixtures. Data collected included cover crop biomass, biomass N, C/N ratio, and cash crop yield. Similarly, the meta-analysis data were compiled from multiple studies that had some form of cover crop mixture and evaluated the agronomic impacts. Data collected were composed of cover crop biomass, biomass N, C/N ratio, and cash crop yield.

Field study results concluded that plots using cover crop mixtures produced greater cover crop biomass than the control plots. Meta-analysis results were similar with polycultures, binary mixtures, and monocultures producing more biomass than weedy or bare ground control plots. Field study and meta-analysis results indicate fall cover crops accumulate more biomass than weeds in control plots.

Biomass N was different in 4 out of the 6 treatments in the field study. Biomass N was greatest in polycultures and lowest in cereal rye and control plots. Different seeding ratios of grass, legumes, and brassicas were associated with varying N concentrations. Meta-analysis of biomass N varied between monocultures and binary mixtures. Similar to the field study, biomass N was related to which species were in the mixture.

Both studies resulted in no difference among C/N ratios. There was such a broad use of species and seeding ratios it seemed to average out the overall ratios. Field study yield did not show any difference among mixtures and the control plots. Edamame was not used as a cash crop in any of the meta-analysis studies but contributes to the large variations in cash crops assessed in the literature. No effects on yield were found over all the studies evaluated in the

meta-analysis. The meta-analysis allowed data to be analyzed across all regions, environments, and crops. The field study only had a local perspective which often occurs in experiments.

Normal regional barriers did not apply to the overall results and a more accurate conclusion can be drawn from the meta-analysis data.

In conclusion, there are many advantages when using cover crops. Cover crops can contribute to an agroecosystem when properly managed. Soil conservation is extremely important and cover crops can effectively reduce erosion. Large amounts of biomass were recorded from both studies when fall cover crops were implemented compared to a bare fallow.

The data presented here suggest that cover crops do not negatively impact yield. Cover crop species, mixture ratios, termination timing, and management all need to be considered to avoid negative impacts to cash crops. The option to implement cover crops is made by each grower and the goals of their business.

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