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Samuel E. Wortman

University of Nebraska-Lincoln

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DIVERSIFICATION OF ORGANIC CROPPING SYSTEMS WITH COVER CROP
MIXTURES: INFLUENCE ON WEED COMMUNITIES, SOIL MICROBIAL
COMMUNITY STRUCTURE, SOIL MOISTURE AND NITROGEN,
AND CROP YIELD

by

Sam E. Wortman

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DIVERSIFICATION OF ORGANIC CROPPING SYSTEMS WITH COVER CROP
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Sam E. Wortman, Ph.D.

University of Nebraska, 2012

Advisor: John L. Lindquist

Organic grain cropping systems typically depend on intensive mechanical cultivation for weed control and manure or compost applications to meet plant nutrient demands. However, cover crops may contribute to weed suppression and soil fertility, potentially increasing crop yield and sustainability of the system. The utility of individual cover crop species have been well documented, but the agronomic benefits of diverse cover crop mixtures have received less attention. Cover crop mixtures are an appealing option for farmers, as increasing species diversity has been shown to increase resource-use efficiency, stability, resiliency, and productivity of plant communities. Despite the growing interest in cover crop mixtures, little is known about the effect of increasing cover crop diversity on cropping system performance. Moreover, organic farmers have questions about the most effective method for cover crop mixture termination.

In an effort to increase knowledge about cover crop mixtures and management for the western Corn Belt, an organic cropping systems trial was initiated in 2009 at the UNL ARDC near Mead, NE. Spring-sown mixtures of cover crops, ranging from two to eight species, were included in a sunflower – soybean – corn crop rotation. Cover crops were

planted in late-March and terminated mechanically with either a field disk or sweep plow undercutter in late-May. Changes in cover crop mixture influenced cover crop productivity and early-season weed biomass, while termination method drove differences in weed community composition, soil microbial community structure, soil moisture and nitrogen, and crop yield. Interestingly, the management of ambient weed communities as a cover crop led to unique shifts in soil microbial community structure, but did not alter soil nitrogen or crop yield when compared to cover crop mixtures. When considering cropping system performance in combination with potential environmental benefits, diverse cover crop mixtures paired with a sweep plow undercutter for termination seems to be a profitable and sustainable management option for organic grain farmers in the western Corn Belt.

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PROLOGUE

Cover crops are most often planted for conservation purposes providing soil coverage between cash crop cycles to reduce soil erosion (Pimentel et al., 1995).

Depending on what species are planted, cover crops may provide additional benefits to crops and surrounding ecosystems. Some of these benefits may help farmers to increase grain yield and profitability, while others are less tangible. For example, cover crops can help to build long-term soil quality that contributes value to farmers, the environment, and society as a whole (Dabney et al., 2001).

Cover Crops Options for the Western Corn Belt

Cover crop plantings in the western Corn Belt are often limited by the length of the growing season. As defined by the average last and first freeze, the growing season in east-central Nebraska begins 27 April and ends 6 October. Corn and soybeans are typically planted prior to the second week of May and harvested in mid- to late-October which leaves only a narrow window, if any, for growing cover crops. In grain-based rotations, the best opportunity for cover crop growth is following a winter annual crop like wheat, which is harvested in July and provides a large window for establishment and growth of a productive cover crop. However, for many agronomic, social, and especially economic reasons the widespread adoption of a corn – soybean – winter wheat crop rotation throughout the Midwest US seems unlikely. Therefore, our challenge as researchers is to work with farmers to create a window for cover crop growth where one does not currently exist in corn – soybean cropping systems.

The most common option for cover crop establishment within the corn – soybean rotation is to plant a winter annual cover crop (e.g., rye or hairy vetch) immediately

following soybean harvest. Soybeans are typically harvested sooner than corn and soybean is a low-residue crop; thus, the soil surface following soybean is far more susceptible to erosion (Kessavalou and Walters, 1997). Therefore, planting cover crops following soybean in a two-year rotation is often the highest priority for farmers.

However, planting a cover crop in the fall can become challenging. Soybean harvest could be delayed or corn harvest may need to be expedited, and typically activities to get crops out of the field will take priority over cover crop planting. Unfortunately, the result is often a late-planted cover crop, resulting in poor establishment and minimal growth prior to corn planting the following spring. Another possibility for cover crop establishment is to broadcast the seed via airplane prior to summer crop harvest. If successful, this option certainly creates a longer period for cover crop growth, but may be a relatively expensive option potentially resulting in a spatially heterogeneous cover crop stand. A similar option attracting recent interest is attaching a broadcast spreader to a “high-boy” spray tractor and spreading cover crop seed after leaf drop in soybean but before harvest.

If none of these fall seeding options is viable for a farmer, the only other possibility for cover crop establishment is in early spring. This option has promise but is often viewed as a less desirable option because of the opportunity to reduce late-fall and early-spring soil erosion with fall-planted winter annual cover crops. Despite the shortcomings of a spring-sown cover crop, this is the most practical cover crop option for many farmers. For example, farmers with integrated crop – livestock operations will graze livestock on crop residue in the winter months, which may damage the cover crop stand and reduce the benefits of fall-sown cover crops. Indeed, spring-sown cover crops

provide farmers the opportunity to receive at least a portion of the ecological and economic benefits of cover crops in rotation while maintaining the flexibility to graze livestock on crop residue post-harvest. Regardless of the desired strategy, inclusion of cover crops in a corn – soybean cropping system will require management changes and potential sacrifices to ensure maximum benefit of the cover crop. For example, traditional corn and soybean planting dates may need to be delayed or harvest may need to occur earlier to allow sufficient growth of cover crops, which may mean that shorter-season corn hybrids and soybean varieties need to be considered. However, this option may cause a reduction in corn and soybean yields. In short, diversification of the corn – soybean rotation with cover crops is possible, but it will present unique management challenges that require further research.

Benefits of Cover Crop Mixtures

Traditionally, cover crop use and management have followed the conventional single species paradigm. Monoculture systems were developed to facilitate ease of mechanical cultural practices including planting, fertilization, weed control, and harvest. However, with the exception of planting, farmers do not have to consider these management factors when growing a cover crop. Realizing this has prompted many farmers to consider using multiple species cover crop mixtures.

Cover crop species are generally chosen to meet specific farmer goals. Not surprisingly, the specific benefits associated with a cover crop vary by species and management method. For example, species in the Fabaceae (legume) family are typically chosen due to their capacity to utilize atmospheric nitrogen through a mutualistic relationship with nitrogen-fixing bacteria. Properly managed legume cover crops can

reduce or eliminate the need for synthetic nitrogen additions for the subsequent cash crop (Biederbeck et al., 1996; Burket et al., 1997). Another benefit often sought in a cover crop species is the capacity for reducing soil compaction. Species with long tap roots, typical of cover crops in the Brassicaceae (mustard) family, can often penetrate compacted soil layers up to six feet deep (Williams and Weil, 2004). The potential for specific cover crop species to suppress weeds is another area of increasing interest, especially in organic cropping systems where the use of synthetic herbicides is prohibited. While many species offer specific benefits, many of the biological advantages associated with a healthy cover crop are not unique to individual species. The potential benefits of most cover crops include reduced topsoil erosion, increased nutrient cycling and reduced nitrate leaching, improved soil aggregation and water retention, increased organic matter content and soil carbon sequestration, and a reduction in the incidence of disease and insect pests (Hartwig and Ammon, 2002).

Given that many cover crop benefits are species- or family-specific, there may be an advantage for farmers to grow multiple species in cover crop mixtures. Moreover, growing mixtures of cover crops should increase resource-use efficiency of the entire community (Tilman et al., 1997). Species with a variety of canopy and root structures, along with variable demands for water and nutrients, will ensure that the entire plant community maximizes productivity given the available resources. The positive relationship between plant community diversity and productivity has been well documented in grassland ecosystems (Tilman et al., 2001). However, certain species may be extremely competitive or antagonistic toward other species when grown in mixed species communities, so cover crop mixtures should not be chosen carelessly. In addition

to the specific benefits of individual species in a mixture and the potential for increased resource-use efficiency and productivity, a multi-species mixture will drastically increase biodiversity within the corn – soybean rotation. The immediate increase in vegetative diversity during cover crop growth will likely lead to increased diversity of other species in associated trophic levels such as beneficial insects, birds, and microorganisms that may use the cover crop community as a source of food, habitat, or refuge (Altieri, 1999). While the benefits of biodiversity are not always immediately realized by the farmer, most agree that conservation of biodiversity is intrinsically valuable (Ghilarov, 2000).

Economic Advantages of Cover Crop Mixtures

There are both immediate and long-term economic incentives for using cover crops. In general, the immediate economic advantages of cover crop use include the cost savings associated with replacing off-farm inputs such as synthetic nitrogen, fuel, herbicides, and labor, as well as any associated yield increases. Cover crop mixtures provide further economic advantage to farmers by reducing economic risk. Depending on annual weather patterns, certain cover crop species perform better than others in a given year and this outcome is somewhat unpredictable. Therefore, a mixture of cover crop species may reduce the economic risk of choosing an unsuccessful single cover crop species and losing the investment of seed and labor associated with establishment. For example, seed of many legume cover crops can be expensive and also more difficult to establish compared to other cover crops. Therefore, combining legumes in mixture with other broadleaf and grass species could reduce the initial cost of seeding the cover crops and also ensure the farmer gains some biological and economic benefit, even if growth of the legume is unsuccessful in a given year. In some cases, competition among species

may increase the productivity of each individual species compared to growth in monoculture. For example, if nitrogen fixation per legume plant can be maximized in a multi-species mixture, the economic return per seed in the form of replaced nitrogen cost would increase. While not directly related to the biology of cover crops, another economic incentive is through USDA Natural Resource Conservation Service (NRCS) conservation programs. Several options provide payments to farmers for individual cover crop use, and there is currently one provision in the Conservation Stewardship Program that provides economic incentive for planting cover crop mixtures (USDA-NRCS, 2012).

In addition to short-term economic incentives associated with cover crop use, there are recognized long-term benefits. The long-term economic advantages are related to reductions in soil erosion and improved soil organic matter. Reducing soil erosion long-term is in the best interest of the farmer, local communities, and society. It has been estimated that soil erosion costs farmers in the US over 27 billion dollars annually. Most of this cost is due to the nutrients lost in eroded topsoil, but this estimate also includes the cost of lost water and thickness of topsoil (Pimentel et al., 1995). Equally troubling is the cost of soil erosion to society, which is estimated at 17 billion dollars per year in the US. The off-farm societal impacts of erosion include costs associated with the siltation of navigable waterways, sewers, and roadways, and the associated clean-up costs (Pimentel et al., 1995). Including cover crop mixtures in the corn – soybean rotation will not eliminate the economic burden of soil erosion, but it would certainly be a step in the right direction. The second long-term economic advantage of cover crop use may be more easily observed by farmers. Cover crop use over time has been shown to increase organic matter content (stable carbon) in soils (Lotter et al., 2003). For the farmer, increasing

organic matter is generally a sign of improved soil quality and productivity, and has also been shown to lead to greater yield stability. Increased organic matter in soils increases soil water retention, which improves the likelihood of yield stability in exceptionally dry years (Lotter et al., 2003). This is an important economic consequence in a climate and society where water for agriculture is increasingly scarce and there is competition from other sectors of the economy.

Conclusions

When considering cover crop use in the western Corn Belt, there is certainly a gradient of environmentally and economically sound options. Adding winter wheat to our current crop rotation would provide the largest window for cover crop growth and environmental benefits, but the threat of short-term economic risk associated with an alternative cash crop will likely limit widespread adoption of this practice. While this option may be part of a long-term vision for our agricultural landscape, in the short-term researchers and policy makers should be developing evidence and incentives to encourage the use of cover crops and cover crop mixtures within the current corn – soybean rotation. Indeed, the demand for science-based evidence regarding the agronomic and economic benefits of cover crop mixtures was the inspiration for this dissertation. Until now, many of the perceived benefits of mixed-species cover crop communities were based on theoretical considerations (Tilman et al., 1997). The goal for this research project was to take the theoretical principles regarding ecological diversity, and integrate them into an intensive crop production system in an effort to boost the stability, resiliency, productivity, profitability, and sustainability of the corn – soybean cropping system.

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Chapter 1

Cover Crop Mixtures for the Western Corn Belt: Opportunities for Increased Productivity and Stability

Abstract

Achieving agronomic and environmental benefits associated with cover crops often depends on reliable establishment of a highly productive cover crop community. The objective of this study was to determine if cover crop mixtures can increase productivity and stability compared to single species cover crops, and to identify those components most active in contributing to or detracting from mixture productivity. A rainfed field experiment was conducted near Mead, NE in 2010 and 2011. Eight individual cover crop species (in either the Brassicaceae [mustard] or Fabaceae [legume] family) and four mixtures of these species (2, 4, 6, and 8 species combinations) were broadcast planted and incorporated in late March and sampled in late May. Shoot dry weights were recorded for sole crops and individual species within all mixtures. Sole crops in the mustard family were twice as productive (2428 kg ha^{-1}) as sole crops in the legume family (1216 kg ha^{-1}), averaged across two years. The land equivalent ratios (LERs) for all mixtures in 2011 were greater than 1.0, indicating mixtures were more productive than the individual components grown as sole crops. Improved performance in mixture may be related to the ecological resilience of mixed species communities in response to extreme weather events, such as hail. Partial LERs of species in the mustard family were consistently greater than those in the legume family, indicating that mustards dominated the mixtures. Results provide the basis for yield-stability rankings of spring-sown cover crop species and mixtures for the western Corn Belt.

Introduction

Cover crops have been shown to provide a variety of benefits within agroecosystems. These include reduced soil erosion, increased biological diversity (e.g., microbes, insects, and birds), increased nutrient cycling and biological nitrogen fixation, increased soil organic matter, improved weed control, and increased crop yields (Pimentel et al., 1992; Pimentel et al., 1995; Sainju and Singh, 1997; Williams II et al., 1998; Altieri, 1999; Reddy et al., 2003; Teasdale et al., 2007). While cover crops may provide a number of agronomic and environmental benefits, achieving these benefits (e.g., weed suppression) often depends on establishing a highly productive cover crop community (Teasdale et al., 1991). Planting multi-species cover crop mixtures may be a viable solution for increasing the ecological stability and resilience of cover crop communities, which can contribute to higher and more consistent productivity.

Production benefits of multi-species plant communities include the potential for increased resource-use efficiency and crop yields (Francis, 1986). Intercropping systems typically include the production of two crop species (e.g., one cereal grain and one legume species) within a given field in the same season, most commonly oriented in alternating rows or strips of rows (e.g., Chen et al., 2004). While there are logistical challenges related to planting and harvesting intercrop systems, the potential for increased yield of the entire system makes these potentially attractive cropping systems when labor and appropriate equipment are available. Indeed, there are many examples of intercropping systems that have demonstrated greater grain or forage yield compared to monoculture systems on an equivalent land area basis (Ikeorgu et al., 1989; Chen et al., 2004; Agegnehu et al., 2006; Ghosh et al., 2006). There are several potential mechanisms

contributing to the increased yield observed in intercropping systems, including increased resource-use efficiency (light and soil resources) and increased ecological stability and resilience (Reddy and Willey, 1981; Tilman, 1996; Trenbath, 1999; Szumigalski and Van Acker, 2008). While two-species intercropping systems are most common, there are potential benefits associated with further increases in plant community diversity including increased productivity, community stability, and nutrient-use efficiency (Tilman, 1996; Tilman et al., 1997; Tilman et al., 2001).

Multi-species cover cropping systems have been tested in previous studies, but most research was not designed to quantify the benefits of increasing cover crop diversity. Typically, cover crop mixture studies compare monoculture species with biculture combinations of those species (Akemo et al., 2000; Creamer and Baldwin, 2000; Odhiambo and Bomke, 2001; Kuo and Jellum, 2002). While there has been some focus on more diverse mixtures of cover crops (Creamer et al., 1997; Teasdale and Abdul-Baki, 1998; Madden et al., 2004), characterization of the benefits associated with increasing diversity are often limited to simple dry weight comparisons.

Many studies have demonstrated increased productivity of cover crop mixtures relative to monoculture cover crops, but the differences were likely due in part to higher seeding rates in the mixtures (Teasdale and Abdul-Baki, 1998; Odhiambo and Bomke, 2001; Kuo and Jellum, 2002). To accurately evaluate benefits of mixtures and the contributions of individual species to the mixtures, seeding rates of the mixtures should be proportional to the monocultures via a substitutive approach to avoid the confounding effects of variable seeding densities (e.g., seeding rate for a component of the mixture should be equal to its monoculture seeding rate divided by the number of species in the

mixture; Joliffe, 2000). It is possible that some other optimum seeding density or mixture proportion exists for cover crop mixtures, but addressing this question requires an additive seeding approach which would limit the utility of intercropping indices like the land equivalent ratio (Joliffe, 2000). Moreover, a fully additive seeding approach to mixture seeding rates (combining 1x rates of each species) would be impractical and cost-prohibitive for farmers.

Many cover crop mixture studies fail to include monoculture control treatments necessary to evaluate the potential benefits or antagonisms of the different mixtures (Creamer et al., 1997; Madden et al., 2004). Similarly, many of these studies do not quantify the productivity of the mixtures, or the individual components of the mixture, relative to sole cropped cover crops on an equivalent land area basis as calculated in traditional intercropping studies (Teasdale and Abdul-Baki, 1998; Creamer and Baldwin, 2000; Odhiambo and Bomke, 2001; Kuo and Jellum, 2002). Instead, the dry weights of each mixture and sole crop are typically reported; such methods provide limited information about the relative contribution or aggressiveness of each species in a cover crop mixture.

The aim of this study was to quantify the productivity and stability of spring-sown cover crop mixtures relative to sole cropped cover crops in the western Corn Belt, and to identify those species contributing to or detracting most from mixture productivity. With respect to this objective, we hypothesized that increasing cover crop diversity will increase cover crop productivity and stability.

Materials and Methods

To accomplish this objective, a rainfed field experiment was conducted at the University of Nebraska – Lincoln Agricultural Research and Development Center near Mead, NE in 2010 and 2011. Dominant soil type at the site is a Sharpsburg silty clay loam (fine, smectitic, mesic typic Argiudoll; pH = 6.3, organic matter content = 3.6%) with 0 to 5% slopes. The experimental layout was a randomized complete block design with four replications and twelve cover crop treatments. Experimental units were 3 x 3 m and randomized to treatment within each replication. Cover crop treatments included eight individual cover crop species and four mixtures of these species (Table 1.1). Cover crops used belong to either the Fabaceae (legume) or Brassicaceae (mustard) plant families. Mixtures were a 1:1 ratio of legume and mustard species where, for example, the eight species mixture included four legume species and four mustard species. The four cover crop mixtures ranged from two to eight species with an objective to quantify the effects of increasing plant diversity. The seeding rates for individual species in a mixture were determined by dividing the recommended seeding rate for that species by the number of species in mixture (Table 1.1), previously described as the substitutive approach. Recommended seeding rates for individual species were obtained from a combination of USDA Natural Resource Conservation Service, Cooperative Extension, cover crop seed distributor, and farmer recommendations. If recommendations among sources differed, values were averaged to determine the most appropriate seeding rate. Most recommendations were based on an assumption of drilled seeding methods. However, cover crops in this study were broadcast seeded; therefore, drilled seeding

recommendations were increased by approximately 20% to compensate for reduced plant stands when using broadcast seeding methods (Clark et al., 1978).

Cover crops were broadcast planted by hand and surface incorporated with a John Deere “cultipacker” (Deere and Company, Moline, IL, USA) on March 30, 2010 and March 21, 2011. Plants received no supplemental irrigation or nutrition throughout the growing period, but large weeds were removed by hand from experimental units on a bi-weekly basis to limit competitive effects from non-cover crop species. Plants were harvested on May 25, 2010 and May 31, 2011 from two randomly placed quadrats (0.19 m²) in each experimental unit. This harvest time was intended to simulate the termination period for a cover crop grown prior to summer annual crop species (e.g., *Glycine max* [soybean], *Sorghum bicolor* [sorghum], *Helianthus annuus* [sunflower], or possibly *Zea mays* [maize]). Shortly following cover crop harvest and sampling, shoot dry weights were determined for sole crops and individual species within all mixtures by drying samples at 54° C to constant mass and weighing each sample.

The land equivalent ratio (LER) was used to compare the productivity of sole cropped cover crops to those cover crops planted in mixture. The LER indicates the relative amount of land required when growing sole crops to achieve the productivity observed in the mixture (Willey and Osiru, 1972). LER is widely considered a robust and useful indicator of mixture productivity relative to sole crops (Bedoussac and Justes, 2011). LER is typically utilized to evaluate marketable yield in intercropping systems, but to our knowledge has not been previously applied in the evaluation of diverse cover crop mixtures. Total LER is calculated as:

$$\text{LER} = \text{LER}_i + \text{LER}_j \dots + \text{LER}_n$$

where LER_i is the partial LER of species i , LER_j is the partial LER of species j , and so forth for n number of species. Partial LER is calculated as:

$$LER_i = Y_{Mi} / Y_{SCi}$$

where Y_{Mi} is the yield of species i planted in mixture and Y_{SCi} is the yield of species i planted as a sole crop. A total LER value greater than 1.0 indicates the mixture was more productive than the component sole crops, whereas a value less than 1.0 suggests sole crops were more productive (e.g., antagonistic effects). For example, a total LER value of 1.5 suggests that 15 hectares of sole cropped cover crops (the components of the mixture) would need to be planted to achieve an equivalent level of productivity (yield) achievable on 10 hectares when all species are grown together in a mixture. The partial LER values for individual species in a mixture were also used to compare the relative contribution or competitive ability of each species (Bedoussac and Justes, 2011).

To accomplish all objectives, shoot dry weight data, LER, and partial LER values were analyzed with ANOVA implemented using the MIXED procedure in SAS (SAS Institute, Inc., NC, USA). Fixed effects in the model included cover crop treatment, year, and the interaction of treatment*year, while the random effect was the interaction of block*year. Least-squares means and standard errors were reported for all cover species and mixtures for statistical comparisons. Ecological stability of cover crop communities was compared using the coefficient of variation (C.V.) for each cover crop treatment pooled across replications ($n=4$) and years ($n=2$). A lower coefficient of variation implies less variation about the mean and greater ecological stability (Tilman et al., 1998). Lastly, mean contrasts were used to compare the productivity (shoot dry weights) and stability (C.V.'s) of mixtures versus sole crops (legumes and/or mustards).

Results and Discussion

Productivity and Stability of Sole Crops and Mixtures

Shoot dry weight of sole cropped cover crop species in 2010 ranged from 397 kg ha⁻¹ ± 252 kg ha⁻¹ (mean ± one standard error) for *Lathyrus sativus* (chickling vetch) to 3175 kg ha⁻¹ ± 252 kg ha⁻¹ for *Sinapis alba* (Idagold mustard; Figure 1.1). Shoot dry weight of mustard cover crop species (2757 kg ha⁻¹ ± 126 kg ha⁻¹) was consistently greater than legumes (1127 kg ha⁻¹ ± 126 kg ha⁻¹) in 2010. However, a contrast of mixtures vs. mustard sole crops indicated that shoot dry weight of mustard sole crops was not different from the average shoot dry weight of mixtures (2709 kg ha⁻¹ ± 126 kg ha⁻¹). Shoot dry weight of sole cropped cover crop species in 2011 ranged from 1076 kg ha⁻¹ ± 252 kg ha⁻¹ for chickling vetch to 2556 kg ha⁻¹ ± 252 kg ha⁻¹ for *Raphanus sativus* (oilseed radish; Figure 1.1). Consistent with 2010, shoot dry weight of mustard cover crop species (2099 kg ha⁻¹ ± 126 kg ha⁻¹) was consistently greater than legumes (1305 kg ha⁻¹ ± 126 kg ha⁻¹) but not different from the average shoot dry weight of the mixtures (2062 kg ha⁻¹ ± 126 kg ha⁻¹). Within the cover crop mixtures, productivity did not increase with diversity as there was no difference in shoot dry weight among any of the four possible mixtures in 2010 or 2011 (Figure 1.1). Overall, the productivity of all cover crops in this study was far greater than the previously reported dry matter yields of spring-sown cover crops in eastern Nebraska (Power and Koerner, 1994). The greater productivity observed in this study may be related to the earlier cover crop planting date used in this study (late-March) compared to the delayed plantings (late-April and early-May) tested by Power and Koerner (1994).

The coefficient of variation, accounting for spatial (replication) and temporal (year) variation differed among individual cover crop treatments. Among legume species, C.V. values ranged from 16.9 to 55.2% (mean = 33.5%) for *Trifolium incarnatum* (crimson clover) and chickling vetch, respectively. Among mustard species, values ranged from 20.6 to 46.6% (mean = 31.6%) for oilseed radish and Idagold mustard, respectively (Figure 1.2). The variability of Idagold mustard was related to its susceptibility to hail damage. While Idagold mustard was the most productive cover crop in 2010, a May 12, 2011 hail storm limited its productivity in 2011. The hail storm was damaging to all cover crop treatments, but Idagold mustard seemed to recover much more slowly than the other species and mixtures. The coefficient of variation for cover crop mixtures only ranged from 19.8 to 30.7% (mean = 25.9%), but a contrast of mixtures vs. monocultures indicated no difference ($p = 0.35$) in the stability of the two cover cropping strategies. Similarly, the coefficient of variation was relatively uninfluenced by increasing diversity within the mixtures (Figure 1.2). It is possible that the number of replications ($n=4$) and years ($n=2$) was insufficient to detect differences in the stability of different monoculture and mixture cover crop strategies. A more robust measure of stability would require data from a long-term or multi-site experiment. Nonetheless, knowledge of the spatial and temporal variability (though limited) may be useful in selecting an appropriate cover crop species or mixture.

Land Equivalent Ratios (LER) for Mixtures and Mixture Components

The land equivalent ratio (LER) was not affected by cover crop mixture or the interaction of mixture by year. However, LER was influenced by year and was greater in 2011 (LER = 1.38 ± 0.09) than in 2010 (LER = 1.05 ± 0.09) for all mixtures (Figure 1.3).

All mixtures across both years were equal to or greater than 1.0, while all mixtures in 2011 were greater than 1.0. A value greater than 1.0 suggests the mixture resulted in more efficient use of land than the alternative of growing the individual mixture components as sole crops. The primary difference between 2010 and 2011 was the May 12, 2011 hail storm that severely damaged all cover crop treatments. Cover crops were not harvested until May 31, 2011 (approximately one week later than the harvest date in 2010), in an effort to allow the cover crops to recover and regrow after the substantial hail damage. While the objective of this study was not to measure the ecological resilience of cover crop mixtures, the 2011 hail storm did provide anecdotal information about the ability of these species and mixtures to recover after extreme perturbation. Given our observations, we hypothesize that the increased LER in 2011 from 2010 is directly related to the potential for increased resilience in mixtures relative to sole crops. Indeed, the ability to quickly recover from disturbance (resiliency) can contribute to productivity and is often a characteristic of diverse plant communities (Lavorel, 1999; Hooper et al., 2005).

The over-yielding potential of plant species grown in mixture for agricultural use is consistent with many previous studies (e.g., Ikeorgu et al., 1989; Chen et al., 2004; Agegnehu et al., 2006; Ghosh et al., 2006). Undoubtedly, over-yielding characteristics have been observed for decades in cover crop mixtures, but the documentation of this phenomenon requires appropriate data collection and indices like the LER. To our knowledge, this is the first reported evidence of over-yielding properties in a mixture of plant species specifically designed for cover crop use. Contrary to our expectations, LER did not increase with diversity of the mixture (from 2 to 8 species). Increasing

community diversity has been shown to increase resource-use efficiency, primary productivity (Tilman et al., 1997; Tilman et al., 2001), and presumably the efficiency of land use (LER), but this was not observed here.

Partial land equivalent ratios were consistently greater for mustards in mixture compared to legumes (Table 1.2). Idagold mustard was the most competitive cover crop species in all mixtures as indicated by the highest (or among the highest) partial LER pooled across both years (0.98, 0.43, 0.48, and 0.33 in the 2CC, 4CC, 6CC, and 8CC mixtures, respectively). In contrast, all legume species were least competitive in all mixtures pooled across both years (0.33, 0.14, 0.10, and 0.07 in the 2CC, 4CC, 6CC, and 8CC mixtures, respectively; Table 1.2). If all species were contributing equally to the productivity of a mixture, we would expect the partial LER of a given species to be 0.5, 0.25, 0.167, and 0.125 in the 2, 4, 6, and 8 species mixtures, respectively. A partial LER greater than these expected values for species *i* within a given mixture suggests species *i* was benefiting from the increased interspecific and reduced intraspecific competitive environment of the multi-species mixture. Conversely, a partial LER less than these expected values would suggest that species *i* is inhibited more by the interspecific competitive interactions in the mixture. Partial LER values for the mustards were always greater than or equal to these expected values, suggesting all mustard species used in this experiment benefited from the mustard-legume mixture combinations. In contrast, the legumes were always less than or equal to these expected values suggesting the legume species used in this experiment tended to be negatively influenced by the competitive interactions in the mustard-legume mixture combinations.

While these results suggest mustards benefited most from the mixture combinations, it is important to note that total LER was always greater than or equal to 1.0. Despite the negative competitive effects on most legume species, the substantial gain in mustard productivity in mixture (relative to monoculture) led consistently to LER values greater than or equal to 1.0. These results are congruent with the results of Szumigalski and Van Acker (2008) who found that canola (a mustard species) was quite competitive and tended to over-yield in mixture with field pea and wheat. The over-yielding effect of the mustards when grown in mixture with legumes may have at least two possible explanations. First, the canopy architecture of mustards compared to legumes may give the mustards a competitive advantage in these mixtures (Tremmel and Bazzaz, 1993). The shoot and canopy architecture of the mustard species used in this experiment is generally erect with large leaves, whereas the legume species are low growing (vine, rosette, or prostrate growth habit) with relatively small leaves. The morphology of mustard species creates a very competitive environment for light resources (Szumigalski and Van Acker, 2008); thus, when the mustard densities were reduced and replaced with a less light competitive species the mustards were released from this strong intraspecific competitive interaction. A second explanation may be that the monoculture seeding densities for the mustard species were too high, and reducing the proportional seeding densities in the mixtures created an over-yielding environment. Many plant species exhibit a quadratic yield response to increasing plant density; therefore, it is possible the seeding densities in this study were beyond optimum (Cox, 1996). However, the recommended seeding rates for the mustard species were consistent

across many information resources, and it is reasonable to assume that the densities used in this study were sufficiently close to optimum.

Cover Crop Choice

When making decisions about which cover crop or mixture of cover crops to plant, one must consider both the potential productivity and ecological stability of all available options. To aid in a simple and effective cover crop selection process, rankings of each cover crop species and mixture were determined for shoot yield in 2010 and 2011, yield stability, and for a combined measure of yield and stability with varying weights distributed between the two variables (1:1, 2:1, and 4:1 for yield:stability). This method and similar ranking methods have been used in the selection of high yielding and stable maize hybrids (Kang, 1988; Kang and Pham, 1991). The ranking system proposed by Kang and Pham (1991), which combines yield and stability ranks, provides an example of how the “best” or highest ranked option can vary depending on the relative importance placed on yield and stability. Consistent with the results of Kang and Pham (1991), the relative ranking of cover crop options in this experiment varied depending on the importance (weight) placed on yield or stability (Table 1.3). Kang and Pham (1991) found that placing more than a 2x weight on yield (relative to stability) results in a ranking that tends to reflect solely the yield ranks. In this study, the 4:1 yield-stability rankings were only slightly different from the yield rankings; however, the 1:1 and 2:1 yield-stability rankings were substantially different from both the 4:1 yield-stability rankings and yield rankings. Therefore, in order to choose a cover crop option that is most likely to demonstrate stability over time, in addition to high productivity, one

should choose a combined yield-stability ranking with a 1:1 or 2:1 relative weight assigned to yield and stability ranks, respectively (Kang, 1988).

When considering productivity and stability, regardless of the relative weight of each, oilseed radish seems to be the most promising cover crop option observed in this study, followed by the six-species mixture (6CC; Table 1.3). In contrast, chickling vetch and *Vicia villosa* (hairy vetch) grown alone seem to be the two least promising cover crop options when considering both yield and stability (Table 1.3). These rather simple categorical rankings do not account for the over-yielding characteristics of cover crop mixtures identified by the LER or the potential for biological nitrogen fixation of legumes. However, depending on the management objective of the farmer, these rankings could be expanded to include additional factors. Thus, the rankings presented here should instead be used as a starting point for recommendations. It is also interesting to note that cover crop mixtures were never ranked higher than second, but never lower than eighth (of twelve). While mixtures may not provide the greatest potential for maximum productivity in a given year, they do seem to buffer against unacceptably low productivity.

Conclusions

The mustard species (Idagold mustard, *Brassica juncea* [Pacific Gold mustard], oilseed radish, and *Brassica napus* [dwarf essex rape]) tested here proved to be fast growing, competitive, and productive cover crops well suited for early spring growth in the western Corn Belt. Conversely, the legume species tested (hairy vetch, *Pisum sativum* [field pea], crimson clover, and chickling vetch) were far less competitive and almost half

as productive as the mustards. While the legume species were generally less impressive, the potential for biological nitrogen fixation and utility as a green manure may compensate for the limited productivity. Though generally lower, yield variability of mixtures was not significantly different from monocultures. Instead, the primary benefit of cover crop mixtures seemed to be the potential for over-yielding (LER values greater than 1.0) that was observed in one year of this research.

This study provides specific recommendations about productive and stable spring-sown cover crop options for the western Corn Belt, but also offers broad evidence and insight regarding the ecological benefits of cover crop mixtures that should be applicable to a variety of cover crop species, mixture combinations, planting dates, seasonal weather, and agroecoregions. Ultimately, cover crop species or mixture choice will depend on the specific management objective and the available threshold for risk. These results provide an example of the information necessary for making these decisions as part of a production package.

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Tables and Figures

Table 1.1. Common name, scientific name, and seeding rates for eight cover crop species planted as sole crops (SC) or mixtures (2CC, 4CC, 6CC, and 8CC) in 2010 and 2011 near Mead, NE.

Common Name	Scientific Name	Cover Crop Seeding Rate				
		SC	2CC	4CC	6CC	8CC
		———— kg ha ⁻¹ ————				
Hairy Vetch	<i>Vicia villosa</i>	44.8	22.4	11.2	7.5	5.6
Idagold Mustard	<i>Sinapis alba</i>	13.4	6.7	3.4	2.2	1.7
Field Pea	<i>Pisum sativum</i>	112.0		28.0	18.7	14.0
Pacific Gold Mustard	<i>Brassica juncea</i>	8.8		2.2	1.7	1.1
Crimson Clover	<i>Trifolium incarnatum</i>	28.2			4.7	3.5
Oilseed Radish	<i>Raphanus sativus</i>	16.8			2.8	2.1
Chickling Vetch	<i>Lathyrus sativus</i>	67.2				8.4
Dwarf Essex Rape	<i>Brassica napus</i>	13.6				1.7

Table 1.2. Partial land equivalent ratios (LER_i) for eight cover crop species in the four possible mixtures (2CC, 4CC, 6CC, and 8CC) pooled across 2010 and 2011. Numbers in parentheses indicate the standard error of the least squares mean. Different letters indicate differences among means within a mixture.

Cover Crop Species	Cover Crop Mixture			
	2CC	4CC	6CC	8CC
Hairy Vetch	0.33 (0.14) b	0.15 (0.06) b	0.08 (0.05) d	0.07 (0.03) c
Idagold Mustard	0.98 (0.14) a	0.43 (0.06) a	0.48 (0.05) a	0.33 (0.03) a
Field Pea		0.13 (0.06) b	0.15 (0.05) cd	0.10 (0.03) c
Pacific Gold Mustard		0.39 (0.06) a	0.33 (0.05) b	0.19 (0.03) b
Crimson Clover			0.07 (0.05) d	0.04 (0.03) c
Oilseed Radish			0.17 (0.05) c	0.21 (0.03) b
Chickling Vetch				0.06 (0.03) c
Dwarf Essex Rape				0.19 (0.03) b
Total LER	1.31 (0.11)	1.10 (0.11)	1.27 (0.11)	1.19 (0.11)

Table 1.3. Rankings for each cover crop option considering shoot yield (2010 and 2011), yield stability (C.V.), and a combination of yield and stability with varying weights (1:1, 2:1, and 4:1) attributed to each.

	Shoot Yield		C.V.	Proportion of Yield to C.V.		
	2010	2011		1:1	2:1	4:1
<i>Legumes</i>						
Crimson Clover	9	8	1	3	7	8
Field Pea	11	11	5	9	10	11
Hairy Vetch	10	9	10	11	11	10
Chickling Vetch	12	12	12	12	12	12
<i>Mustards</i>						
Oilseed Radish	2	1	3	1	1	1
Dwarf Essex Rape	8	2	6	5	3	5
Pacific Gold Mustard	4	4	9	7	6	4
Idagold Mustard	1	10	11	10	9	7
<i>Mixtures</i>						
2CC	6	6	4	4	3	6
4CC	7	7	7	8	8	8
6CC	3	4	2	2	2	2
8CC	5	3	8	6	3	3

Figure 1.1. Shoot dry weights (kg ha^{-1}) of eight cover crop species and four possible mixtures of the eight species in 2010 and 2011 (see Table 1.1 for species and mixture components and seeding rates). Pooled means of monoculture treatments vs. mixture treatments is presented for each year. Error bars represent the standard error of the mean.

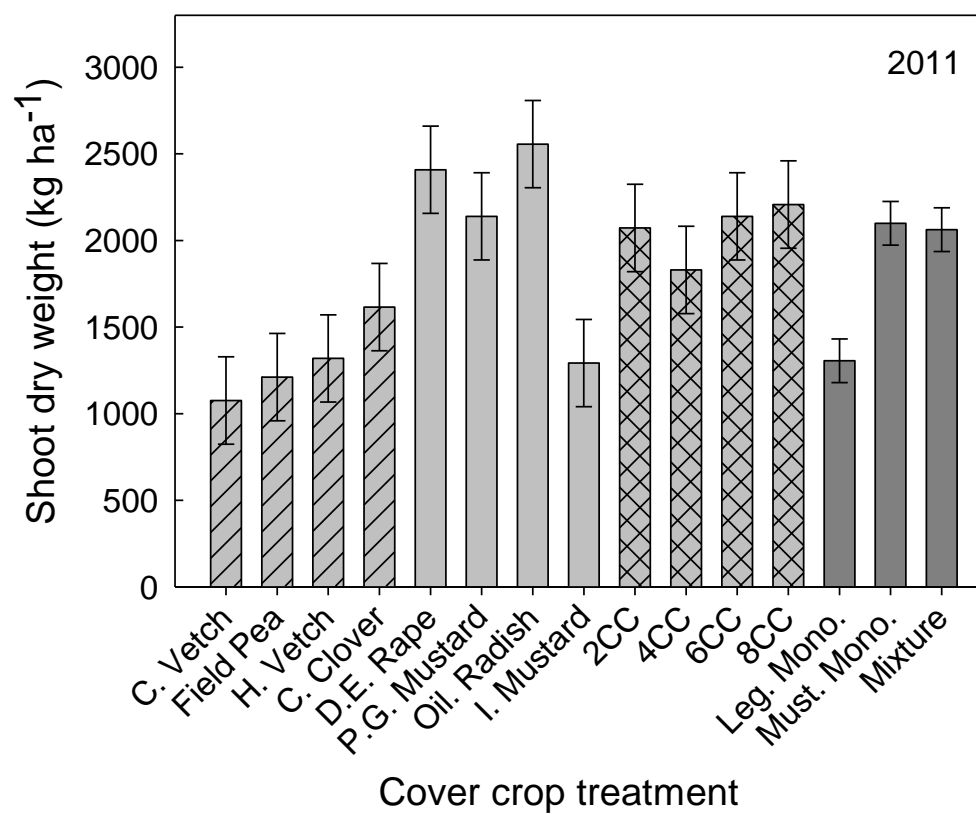
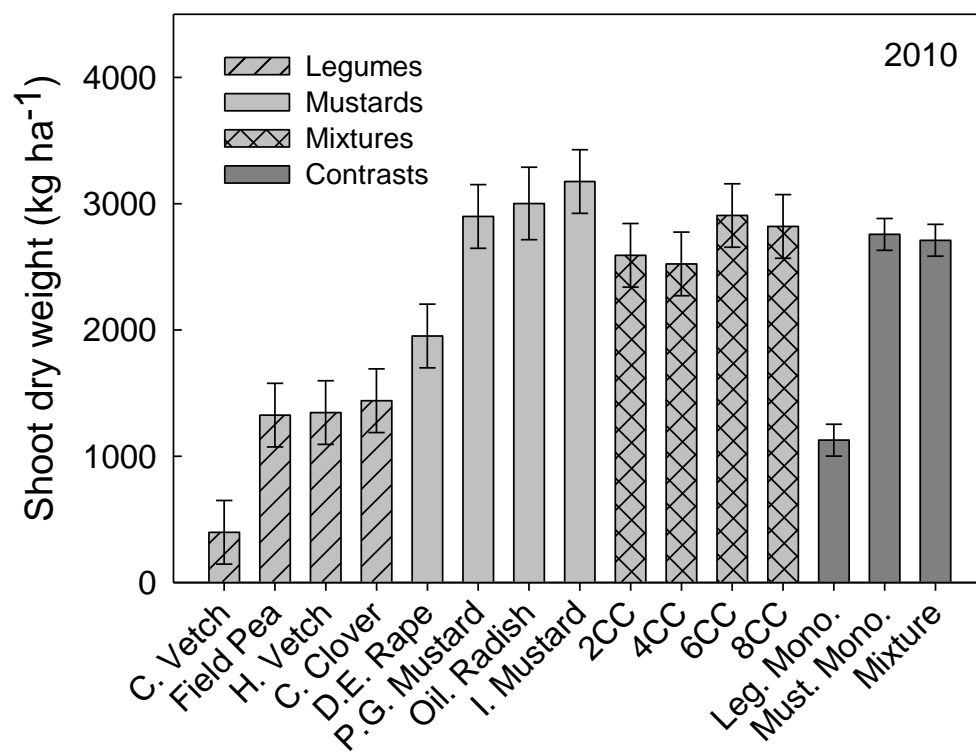


Figure 1.2. Coefficient of variation (C.V. %) for each cover crop monoculture and mixture combination (2, 4, 6, and 8 species) pooled across replications ($n=4$) and years ($n=2$). The mean and standard error of C.V.'s pooled within monoculture treatments ($n=8$) and within mixture treatments ($n=4$) is also presented.

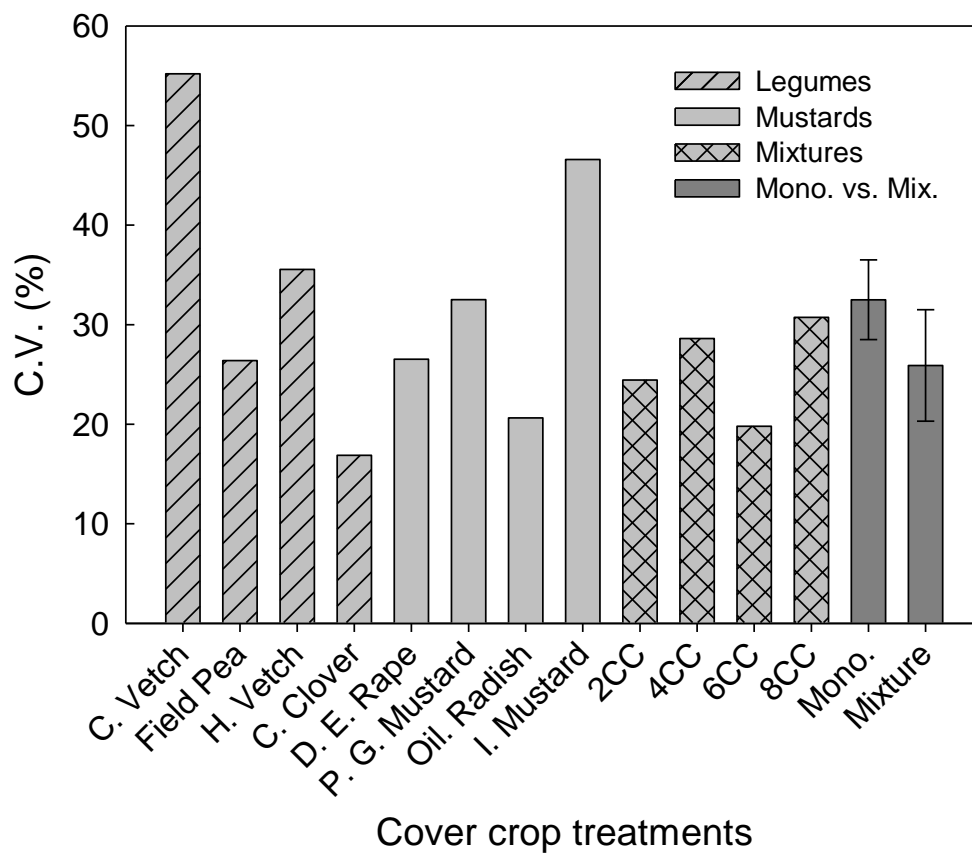
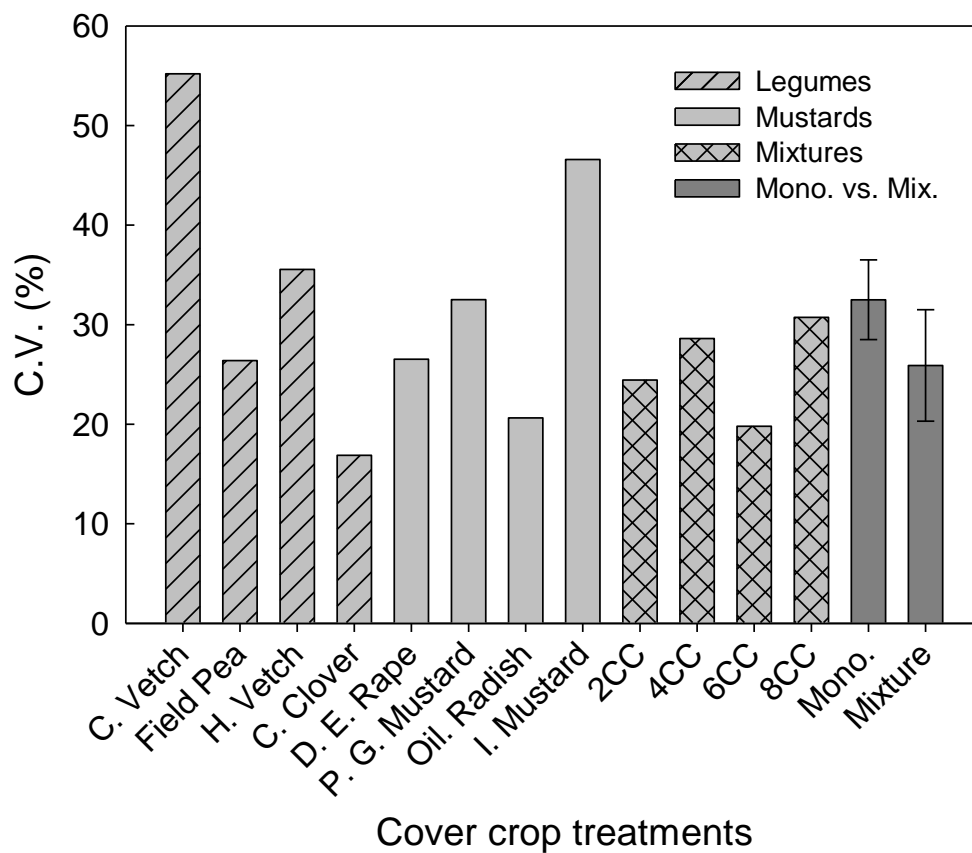


Figure 1.3. Total land equivalent ratios (LER) for the four cover crop mixtures (combinations of 2, 4, 6, and 8 species) in 2010 and 2011. Error bars represent the standard error of the mean. A LER value greater than 1.0 suggests a given mixture is more productive than its component sole crops.



Chapter 2

Weed Biomass, Density, and Community Response to Cover Crop Mixtures and Mechanical Termination Method

Abstract

Cover crops can provide many benefits in agroecosystems, including the opportunity for improved weed control. However, the weed suppressive potential of cover crops may depend on the species (or mixture of species) chosen, and the method of cover crop termination and residue management. The objective of this study was to determine the effects of increasing cover crop species diversity and mechanical termination method on weed biomass, density, and community composition, and relative crop yield in an organic cropping system. A field experiment was conducted from 2009 through 2011 near Mead, NE where spring-sown mixtures of 2, 4, 6, and 8 cover crop species were included in a sunflower – soybean – corn crop rotation. Cover crops were planted in late-March, terminated in late-May using a field disk or sweep plow undercutter and main crops were planted within one week of termination. Terminating cover crops with the undercutter consistently reduced early-season grass weed biomass and late-season broadleaf weed cover, whereas termination with the field disk typically stimulated grass weed biomass and total weed cover. The effects of cover crop mixture were not evident in 2009, but the combination of the undercutter and the most diverse mixture reduced early-season weed biomass by 48% relative to the no cover crop control in 2010. Cover crops provided less weed control in 2011, where only the combination of the undercutter and the two-species mixture reduced weed biomass (by 31%) relative to the control. Weed community composition and species diversity were not influenced by

cover crop mixture. However, termination with the undercutter reduced abundance of later-emerging summer annual weeds (velvetleaf and redroot pigweed) and promoted the presence of common lambsquarters – an earlier-emerging summer annual weed.

Termination with the undercutter resulted in relative yield increases of 16.6 and 22.7% in corn and soybean, respectively. In contrast, termination with the field disk resulted in a relative yield reduction of 13.6% in soybean. The strong influence of termination method highlights the importance of appropriate cover crop residue management in maximizing potential agronomic benefits associated with cover crops.

Introduction

Cover crops can provide many benefits to agroecosystems, and there is growing interest in cover crop use among a diverse range of agricultural stakeholders. The potential for weed suppression is one benefit of cover crops of particular interest to farmers in the Corn – Soybean Belt of the USA (Corn and Soybean Digest, 2010). Cover crops have been shown to suppress weeds through physical interference (Teasdale et al., 1991), light interception (Teasdale et al., 2007), buffered soil temperatures (Teasdale and Mohler, 1993), increased habitat for weed seed predators (Gallandt et al., 2005), delayed release of plant available nitrogen (Dyck et al., 1995; Moonen and Barberi, 2004), and release of allelopathic phytotoxins (Blackshaw et al., 2001; Sarrantonio and Gallandt, 2003). The capacity for cover crops as a long-term weed management tool will depend on a combination of these factors, but the mechanisms of physical interference and allelopathy are often viewed as near-term weed management solutions.

Regardless of the mechanism, the success of cover crops as a weed management tool will depend on the high-level production of biomass and resulting soil coverage (Teasdale et al., 2007). Relative to light interception, it may be necessary to achieve 97% soil coverage with cover crop residue to reduce weed density by 75% (Teasdale et al., 1991). However, many cover crops are not grown to full maturity, so achieving maximum biomass and soil coverage is difficult. Therefore, it is necessary to choose cover crop species that provide additional mechanisms of weed control through allelopathic activity or effects on germination cues (Teasdale et al., 2007). When cover crop residue is decomposed in the soil, phytotoxins may be released that can inhibit the emergence and growth of many weed species (Blackshaw et al., 2001; Dabney et al.,

1996; Davis and Liebman, 2003; Sarrantonio and Gallandt, 2003). There are many cover crop species with demonstrated phytotoxicity such as rye (*Secale cereale*), crimson clover (*Trifolium incarnatum*), hairy vetch (*Vicia villosa*), and members of the Brassicaceae family (Putnam and Barnes, 1986; White et al., 1989; Norsworthy et al., 2007).

All Brassicaceae spp. contain glucosinolates (Rosa et al., 1997), which are hydrolyzed upon decomposition releasing biologically active compounds, such as isothiocyanates, that inhibit weed seed germination (Petersen et al., 2001; Teasdale and Taylorson, 1986, Norsworthy et al., 2007). The potential of glucosinolates to suppress weed emergence and growth has been widely demonstrated in the greenhouse; thus, Brassicaceae spp. are increasingly popular cover crops (Bialy et al., 1990; Boydston and Hang, 1995; Al-Khatib et al., 1997; Eberlein et al., 1998; Krishnan et al., 1998; Petersen et al., 2001; Norsworthy, 2003). Phytotoxin composition differs among and within species and total production may depend on a variety of biotic and abiotic stresses (Ju et al., 1980; Louda and Rodman, 1983; Branca et al., 2002). Moreover, the specific allelopathic effects of individual phytotoxic compounds may be weed species specific (Norsworthy et al., 2007). Therefore, a diverse mixture of allelopathic cover crop species may be effective in targeting a broad range of weed species. Moreover, mixed species communities may help to ensure stable, resilient, and productive cover crop yields that will contribute to improved soil coverage and physical mechanisms of weed suppression (Tilman et al., 2001; Teasdale et al., 2007; Wortman et al., 2012).

Cover crop choice is important, but appropriate cover crop termination method and residue management may be the most critical factors in successfully using cover

crops for weed suppression. Cover crops can be terminated climatically (e.g., winterkill), chemically, or through various mechanical measures (e.g., plowing, disking, mowing, roller-crimping, or undercutting). The most appropriate termination method will depend on the farm management objective. When managing for improved weed control, previous studies have shown that termination methods resulting in maximum surface residue and minimal soil disturbance have the greatest potential to inhibit weed germination and growth (Teasdale et al., 1991; Teasdale et al., 2007). To this end, a sweep plow undercutter may have great potential, especially in organic cropping systems where chemical termination is prohibited. Creamer et al. (1995) demonstrated that cover crop termination with a sweep plow undercutter created a thick and uniform cover crop mulch and subsequent weed suppression was greater than when cover crops were terminated via mowing (which finely shredded the cover crop). While other mechanical termination methods such as the roller-crimper have shown great promise for weed control (Davis, 2010; Mischler et al., 2010), the sweep plow undercutter may be more effective in killing cover crops at less mature growth stages (Creamer et al., 1995; Mirsky et al., 2009). Moreover, the sweep plow undercutter is a traditional tillage implement in the US Great Plains that may be more easily accessible compared to newer implements such as the roller-crimper not yet widely distributed.

A three-year field experiment was conducted to determine the capacity of cover crop mixtures to contribute to weed management in organic cropping systems. More specifically, the objectives of this study were to (1) quantify the weed suppressive potential of four cover crop mixtures of different levels of species diversity and two cover crop termination methods; (2) determine the effects of cover crop mixture and

termination method on weed community composition and species diversity; and (3) quantify the effects of cover crop mixture and termination method on crop yields relative to a traditional organic cropping system with no cover crops. With respect to these objectives, we hypothesized that increasing cover crop mixture diversity coupled with termination via the undercutter would suppress a broad range of weed species leading to distinct shifts in weed community composition and increased crop yield.

Materials and Methods

Experimental Site and Treatment Design

A field experiment was conducted in 2009, 2010 and 2011 at the University of Nebraska-Lincoln Agricultural Research and Development Center (ARDC) near Mead, Nebraska. Dominant soil type at the site is a Sharpsburg silty clay loam (fine, smectitic, mesic typic Argiudoll) with 0 to 5% slopes. The experimental 2.8 ha field is certified for organic production (OCIA International, Lincoln, NE), and is managed without irrigation. This field was in organic alfalfa hay production for the five seasons prior to 2009. In the fall of 2008 the experimental area was amended with 50 Mg ha⁻¹ of liquid beef feedlot manure that was incorporated via field disk. On March 15, 2009, the entire field (excluding a weed-free control treatment) was seeded with 8.1 kg ha⁻¹ of velvetleaf (*Abutilon theophrasti*; ABUTH) seed, 2.6 kg ha⁻¹ of common lambsquarters (*Chenopodium album*; CHEAL) seed, 1.2 kg ha⁻¹ of redroot pigweed (*Amaranthus retroflexus*; AMARE) seed, and 3.7 kg ha⁻¹ of green foxtail (*Setaria viridis*; SETVI) seed to establish a common weed seedbank throughout the field.

The experiment was designed as a split-plot randomized complete block design within 4 replications of a 3-year crop rotation. The rotation sequence consisted of confectionery sunflower (*Helianthus annuus* L. ‘Seeds 2000 Jaguar’) – soybean (*Glycine max* L. Merr. ‘Blue River Hybrids 2A71’) – corn (*Zea mays* L. var. ‘Blue River Hybrids 57H36’). Within each crop species, whole-plots (9.1 x 21.3 m; 12 crop rows spaced 0.76 m apart) were defined by cover crop mixture, while split-plots (4.6 x 21.3 m; 6 crop rows spaced 0.76 m apart) were defined by cover crop termination method. Each “crop x cover crop mixture x termination method” treatment combination was replicated within each block so that each phase of the 3-year crop sequence was present each year within each block. There were six whole-plot cover crop treatments: 1) two-species cover crop mixture (2CC), 2) four-species cover crop mixture (4CC), 3) six-species cover crop mixture (6CC), 4) eight-species cover crop mixture (8CC), 5) weedy but cover crop-free (prior to main crop planting) control (WD), and 6) weed-free and cover crop-free (prior to main crop planting) control (NC). The NC whole-plots were field disked and hand-hoed twice prior to main crop planting, while the WD whole-plots were left unmanaged until cover crop termination. Details on the individual species and seeding rates included in each cover crop treatment whole-plot are included in Table 2.1.

Split-plot cover crop termination methods included either disking or undercutting. Termination method was randomized within the first replication (southernmost) and duplicated in the remaining three replications (north of the first replication) to facilitate adequate speed for effective tillage operations driving north-south through the field. Disking was conducted with a 4.6 m wide Sunflower 3300 (Sunflower Mfg., Beloit, KS, USA) disk to an approximate depth of 15 cm. Undercutting was conducted with either a

Buffalo 6000 (Buffalo Equipment, Columbus, NE, USA) cultivator (modified for undercutting) with seven overlapping 0.75 m wide sweep blades (2009) or a Miller Flex-Blade sweep plow undercutter (2010 and 2011) with three overlapping 1.5 m sweep blades. The undercutter sweeps are designed to cut a level plane through the soil at an approximate depth of 10 cm, severing plant roots and minimizing soil inversion, resulting in a layer of intact surface residue. Details on the design of the undercutter can be found in Creamer et al. (1995).

Cover crop mixtures were planted via hand-crank broadcast seeding followed by light incorporation with a John Deere 950 cultipacker (Deere and Company, Moline, IL, USA). Generally, cover crops were planted in late-March, terminated in late-May, and the main crop was planted within one week of termination. Specific dates for field operations across all years are detailed in Table 2.2. Seeding rates for confectionery sunflower, soybean, and corn were 62,000, 556,000, and 86,000 seeds ha⁻¹, respectively. All crops were inter-row cultivated once (2009) or twice (2010 and 2011) approximately 30 days after planting the main crop. Seeds of all legume cover crop and crop species were inoculated with appropriate rhizobia bacterial species prior to planting in 2009 and 2010.

Data Collection

Three (2009) or four (2010 and 2011) aboveground plant samples were taken from each whole-plot experimental unit prior to cover crop termination to determine productivity of the cover crop mixtures and weed communities. Samples were combined within each experimental unit, dried at 60° C to constant mass and weighed. Three (2009) or four (2010 and 2011) aboveground plant samples were taken from each split-plot

experimental unit approximately 30 days after planting the main crop (DAP) to quantify weed species density and aboveground biomass. Samples were combined within each split-plot experimental unit, sorted by species, and each component counted. In 2010 and 2011, the samples were then divided by broadleaf and grass weeds, dried at 60° C to constant mass and weighed. The 2009 samples were divided by broadleaf and grass weeds, fresh weights were recorded, and one composite sample (containing all weeds) was dried to constant mass and weighed. A second sampling interval was conducted in 2010 at 50 DAP to quantify mid-season grass and broadleaf weed suppression.

The sampling quadrat area in 2009 consisted of three 0.3 x 0.3 m samples per experimental unit. The sampling quadrat area in 2010 and 2011 was increased to four 0.3 x 0.6 m samples per experimental unit. Quadrats were placed at random locations between (2009 and 2010) or within (2010 sampling at 50 DAP and 2011) crop rows of each split-plot. Sample quadrats were placed within crop rows at the 2010 50 DAP interval and in 2011 to avoid the inter-row area that was previously cultivated. The second plant sampling interval (at 50 DAP in 2010) was replaced with a mid-season visual rating of weed cover in 2009 and 2011. Ratings were determined by walking through three rows of each split-plot experimental unit and assessing the proportion of the plant canopy occupied by each of the four weed species planted prior to the 2009 growing season (ABUTH, ALBUM, AMARE, and all grass species combined including SETVI). The visual rating was conducted in each experimental unit by three (2009) or four (2011) individuals, and the mean of all ratings was used to estimate weed cover for each species.

Grain yield was determined for each main crop by harvesting the middle 4 rows of each split-plot experimental unit. Contents were weighed using a Weigh-Tronix 400 combine scale (Avery Weigh-Tronix, Fairmont, MN, USA) and adjusted for moisture content in the lab. Corn grain yields were adjusted to 0.155, soybean to 0.130, and sunflower to 0.10 g kg⁻¹ moisture. Relative yield for each experimental unit was calculated as:

$$\text{Relative Yield} = ((\text{CCE} - \text{NC}) / (\text{NC})) * 100\%$$

where CCE is the grain yield from one split-plot cover crop experimental unit and NC is the grain yield from the no cover crop control (NC) experimental units averaged across all replications within a given year.

Data Analysis

Weed biomass and weed cover data were either log- or root-transformed prior to statistical analysis to improve normality and homogeneity of variances when necessary. Least square means obtained from these analyses were back-transformed for presentation in all tables and figures. After transformation (if necessary), values for weed biomass, weed cover, and relative yield were compared among treatments using a linear mixed model analysis of variance in the GLIMMIX procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Weed species density data were compared among treatments using a generalized Poisson mixed model for overdispersed count data, also using the GLIMMIX procedure (SAS 9.2 User's Guide, 2nd ed.). Fixed effects in both models included main crop, cover crop mixture, termination method and all possible interactions of these effects. The random effects were block and the interaction of block by current crop by cover crop mixture. Effects were tested within individual years due to experimental

changes in the cover crop mixture (buckwheat was replaced in all mixtures with Idagold mustard after 2009) and interactions with year when initially included as a fixed effect. Least square means and standard errors were calculated for all significant fixed effects at an alpha level of 0.05. Lastly, a simple linear regression of cover crop biomass and weed biomass at the first sampling interval between 2009 and 2011 was conducted using the REG procedure in SAS 9.2 to quantify the potential role of physical interference in the weed suppressive capacity of cover crop residue.

To aid in the visualization of statistical interactions, data were often plotted as lines with cover crop mixture on the x-axis (Sosnoskie, 2006). The cover crop treatments were arranged in order (left-to-right) of increasing species diversity (from zero in the NC treatment to eight species in the 8CC treatment) along the x-axis, similar to the figures presented by Tilman et al. (2001). However, we recognize that these data are not truly continuous as is traditionally expected in line plots.

To further characterize weed species community composition, broadleaf weed species density data were used to calculate indices of weed species diversity, evenness, and richness for each split-plot experimental unit. Diversity (H') was calculated using the Shannon diversity index:

$$H' = - \sum P_i (\ln P_i), \text{ where } P_i = N_i / N_{total}$$

where N_i = number of individuals of species i (plants m^{-2}) and N_{total} = total number of individuals (plants m^{-2}). Evenness (J) was then calculated as:

$$J = H' / \ln (S)$$

where S = species richness calculated as the total number of species per plot (Sosnoskie et al. 2006; Wortman et al. 2010). Estimates of H' , J , and S for broadleaf weeds were

compared among management treatments using the GLIMMIX procedure in SAS 9.2 as described previously.

Results and Discussion

Early-Season Weed Suppression

Grass weed biomass (fresh shoot weight) was influenced by the effects of main crop and termination method at 32 DAP in 2009 (Table 2.3). Grass biomass was lowest following termination with the undercutter (1137 g m^{-2}) compared to both disk incorporation and the NC control (1254 g m^{-2} and 1279 g m^{-2} , respectively). In addition, grass weed biomass was lowest in sunflower (1115 g m^{-2}) and greatest in corn (1288 g m^{-2}). Indeed, sunflower may be a competitive crop choice, especially in organic systems, due to its capacity for early light interception (Geier et al., 1996) and allelopathic effects on weed seed germination and growth (Leather, 1983). In 2010 (at 23 DAP), grass weed biomass was influenced by the interaction of mixture and termination method (Table 2.3). Termination with the undercutter in the 4CC and 8CC mixtures reduced biomass by 39 and 45%, respectively, relative to the NC control (Figure 2.1a). In contrast, termination with the disk in the 6CC and 8CC mixtures stimulated grass weed biomass by 56 and 32%, respectively, relative to the NC control (Figure 2.1a). While grass weed biomass was generally not influenced by the effect of increasing cover crop diversity, the differences among mixtures within termination methods suggests there may be unique characteristics associated with each mixture (e.g., biomass quantity, quality, biochemical composition, or phytotoxins) driving this variable response.

Grass weed biomass was influenced by the effects of mixture and the interaction of termination method by crop at 36 DAP in 2011 (Table 2.3). In general, grass weed biomass was stimulated by the presence of cover crops (not weeds) regardless of termination method (data not shown). However, the termination method by crop interaction indicated that disk termination stimulated grass weed biomass in all crops while termination with the undercutter reduced grass weed biomass only in soybean (data not shown). The results in 2011 highlight the challenges of using high quality (low C:N ratio) residue to suppress weeds regardless of termination strategy. As cover crops increase nutrient availability, both crops and weeds are likely to respond with greater growth if the weeds are not managed properly (Liebman and Davis, 2000). Moreover, low quantities of legume cover crop residue (perhaps similar to levels found in a diverse mixture) have been shown to stimulate weed seed germination and radicle elongation (Teasdale and Pillai, 2005; Hill et al., 2006).

Broadleaf weed biomass was not affected by any of the fixed effects or interactions at 32 DAP in 2009 (Table 2.3). Similarly, only the effect of main crop influenced broadleaf weeds at 23 DAP in 2010, where weed biomass in sunflower was reduced by 53 and 44% relative to weeds in corn and soybean. This is consistent with grass weed response, and provides further support for the alleged competitiveness of the sunflower crop. In 2011 (at 36 DAP), broadleaf weed biomass was again influenced by the effect of crop, but also by the interaction of mixture by termination method (Table 2.3). In contrast to the 2010 results, broadleaf weed biomass was lowest in soybean (13.5 g m^{-2}) and greatest in corn and sunflower (26.2 and 22.2 g m^{-2} , respectively). This may be related to the low level of weed biomass seen in the 2010 sunflower crop, which precedes

soybean in the rotation. This conclusion is based on the assumption that lower biomass at 23 DAP resulted in lower fecundity of broadleaf weeds and reduced emergence the following year (Aarssen and Taylor, 1992). Managing weed populations for reduced biomass and seed production is an essential component of integrated weed management strategies in low-external-input cropping systems, especially when growing less competitive crops like soybean (Kegode et al., 1999).

The interaction of mixture by termination method in 2011 was the result of exceptionally high broadleaf weed biomass (51.8 g m^{-2}) in the WD/undercutter treatment combination, relative to all other treatments combined (18.8 g m^{-2}). The large amount of broadleaf weed biomass in the WD/undercutter treatment combination was related to the ineffectiveness of the undercutter in terminating small weed seedlings. Creamer et al. (1995) also found that plants were difficult to terminate with the undercutter if they had not yet reached the mid- to late-bloom stage of maturity. The continuous and unmanaged emergence of weed seedlings throughout the spring in the WD treatment resulted in a weed community representing various growth stages. The undercutter sweeps travel at a depth of 10 cm beneath the soil surface; thus, recently emerged weed seedlings with shallow root systems may not have been effectively killed by the undercutting operation. Presumably, this was not an issue in the cover crop mixtures as there were fewer weeds growing in the mixtures, and those that were established were likely mature enough to compete with the mixtures; thus, the root systems would be mature enough to be effectively terminated by the undercutter.

Broadleaf weed density during the first sampling interval was influenced by crop and termination method (2010 and 2011) or the interaction of termination by crop (2009;

Table 2.4). With regard to termination method, broadleaf weed density following termination with the undercutter was always at least 36% less than the densities observed following termination with the disk or the NC control (Table 2.5). Broadleaf weed density spiked upward in 2010, where 115.2 plants m^{-2} were observed in the NC control compared to 38.6 and 24.7 plants m^{-2} following termination with disk and undercutter, respectively (Table 2.5). The interaction effect in 2009 was due to the lack of a termination effect in sunflower, whereas trends in corn and soybean were consistent with those observed across all other years and crops. It is possible that the competitive effects of sunflower masked any additional weed suppressive potential of termination with the undercutter. With regard to the influence of crop, broadleaf weed density was always greatest in corn and lowest in either sunflower (2010) or soybean (2011; Table 2.5). Consistent with the response of broadleaf biomass, reduced broadleaf weed density in 2011 soybean may be related to the strong competitive effects and reduced weed pressure observed in sunflower in 2010. Indeed, sunflower is typically a more competitive crop species than soybean (Geier et al., 1996).

When pooling grass and broadleaf weed biomass into a measure of total weed biomass, results were similar to those for grass weed biomass in 2009 and 2010, as these weeds dominated the community (Table 2.3; Figure 2.1b). However, a more even distribution of grass and broadleaf weeds led to unique results for total weed biomass in 2011. Total weed biomass was influenced by the interactions of termination by main crop and also termination by cover crop mixture at 36 DAP in 2011. Undercutting cover crop mixtures for weed suppression was most effective in soybean, which led to the termination by crop interaction. Overall, the undercutter was less effective in suppressing

weeds in 2011 as only the 2CC/undercutter treatment combination successfully reduced total weed biomass relative to the NC control (Figure 2.2). While the undercutter was less beneficial in 2011, using the field disk for termination was largely detrimental as total weed biomass was stimulated by 58, 52, and 51% in the 2CC, 6CC, and 8CC mixtures, respectively (Figure 2.2). Consistent with the results for broadleaf weed biomass, total weed biomass in the WD/undercutter treatment combination was greater than that in the WD/disk treatment combination. As observed in 2010, total weed biomass was greater in the 6CC mixture regardless of termination method. Given the consistency of this result across two consecutive years, it appears likely that the composition of species in the 6CC mixture (Table 2.1) is uniquely beneficial to weed growth. Whereas increasing cover crop diversity did not predictably decrease weed biomass and density as we hypothesized, we did observe variable levels of weed suppression or stimulation across the four mixtures of cover crops. The consistency of these trends (i.e., weed stimulation following the 6CC mixture) suggests there is something unique to each mixture driving these differences. There may be species interactions between/among cover crops in mixtures or between/among cover crop mixtures and main crops that we could not detect in this experimental design.

Variability in the weed suppressive capacity of cover crops is most often related to cover crop biomass and productivity, especially when the residue is managed on the soil surface to promote physical interference with weed seed emergence and growth (Teasdale et al., 1991; Teasdale et al., 2007; Mirsky et al., 2011). Therefore, using regression analysis we tested the hypothesis that the observed variability in weed suppression among cover crop mixtures was related to variability in the biomass

productivity of the mixtures. However, we observed no relationship between these two factors in any year of this study, regardless of termination method (data not shown). This result suggests that the variability in weed suppression observed among mixtures is related to the biochemical composition and quality of the mixture residue, and largely independent of the quantity of mixture residue. This finding offers support for an allelopathic (or facilitative in the case of the 6CC mixture), rather than a physical mechanism of weed suppression for these cover crop mixtures, and opens the door for further research on inter-specific allelopathic interactions.

The composition and concentration of individual allelopathic plant compounds is often species and variety dependent (Branca et al., 2002); thus, it is possible that a diversity of allelopathic interactions between cover crops and the numerous target weed species resulted in lower weed emergence and growth for various mixtures (Norsworthy et al., 2007). Though often documented in greenhouse studies, allelopathic effects of cover crop residue on weed seed emergence and growth has been difficult to observe in field studies (Haramoto and Gallandt, 2005). While we do not have the biochemical analyses to directly support an allelopathic mechanism of suppression, elimination of the physical interference hypothesis seems to leave few other logical alternatives. However, one additional explanation for these results may be the potential for negative soil microbial feedback effects. The negative soil feedback hypothesis suggests that changes in the soil microbial community during cover crop growth create a soil environment less suitable for germination and growth of certain weed species (Klironomos, 2002). Unfortunately, elucidation of these mechanisms will require fundamental research beyond the scope of this study.

Early-Season Weed Community Composition

Density of ABUTH was influenced by the three-way interaction of mixture, termination method, and current crop at 32 DAP in 2009 (Table 2.4), but there were few consistent differences among crops or cover crop mixtures. Despite this interaction, the most noticeable trend in ABUTH density was driven by termination method where density was greatest following termination with the disk (12.2 plants m⁻²), followed by the NC control (9.9 plants m⁻²), and lowest following termination with the undercutter (4.3 plants m⁻²). Termination method strongly influenced ABUTH at 23 DAP also in 2010, but density was greatest in the NC treatment (55.5 plants m⁻²), followed by termination with the disk (19.9 plants m⁻²), and lowest after termination with the undercutter (10.3 plants m⁻²). ABUTH density also was influenced by current crop with the lowest densities occurring in the sunflower crop (10.7 plants m⁻²) – substantially less than the densities found within the corn crop (27.2 plants m⁻²). Similar to results for 2009, ABUTH density was influenced by the three-way interaction of mixture, termination method, and current crop at 36 DAP in 2011 (Table 2.4). Again, the only consistent trend was the effect of termination method, where the undercutter reduced ABUTH density by 51 and 60% relative to termination with the disk and the NC control, respectively. Suppression of ABUTH density with cover crop surface mulch is consistent with previous findings (Liebl et al., 1992). Moreover, the reduction in soil mixing with conservation tillage implements like the undercutter can aid in reduced emergence of dicot weed species like ABUTH (Buhler and Daniel, 1988; Liebl et al., 1992).

Density of AMARE was not different among cover crop mixtures, termination methods, or crops at 32 DAP in 2009, but was influenced by the three-way interaction of

these factors at 23 DAP in 2010 (Table 2.4). This interaction was the result of extraordinarily high densities of AMARE in the NC control and WD/disk treatment combination in soybean (123.2 and 78.2 plants m⁻², respectively), along with elevated densities in the NC control and the WD/disk treatment combination in corn (50.1 and 77.8 plants m⁻², respectively). In contrast, AMARE density was relatively low in the sunflower crop with minor differences among mixtures and termination methods (Figure 2.3). Again, the strong competitive effects of sunflower seem to have masked any potential effects of mixture or termination method on weed suppression. However, reduced densities of AMARE in the cover crop mixtures (regardless of termination method) provide evidence for the utility of cover crop mixtures as a weed management tool. While the weed suppressive effects of cover crops are often inconsistent and species specific, these results suggest that cover crop mixtures may be most effective when used as a component of more diversified and integrated approaches to weed management (Liebman and Davis, 2000).

Differences in AMARE densities were influenced by the interaction of termination method by current crop at 36 DAP in 2011 (Table 2.4). Termination with the undercutter reduced AMARE density by 61% relative to termination with the disk in soybean. In sunflower, termination with the undercutter reduced AMARE density by 55 and 54% relative to the NC control and termination with the disk, respectively. In contrast, AMARE density was elevated in corn but not influenced by the effect of termination method. Similar to ABUTH, AMARE density was most effectively suppressed with conservation tillage and the associated reduction in soil mixing (Liebl et al., 1992).

Density of CHEAL was not different among cover crop mixtures, termination methods, or crops at the first sampling intervals in 2009 or 2010; however, CHEAL was influenced by the interaction of mixture by termination and the interaction of mixture by crop at 36 DAP in 2011 (Table 2.4). The interaction of mixture by termination method was strongest, where termination with an undercutter in the WD, 6CC, and 8CC treatments led to increased CHEAL densities of 6.2, 2.2, and 1.5 plants m⁻², respectively (Figure 2.4). Densities of CHEAL in all other mixture/termination method treatment combinations were essentially zero. This result is consistent with the increased broadleaf biomass following termination with the undercutter in 2011. Of the dominant broadleaf weeds observed in this study, CHEAL was consistently the earliest emerging species in the spring (Myers et al., 2004). Therefore, this was the most abundant weed species at the time of cover crop/weed termination and thus the most probable species to survive the undercutting operation. In contrast, ABUTH and AMARE typically emerged after the termination operation, which explains improved suppression of these weeds following termination with the undercutter (Liebl et al., 1992; Myers et al., 2004).

Broadleaf weed species richness, evenness, and diversity at the first sampling interval (approximately 30 DAP) were not influenced by cover crop mixture in any year of this study (data not shown). It was hypothesized that increasing the diversity of allelopathic cover crop species in a mixture would suppress a broad range of weed species, resulting in a more diverse but less dense weed community. Initially diverse plant communities (polycultures) have previously been shown to beget subsequently diverse weed communities (Palmer and Maurer, 1997). In contrast, increasing diversity of crop rotations has been shown to reduce weed species diversity (Smith and Gross, 2007).

It is possible that spatial and temporal diversity of crop communities have unique influences (potentially opposite) on weed community composition and species diversity. The lack of differences observed in this study may be related to the short-term duration of this cropping systems trial; typically, changes in weed community composition and diversity have been observed in longer-term trials (e.g., Menalled et al., 2001; Sosnoskie et al., 2006; Wortman et al., 2010).

Mid-Season Weed Biomass, Cover, and Community Composition

Weed cover (%) for grasses, ABUTH, CHEAL, AMARE, and total broadleaf weeds was most commonly influenced by the effects of current main crop and termination method at 74 DAP in 2009 (Table 2.6). Weed cover of most broadleaf and grass weed species was lowest in the sunflower crop compared to both corn and soybean, while broadleaf cover was typically greatest in soybean (Table 2.8). This is consistent with the levels of weed biomass and densities observed in these crops at 32 DAP. With regard to termination method, broadleaf weeds were typically greatest in the NC control and lowest following termination with the undercutter (Table 2.8).

Grass biomass at 50 DAP in 2010 was influenced by the interaction of mixture by current main crop, as cover crop mixtures tended to stimulate weed growth (relative to the WD and NC controls) in corn and soybean, but not sunflower (Table 2.7). While cover crops may aid in early season weed suppression via physical interference or allelopathic effects, decomposition and mineralization of the cover crop residue may lead to increased growth of both crops and weeds later in the growing season. This is consistent with results of Teasdale et al. (1991), who found no difference in late-season weed biomass despite early-season reductions in weed density with cover crop residue.

Broadleaf and total weed biomass responded similarly, where both were influenced by the effect of current crop and the interaction of mixture by termination method (Table 2.7). Broadleaf and total weed biomass were consistently lowest in sunflower, followed by corn, and greatest in soybean (data not shown). The interaction of mixture by termination method was largely the result of a 33 and 220% increase in the WD/disk relative to the WD/undercut treatment combination for total and broadleaf weed biomass, respectively (Figure 2.5).

Despite several interactions, weed cover was most strongly influenced by current crop and termination method at 57 DAP in 2011 (Table 2.6). Results in 2011 were similar to those in 2009, where weed cover (both grasses and broadleaves) was lowest in sunflower (Table 2.8). However, in contrast to 2009, weed cover was greater in corn compared to soybean in 2011 (Table 2.8). Also similar to 2009 results, weed cover was greatest following termination with the disk and lowest following termination with the undercutter or in the NC control (Table 2.8). However, CHEAL cover was greatest following termination with the undercutter (6.7%) compared to termination with the disk (0.8%) and the NC control (0.5%; Table 2.8). This result is consistent with the increase in CHEAL density at 36 DAP in 2011 following termination with the undercutter, which is likely related to the early emergence timing of CHEAL relative to ABUTH and AMARE (Myers et al., 2004).

Relative Crop Yield

Despite the effect of cover crop mixtures on weed biomass early in the growing season, relative crop yield was only influenced by termination method in this study. Relative to a traditional organic cropping system (NC control), cover crop termination

with the undercutter increased corn yield by 16.6%, while termination with the disk did not alter yield (Figure 2.6). In soybean, the effect of cover crop termination method was more pronounced. Termination with the undercutter increased yield by 22.7%, while termination with the disk reduced yield by 13.6% relative to the NC control (Figure 2.6). Despite an apparent yield benefit following the disk and undercutter for termination in sunflower, the increase was not statistically different from the NC control due to substantial variation in relative yield within and among years (Figure 2.6).

Many studies have demonstrated peripheral benefits of including cover crops in agroecosystems, but yield gains are often difficult to detect (Reddy et al., 2003; Haramoto and Gallandt, 2005; Russo et al., 2006). However, recent studies have demonstrated the potential for cover crop mulches to increase or maintain grain yield relative to a no cover crop control (Mischler et al., 2010). Many of these systems have depended on herbicides for termination of cover crops and weeds (Swanton et al., 1999; Shrestha et al., 2002; Teasdale et al., 2007), which has limited applicability for organic farmers. The results of this study demonstrate the potential of cover crop mixtures to increase crop yield in organic cropping systems when combined with a sweep plow undercutter for termination.

Conclusions

Changes in weed biomass, density, and community composition were largely driven by the current main crop and differences in cover crop termination strategies. Reduced weed pressure following termination with the undercutter observed here is congruent with the results of Creamer et al. (1995), who found reduced weed biomass

following cover crop termination with an undercutter compared to a flail mower. Moreover, the stimulation of weed growth commonly observed following termination with the disk and in the no cover control is consistent with previous work demonstrating the risks of using intensive tillage for early-season weed control and seedbed preparation (Liebl et al., 1992; Yenish et al., 1992; Mulugeta and Stoltenberg, 1997). Use of the undercutter for weed control and cover crop termination has typically been limited to sandier soils of the western US Great Plains. However, these results demonstrate potential for this unique conservation tillage implement in the silty clay loam soils of eastern Nebraska to aid in profitable cover crop and weed management for increased crop yields in organic systems.

The influence of cover crop mixture and increasing cover crop diversity in this study were far more subtle than the impacts of current main crop and termination method. However, changes in weed biomass among cover crop mixtures were detectable early in the growing season in two of three years. The lack of a relationship between cover crop biomass and early-season weed biomass suggests that allelopathic or negative soil microbial feedback mechanisms contributed to weed suppression in this study. While allelopathic mechanisms of weed suppression are well understood for individual cover crop species (e.g., Norsworthy et al., 2007), future studies should focus on the complex interactions occurring at the plant-soil interface between diverse cover crop communities and weed seed germination and growth.

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Tables and Figures

Table 2.1. Cover crop species and seeding rates used in individual cover crop mixtures for 2009 and 2010-11 (2CC = 2 species mixture; 4CC = 4 species mixture; 6CC = 6 species mixture; 8CC = 8 species mixture).

Common Name	Scientific Name	Cover Crop Seeding Rate			
		2CC	4CC	6CC	8CC
		kg ha ⁻¹			
Hairy Vetch	<i>Vicia villosa</i>	22.4	11.2	7.5	5.6
Buckwheat (2009)	<i>Fagopyrum sagittatum</i>	28.0	14.0	9.3	7.0
Idagold Mustard (2010-11)	<i>Sinapis alba</i>	6.7	3.4	2.2	1.7
Field Pea	<i>Pisum sativum</i>		28.0	18.7	14.0
Pacific Gold Mustard	<i>Brassica juncea</i>		2.2	1.7	1.1
Oilseed Radish	<i>Raphanus sativus</i>			2.8	2.1
Crimson Clover	<i>Trifolium incarnatum</i>			4.7	3.5
Dwarf Essex Rape	<i>Brassica napus</i>				1.7
Chickling Vetch	<i>Lathyrus sativus</i>				8.4

Table 2.2. Timing of field operations and data collection for each year of the study.

Operation	Year		
	2009	2010	2011
Cover Crop Planting	20 March	30 March	21 March
Cover Crop Termination	22 May	28 May	3 June
Main Crop Planting	28 May	1-3 June	6 June
1st Weed Biomass Sampling	29-30 June	24-25 June	12-13 July
1st Inter-row Cultivation	1 July	28 June	30 June
2nd Inter-row Cultivation		1 July	8 July
2nd Weed Biomass Sampling/Visual Rating	10 August	19-26 July	2 August

Table 2.3. *F*-values from linear mixed model analyses of variance for fixed effects and all possible interactions of cover crop mixture, termination method, and current crop on grass, broadleaf, and total weed biomass at 32, 23, and 36 DAP for the years 2009, 2010, and 2011, respectively. Significance of *F*-values is designated as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Source	df ^a	Grass biomass	Broadleaf biomass	Total biomass
2009				
Mixture	4	0.81	1.47	0.72
Termination	1	5.59*	1.70	6.47*
Crop	2	4.94*	0.07	2.50
Mixture x termination	4	1.55	1.08	2.55
Mixture x crop	8	0.93	0.78	0.74
Termination x crop	2	0.85	2.74	0.60
Mixture x termination x crop	8	1.74	1.91	1.70
2010				
Mixture	4	2.75*	2.54	2.18
Termination	1	95.84***	0.39	94.98***
Crop	2	0.17	5.07**	0.41
Mixture x termination	4	3.30*	0.70	4.13**
Mixture x crop	8	0.42	0.50	0.59
Termination x crop	2	2.24	0.38	1.97
Mixture x termination x crop	8	1.62	0.33	1.48
2011				
Mixture	4	3.32*	2.11	0.64
Termination	1	69.45***	0.76	16.76***
Crop	2	3.19*	5.04**	5.04**
Mixture x termination	4	0.04	3.91**	4.61**
Mixture x crop	8	0.98	0.59	0.73
Termination x crop	2	3.65*	1.85	4.28*
Mixture x termination x crop	8	0.93	1.30	1.51

^a Abbreviation: df, degrees of freedom.

Table 2.4. *F*-values from linear mixed model analyses of variance for fixed effects and all possible interactions of cover crop mixture, termination method, and current crop on ABUTH, CHEAL, AMARE, and total broadleaf weed density at 32, 23, and 36 DAP for the years 2009, 2010, and 2011, respectively. Significance of *F*-values is designated as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Source	df ^a	ABUTH	CHEAL	AMARE	Broadleaves
2009					
Mixture	4	1.09	1.12	0.03	1.67
Termination	1	23.55***	1.29	0.01	27.68***
Crop	2	0.59	0.16	0.01	0.07
Mixture x termination	4	0.94	0.80	0.28	0.68
Mixture x crop	8	1.69	0.82	0.15	0.76
Termination x crop	2	4.37*	0.06	0.01	3.90*
Mixture x termination x crop	8	2.57*	0.47	1.10	1.43
2010					
Mixture	4	1.07	0.03	3.08*	0.68
Termination	1	41.58***	0.01	1.75	17.65***
Crop	2	7.20**	0.01	11.10***	10.85***
Mixture x termination	4	0.49	0.01	3.69*	1.36
Mixture x crop	8	1.01	0.04	0.37	0.44
Termination x crop	2	0.03	0.01	1.71	0.64
Mixture x termination x crop	8	0.82	0.25	2.30*	1.46
2011					
Mixture	4	1.94	108.80	1.92	1.00
Termination	1	40.18***	0.04	9.31**	13.17***
Crop	2	11.98***	0.01	25.70***	23.12***
Mixture x termination	4	1.28	1.7 E +31***	1.18	0.97
Mixture x crop	8	1.79	2.62*	1.27	1.12
Termination x crop	2	3.33*	0.01	3.35*	2.81
Mixture x termination x crop	8	2.91*	1.39	0.56	1.16

^a Abbreviation: df, degrees of freedom.

Table 2.5. Total broadleaf weed density (plants m⁻²) in response to current crop and cover crop termination method at 32, 23, and 36 DAP for the years 2009, 2010, and 2011, respectively. Data shown are back-transformed LS means, which eliminated the possibility to present error terms. Instead, differences ($\alpha = 0.05$) among transformed LS means are indicated by different letters adjacent to the back-transformed value.

Effect	Year		
	2009	2010	2011
Total broadleaf weed density (plants m ⁻²)			
<i>Crop</i>			
Corn	21.7 a	53.4 a	26.3 a
Soybean	19.3 a	37.9 b	8.3 b
Sunflower	18.5 a	20.8 c	21.7 a
<i>Termination</i>			
No cover	25.3 a	115.2 a	24.2 a
Disk	24.1 a	38.6 b	20.3 a
Undercutter	10.1 b	24.7 c	12.9 b

Table 2.6. *F*-values from linear mixed model analyses of variance for fixed effects and all possible interactions of cover crop mixture, termination method, and current crop on grass, ABUTH, CHEAL, AMARE, and total broadleaf weed cover at 74 and 57 DAP for the years 2009 and 2011, respectively. Significance of *F*-values is designated as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Source	df ^a	Grasses	ABUTH	CHEAL	AMARE	Broadleaves
2009						
Mixture	4	0.95	0.92	1.37	1.27	1.44
Termination	1	3.87	21.69***	2.18	5.00*	8.21**
Crop	2	10.60***	14.85***	4.50*	2.98	19.92***
Mixture x termination	4	0.71	0.09	0.92	0.84	0.12
Mixture x crop	8	0.41	0.95	0.57	0.97	1.29
Termination x crop	2	0.73	0.96	1.20	1.76	1.73
Mixture x termination x crop	8	1.47	1.66	0.44	0.46	0.68
2011						
Mixture	4	2.83*	3.77**	1.87	0.18	0.90
Termination	1	93.49***	123.09***	64.71***	6.24*	20.06***
Crop	2	13.92***	22.18***	2.02	26.61***	34.37***
Mixture x termination	4	0.74	1.28	2.28	0.30	1.62
Mixture x crop	8	1.29	0.72	1.46	0.56	0.68
Termination x crop	2	5.75**	4.18*	0.85	1.64	3.48*
Mixture x termination x crop	8	1.23	1.72	0.49	0.76	2.64*

^a Abbreviation: df, degrees of freedom.

Table 2.7. *F*-values from linear mixed model analyses of variance for fixed effects and all possible interactions of cover crop mixture, termination method, and current crop on grass, broadleaf, and total weed shoot biomass at 50 DAP in 2010. Significance of *F*-values is designated as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Source	df ^a	Grass biomass	Broadleaf biomass	Total biomass
Mixture	4	4.07**	0.51	2.95*
Termination	1	2.08	0.05	3.32
Crop	2	22.48***	13.98***	40.21***
Mixture x termination	4	1.69	3.22*	4.11**
Mixture x crop	8	2.33*	0.65	1.96
Termination x crop	2	0.98	0.85	0.34
Mixture x termination x crop	8	0.46	0.64	0.87

^a Abbreviation: df, degrees of freedom.

Table 2.8. Percent weed cover in response to main crop and cover crop termination method at 74 and 57 DAP for the years 2009 and 2011, respectively. Data shown are back-transformed LS means, which eliminated the possibility to present error terms. Instead, differences ($\alpha = 0.05$) among transformed LS means are indicated by different letters adjacent to the back-transformed value.

Effect	Grasses	ABUTH	CHEAL	AMARE	Broadleaves
————— % weed cover —————					
2009					
<i>Crop</i>					
Corn	87.2 a	10.8 a	3.2 b	4.6 b	23.1 b
Soybean	85.8 a	10.7 a	5.7 a	5.7 a	27.6 a
Sunflower	82.5 b	5.4 b	3.4 b	3.8 b	15.6 c
<i>Termination</i>					
No cover	82.6 b	13.6 a	2.3 b	6.0 a	27.8 a
Disk	86.1 a	9.9 b	3.8 a	4.8 ab	22.4 b
Undercutter	84.8 b	6.9 c	4.7 a	4.1 b	19.8 c
2011					
<i>Crop</i>					
Corn	47.7 a	28.8 a	2.9 a	16.1 a	54.5 a
Soybean	36.7 b	23.6 b	2.9 a	11.2 b	46.8 b
Sunflower	32.5 c	14.4 c	1.8 b	6.1 c	26.8 c
<i>Termination</i>					
No cover	28.2 b	20.3 b	0.5 b	11.2 ab	37.8 b
Disk	48.2 a	29.5 a	0.8 b	12.2 a	50.1 a
Undercutter	31.8 b	15.6 c	6.7 a	9.4 b	37.4 b

Figure 2.1. Grass (a) and total (b) weed shoot biomass (g m^{-2}) as influenced by the interaction of cover crop mixture and termination method at 23 DAP in 2010. Data shown are back-transformed LS means, which eliminated the possibility to present error terms. Instead, differences ($\alpha = 0.05$) among transformed LS means are indicated by different letters above back-transformed data points. NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 2.1).

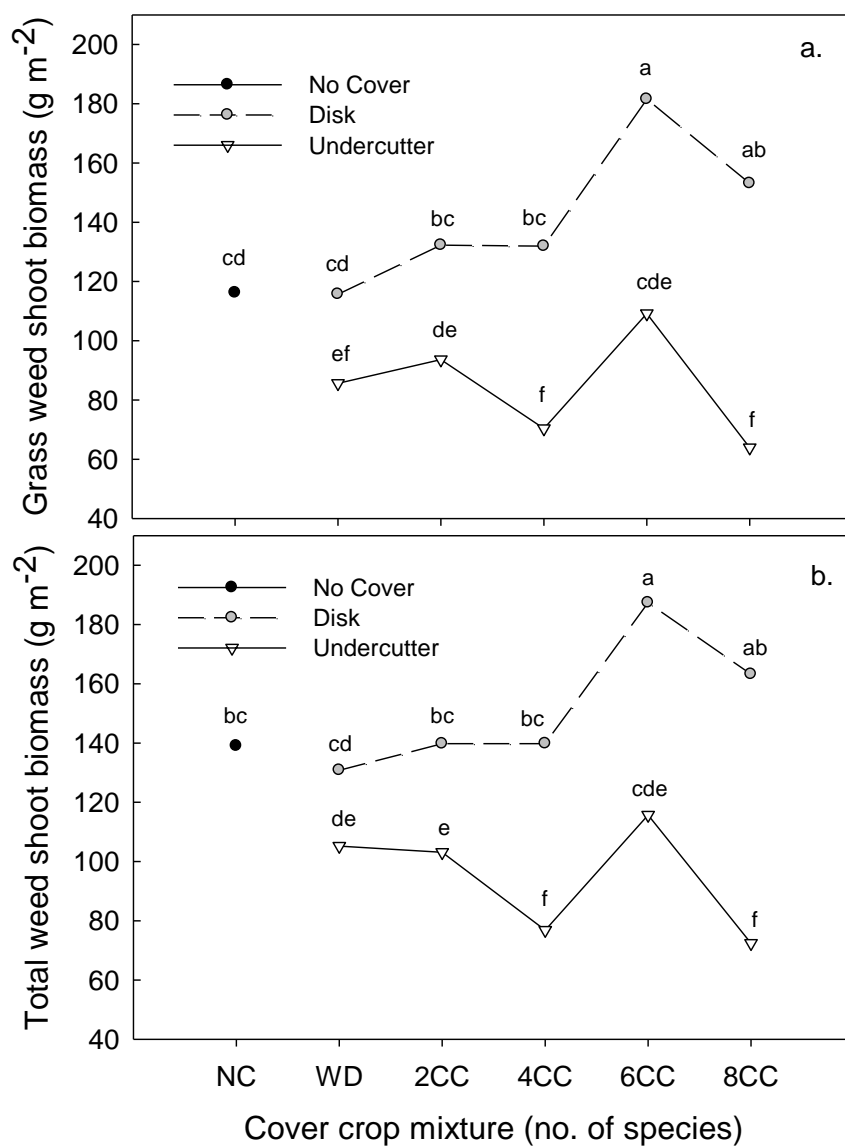


Figure 2.2. Total weed shoot biomass (g m^{-2}) as influenced by the interaction of cover crop mixture and termination method at 36 DAP in 2011. Data shown are back-transformed LS means, which eliminated the possibility to present error terms. Instead, differences ($\alpha = 0.05$) among transformed LS means are indicated by different letters above back-transformed data points. NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 2.1).

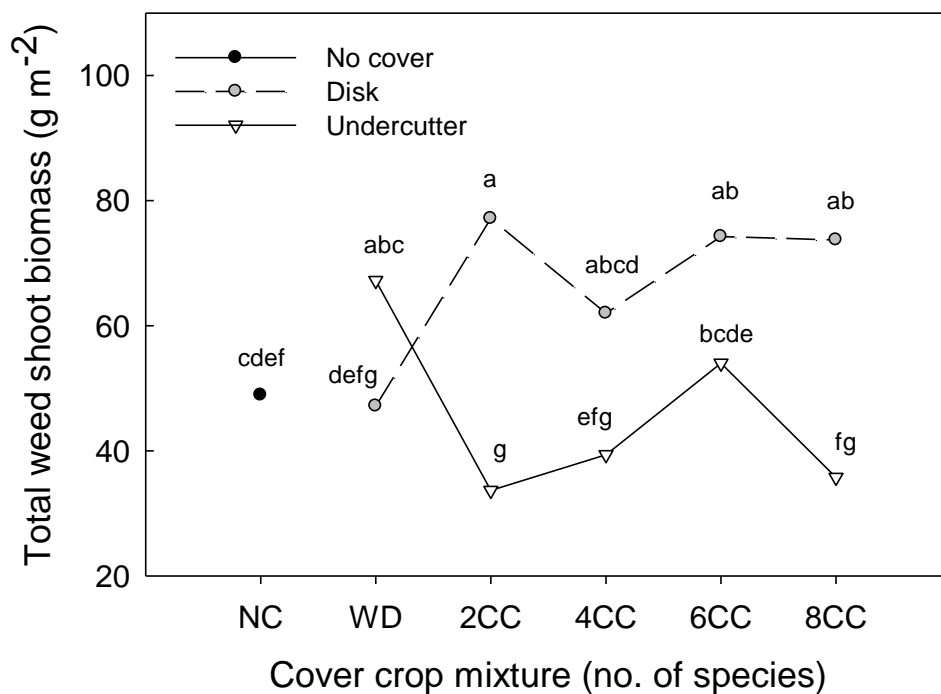


Figure 2.3. Total AMARE weed density (plants m⁻²) as influenced by the interaction of cover crop mixture, termination method, and current crop at 23 DAP in 2010. Data shown are LS means and standard errors with a generalized Poisson distribution; thus, the standard error varies with the mean for each treatment. NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 2.1).

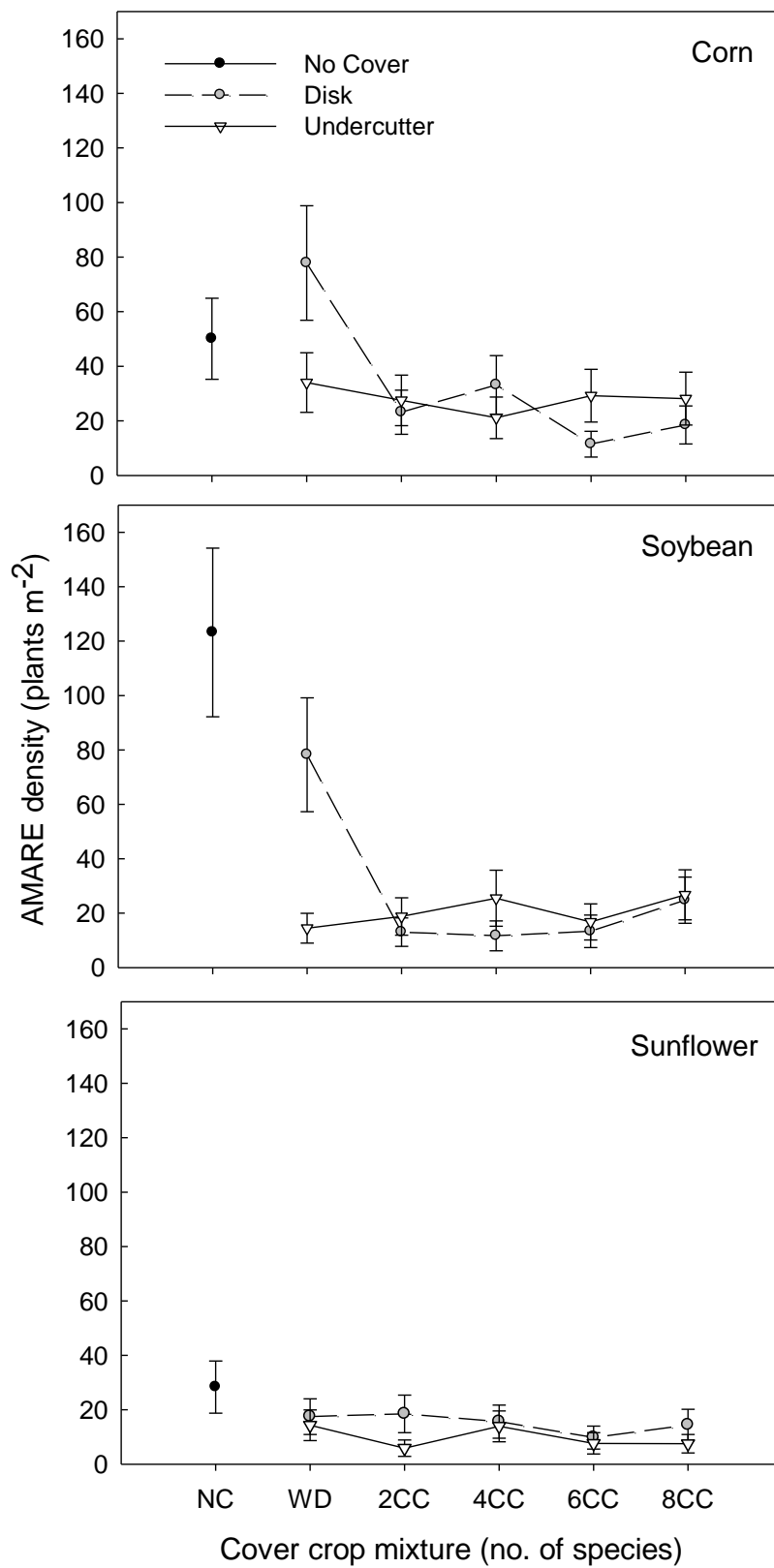


Figure 2.4. Total CHEAL weed density (plants m^{-2}) as influenced by the interaction of cover crop mixture and termination method at 36 DAP in 2011. Data shown are LS means and standard errors with a generalized Poisson distribution; thus, the standard error varies with the mean for each treatment. NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 2.1).

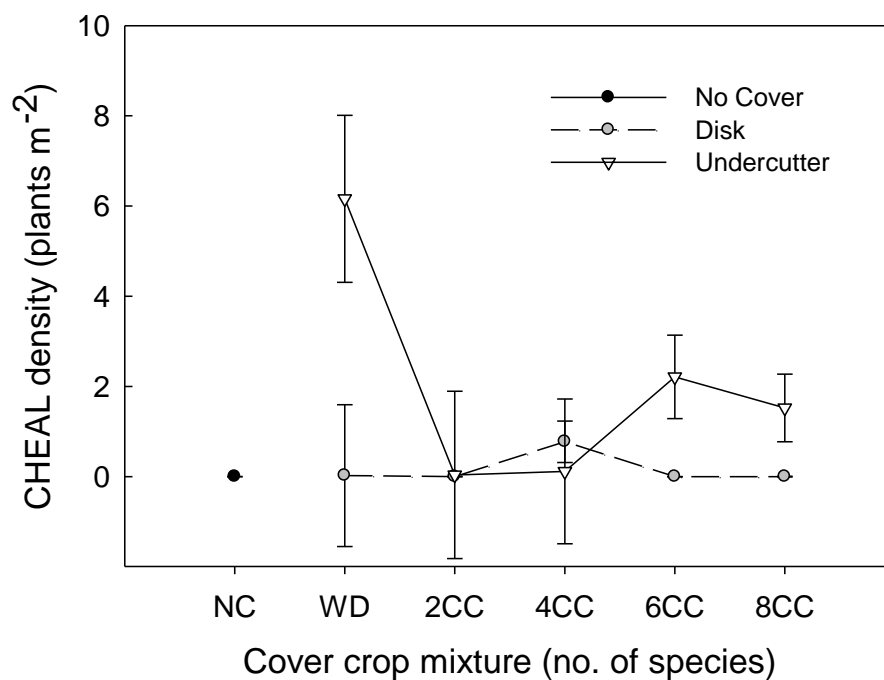


Figure 2.5. Broadleaf (a) and total (b) weed shoot biomass (g m^{-2}) as influenced by the interaction of cover crop mixture and termination method at 50 DAP in 2010. Data shown are back-transformed LS means, which eliminated the possibility to present error terms. Instead, differences ($\alpha = 0.05$) among transformed least square means are indicated by different letters above back-transformed data points. NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 2.1).

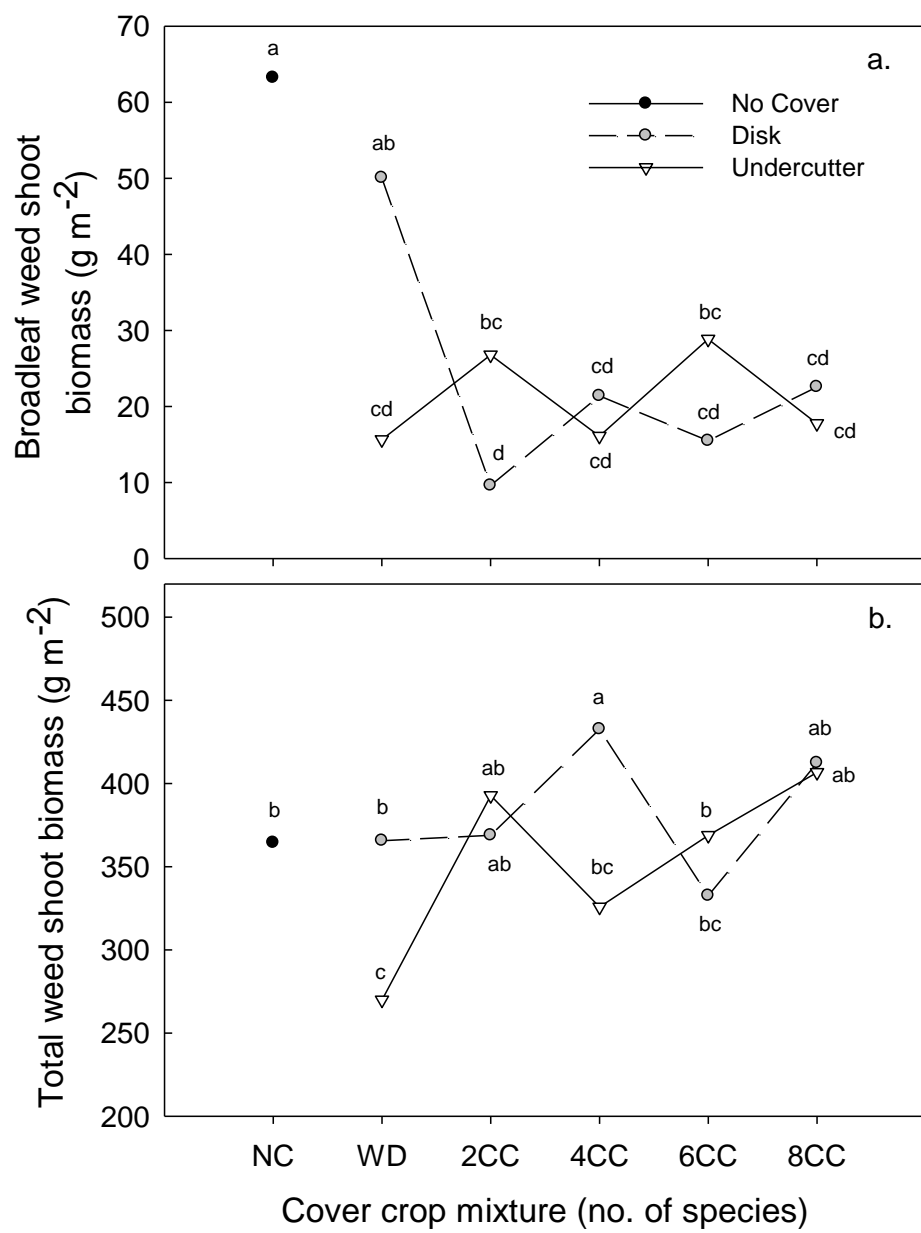
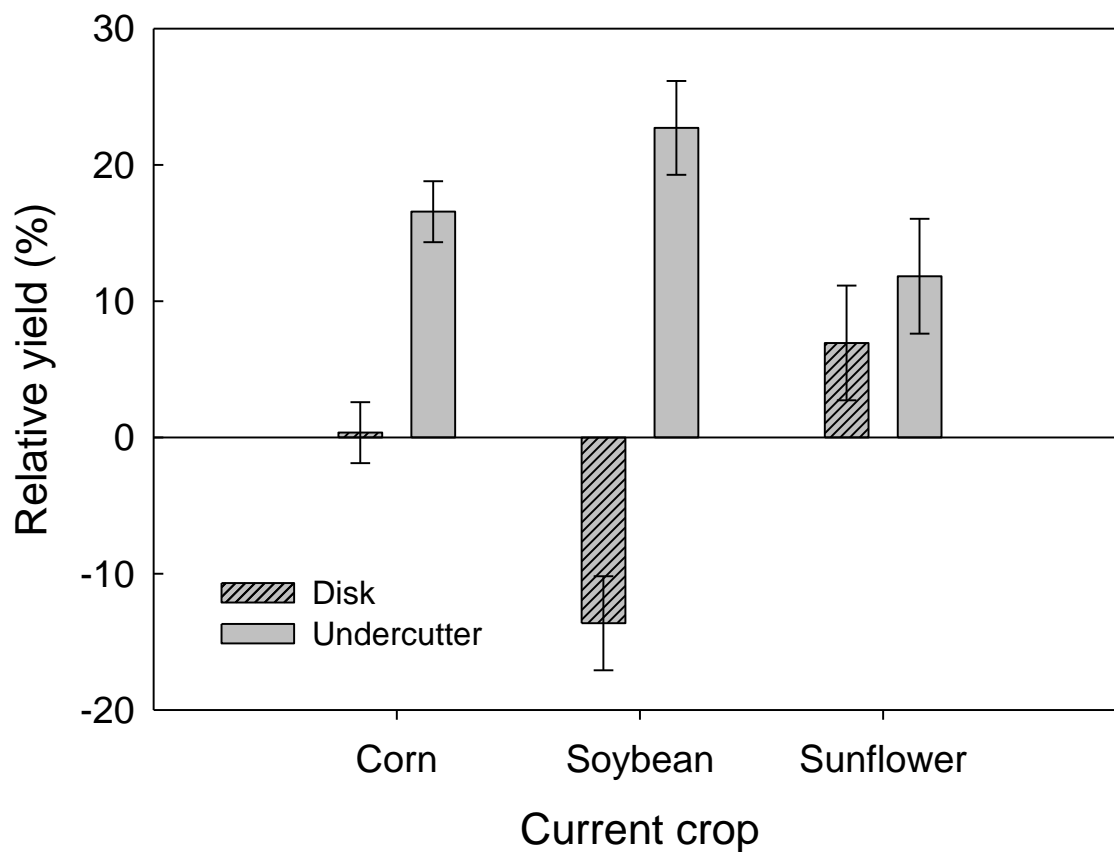


Figure 2.6. The effect of cover crop termination method (disk or undercutter) on crop yield relative to the no cover crop control treatment pooled across 2009, 2010, and 2011 for each crop. Error bars indicate \pm one standard error of the LS means.



Chapter 3

Relative influence of cover crop diversity, weed communities, and termination method on soil microbial community structure

Abstract

Many studies have demonstrated microbial community response to individual cover crop species, but the effect of increasing cover crop diversity has received less attention. Moreover, the relationship between agricultural weeds and soil microbial communities is not well understood. The objective of this study was to determine the relative influence of cover crop diversity, spring weed communities, and plant termination method on soil microbial community structure in an organic cropping system through the extraction of fatty acid methyl esters (FAMES). A field experiment was conducted in 2009 and 2010 near Mead, NE where spring-sown mixtures of zero (control), two, and eight cover crop species were included in a sunflower – soybean – corn crop rotation. A mixture of four weed species was planted in all experimental units (excluding the no-cover control), and also included as an individual treatment (e.g., weeds as a potential cover crop). Cover crops and weeds were planted in late-March, terminated in late-May using a field disk or sweep plow undercutter, and main crops were planted within one week of termination. Three (2009) or four (2010) soil cores were taken to a depth of 20 cm in all experimental units at 45 and 32 days following cover crop termination in 2009 and 2010, respectively. Total FAMES were greatest in the 2 species mixture – undercutter treatment combination ($140.8 \pm 3.9 \text{ nmol g}^{-1}$) followed by the 8 species mixture – undercutter treatment combination ($132.4 \pm 3.9 \text{ nmol g}^{-1}$). Five FAME

biomarkers (iC16:0, i10MeC17:0, i10MeC18:0, C16:1(*cis*11), C18:1(*cis*11)) were reduced in the weedy treatment relative to both cover-cropped treatments and the no-cover control. Termination with the undercutter reduced abundance of most actinomycete biomarkers while termination with the field disk reduced abundance of C18:1(*cis*11) and iC16:0. Canonical discriminant analysis of the microbial community successfully segregated most cover crop mixture by termination method treatment combinations. Segregation was most pronounced between the cover-cropped and weedy treatments, which was due in part to reduced abundance of the biomarkers C18:1(*cis*11) and i10MeC18:0 in the weedy treatment. While termination method did impact actinomycete abundance, microbial communities were most strongly influenced by the presence and type of early-spring plant communities (i.e., weeds vs. cover crops). Weeds may alter soil microbial community structure as a means of increasing competitive success and this relationship warrants further investigation.

Introduction

Soil microbial community composition is often responsive to a broad range of ecosystem and management factors. Knowledge of microbial community composition and diversity can provide valuable insight into soil function such as soil organic carbon and nitrogen retention, nutrient cycling and overall soil stability and health (van Bruggen and Semenov, 2000; Jackson et al., 2003). Several specific factors that may influence soil microbial community structure include soil type, plant community composition, climatic conditions, soil water availability, and soil management (Waldrop et al., 2000; Drenovsky et al., 2004; Cookson et al., 2008). In agricultural management systems both tillage and cover cropping are thought to influence microbial community structure, though these changes to the community are likely the result of complex interactions (Drijber et al., 2000; Buckley and Schmidt, 2001; Carrera et al., 2007). For example, one management decision (e.g., cover cropping) can substantially alter the subsequent weed community, labile soil carbon, and soil moisture; all of which may have unique impacts on microbial community structure (Buyer et al., 2010).

One management factor that consistently alters microbial community composition is the addition of organic carbon substrates, typical of organic cropping systems (Bossio et al., 1998). Microbial communities are often limited by organic carbon availability; thus, it is not surprising that the addition of labile organic matter (e.g., compost, manure, and plant residue) will result in changes to community structure (Drenovsky et al., 2004). In the short-term, organic management (e.g., cropping systems dependent on organic carbon substrates for soil fertility) selects for microbial species that have the highest growth rate and ability to absorb nutrients (Alden et al., 2001). Among other changes,

previous studies have reported increased abundance and diversity of bacteria and arbuscular mycorrhizal fungi (AMF), as well as greater physiological diversity of microbes in organically managed soils (Shannon et al., 2002; Oehl et al., 2003; van Diepeningen et al., 2006). Cover crops are a common source of labile organic carbon in organic cropping systems and have been shown to increase abundance of gram-negative bacteria, fungi and AMF, actinomycetes, and protozoa for several months following cover crop termination (Schutter et al., 2001; Carrera et al., 2007; Buyer et al., 2010). Moreover, the presence of cover crops has been identified as the primary factor affecting microbial community composition, despite differences in soil moisture and temperature (Buyer et al., 2010). In contrast, one recent study found that species of cover crop (rye vs. vetch) had little effect on community composition (Buyer et al., 2010); rather, the addition of any labile organic matter (e.g., cover crops or compost) will likely result in similar community changes (Drenovsky et al., 2004). However, differences in the biochemical composition of plant species and the subsequent organic compounds available to microbes may alter the composition of microbial communities (Zak et al., 2003).

Several recent studies have reported plant species-dependent changes in microbial communities of either the root rhizosphere or bulk soil (Germida et al., 1998; Kowalchuk et al., 2002; Zak et al., 2003; van Diepeningen et al., 2006). Individual plant species and communities have been shown to foster different levels of bacterivorous nematode species (van Diepeningen et al., 2006), bacterial diversity (Germida et al., 1998), microbial group abundance and overall community composition (Zak et al., 2003). Zak et al. (2003) found that increasing plant community diversity reduced the abundance of soil

bacteria and actinomycetes, and increased abundance of soil fungi, though the effects were confounded by differences in plant primary productivity (productivity increased with diversity). Nonetheless, these studies suggest that a diverse plant community and the individual species therein have the capacity to influence the composition of the soil microbial community.

Weed communities may also exert species-specific impacts on soil microbial community composition. Indeed, previous studies have demonstrated substantial effects of weed species (e.g., *Centaurea maculosa*) on soil microbial functional group abundance and community composition (Marler et al., 1999; Lutgen and Rillig, 2004; Batten et al., 2006). These changes in microbial community composition are often viewed as a novel competitive strategy and defense mechanism adapted by certain weedy and invasive species (Marler et al., 1999; Callaway and Ridenour, 2004). However, many of these observations have been limited to invasive weeds of unmanaged ecosystems and studies on the effects of agricultural weeds on soil microbial community composition are rare.

Soil tillage is another agricultural management factor that results in immediate and long-term changes to microbial community structure (Drijber et al., 2000; Jackson et al., 2003). In general, tillage shifts soil microbial communities toward aerobic species with high metabolic rates typical of bacteria species (Roper and Gupta, 1995). Indeed, several studies have shown that switching from a no-tillage system to a disk or plow management system reduces the ratio of fungi to bacteria (Frey et al., 1999; Pankhurst et al., 2002). Soil tillage has also been shown to reduce diversity of soil bacteria and abundance of microeukaryotes (Lupwayi et al., 1998; Jackson et al., 2003). While general soil disturbance often results in predictable changes to the microbial community, there is

some evidence that different tillage practices (e.g., disk, moldboard plow, chisel plow) will have variable effects on community structure, as one study demonstrated unique community differences between a moldboard plow and a sub plow undercutter tillage system (Drijber et al., 2000). With regard to cover crops, soil tillage associated with different plant termination methods may influence microbial community structure.

Typically, cover crops are used as green manures for increasing soil nitrogen, especially in organic cropping systems. To this end, soil incorporation of the cover crop with a field disk or moldboard plow is usually most effective. This management practice has been shown to increase abundance of total bacteria and gram-negative bacteria, while the abundance of actinomycetes and fungi either decrease or remain stable (Zelles et al., 1992; Lundquist et al., 1999; Drenovsky et al., 2004; Elfstrand et al., 2007). In contrast, utilizing cover crops for weed control may require that residue be mulched and left on the soil surface (Teasdale and Mohler, 1993). In general, residue placement on the soil surface leads to greater abundance of fungi and AMF compared to soil incorporation of residue (Doran, 1980; Holland and Coleman, 1987; Roper and Gupta, 1995; Elfstrand et al., 2007). In addition to weed suppressive benefits, maintenance of cover crop residue on the soil surface appears to create a favorable habitat for fungal growth characterized by greater soil moisture and limited soil disturbance (Elfstrand et al., 2007). Fungal species generally have a greater efficiency of carbon assimilation; thus, increasing the abundance of fungi may increase soil carbon storage in agricultural systems (Holland and Coleman, 1987).

The objectives of this study were to quantify changes in total microbial community structure and individual functional group abundance in response to increasing

cover crop diversity, spring weed communities, and different weed/cover crop residue termination methods. To accomplish these objectives, we used soil extractions of fatty acid methyl esters (FAMES) to quantify the relative abundance of soil microbial functional groups and changes in total community structure. We hypothesized that the combined effects of increasing cover crop diversity and the management of residue on the soil surface (via termination with a sweep plow undercutter) will result in a unique microbial community structure characterized by an increased abundance of fungal biomarkers.

Materials and Methods

Experimental Site and Design

A field experiment was conducted in 2009 and 2010 at the University of Nebraska-Lincoln Agricultural Research and Development Center (ARDC) near Mead, Nebraska. Dominant soil type at the site is a Sharpsburg silty clay loam (fine, smectitic, mesic typic Argiudoll) with 0 to 5% slopes. The experiment was conducted in a 2.8 ha field that is certified for organic production (OCIA International, Lincoln, NE), and is managed without irrigation. This field was in organic alfalfa hay production between the 2004 and 2008 cropping seasons. In the fall of 2008 the experimental area was amended with 50 Mg ha⁻¹ of liquid beef feedlot manure that was incorporated with a field disk.

The experiment was designed as a split-plot randomized complete block design within a 3-year crop rotation with 4 replications. The rotation sequence consisted of confectionery sunflower (*Helianthus annuus*) – soybean (*Glycine max*) – corn (*Zea mays*). Within each crop species, whole-plots (9.1 x 21.3 m; 12 crop rows spaced 0.76 m

apart) were defined by cover crop treatment, while split-plots (4.5 x 21.3 m; 6 crop rows spaced 0.76 m apart) were defined by plant termination method. Each “crop x cover crop mixture x termination method” treatment combination was replicated within each block so that each phase of the 3-year crop sequence was present each year within each block. There were four whole-plot cover crop treatments: 1) two-species cover crop mixture (2CC), 2) eight-species cover crop mixture (8CC), 3) weedy but no cover crop prior to main crop planting (WD), and 4) no cover crop and weed-free prior to main crop planting (NC control). The NC whole-plots were field disked and hand-hoed twice prior to main crop planting to remove emerged weed seedlings, while weeds in the WD whole-plots were left unmanaged until cover crop termination. Details on the individual species and seeding rates included in each cover crop treatment whole-plot are included in Table 3.1. On March 15, 2009, the 2CC, 8CC, and WD treatments were seeded with 8.1 kg ha⁻¹ of velvetleaf (*Abutilon theophrasti*) seed, 2.6 kg ha⁻¹ of common lambsquarters (*Chenopodium album*) seed, 1.2 kg ha⁻¹ of redroot pigweed (*Amaranthus retroflexus*) seed, and 3.7 kg ha⁻¹ of green foxtail (*Setaria viridis*) seed to establish a common weed seedbank for weed suppression data collection.

Split-plot cover crop residue management methods included either disking or undercutting. Management method was randomized within the first replication (southernmost) and duplicated in the remaining three replications (north of the first replication) to facilitate adequate speed for effective tillage operations driving north-south through the field. Disking was conducted with a 4.6 m wide Sunflower 3300 (Sunflower Mfg., Beloit, KS, USA) disk to an approximate depth of 15 cm. Undercutting was conducted with either a Buffalo 6000 (Buffalo Equipment, Columbus, NE, USA)

cultivator (modified for undercutting) with seven overlapping 0.75 m wide sweep blades (2009) or a Miller Flex-Blade sweep plow undercutter (2010 and 2011) with three overlapping 1.5 m sweep blades. The undercutter sweeps are designed to cut a level plane through the soil at an approximate depth of 10 cm, severing plant roots and minimizing soil inversion, resulting in a layer of intact surface residue. Details on the design of the undercutter can be found in Creamer et al. (1995).

Cover crop mixtures were planted via hand-crank broadcast seeding followed by light incorporation with a John Deere 950 cultipacker (Deere and Company, Moline, IL, USA). Generally, cover crops were planted in late-March, terminated in late-May, and main crops were planted within one week of termination. Specific dates for field operations across both years are detailed in Table 3.2. Seeding rates for confectionery sunflower, soybean, and corn were 62,000, 556,000, and 86,000 seeds ha⁻¹, respectively. All crops were inter-row cultivated once (2009) or twice (2010) approximately 30 days after planting the main crops. Seeds of all legume cover crop and crop species were inoculated with appropriate rhizobia bacterial species prior to planting in 2009 and 2010.

Soil Sampling

Soil samples were taken for fatty acid methylated esters (FAME) soil microbial analysis from 84 experimental units at 45 and 32 days after cover crop termination (DAT) in 2009 and 2010, respectively. These samples represented four whole-plot treatments, two split-plot treatments, three main crops, and four replications in each of two years. The NC control treatment did not include split-plots, as there were no plants to terminate and compare methods. This resulted in 168 composite samples for extraction and analysis.

Soil sampling was conducted in an aseptic manner whenever possible. To this end, nitrile gloves were worn during sampling and all supplies (soil probe, buckets, and gloves) were sprayed with 91% isopropyl alcohol between sampling each experimental unit. Three (2009) or four (2010) soil cores (3.2 cm diameter by 20 cm depth) were taken within crop rows in each experimental unit. Cores were sampled from undisturbed soil within crop rows to avoid the effects of inter-row cultivation that occurred prior to sampling. Cores from each experimental unit were pooled in a plastic bucket and mixed by hand to break up large aggregates and create a homogenous mixture of soil profiles. A subsample of approximately 300 grams was then placed in a plastic freezer bag, sealed and placed in an iced cooler for no more than 2 hours. When soil sampling was complete, subsamples were stored in a refrigerator at 2° C for less than 24 hours until processing.

Soil samples were then sieved with a 0.47 cm sieve to remove large organic residues. Similar to the sampling process, sieving was conducted aseptically by wearing nitrile gloves, and spraying all equipment (gloves and sieves) with 91% isopropyl alcohol between each sample. After sieving, 100 g of soil was weighed and placed back in each plastic freezer bag. Sieved samples were then stored at -20° C until the time of FAME extraction (approximately 6 months after sampling).

FAME Extraction

Microbial community composition was determined from fatty acid methyl esters (FAMEs). The method, adapted from White et al. (1979), results in a direct hydrolysis, derivatization, and extraction of FAMEs from soil microorganisms *in situ*. First, 10 g of each frozen soil sample was weighed and placed in a 50 ml Teflon centrifuge tube. Twenty ml of MeOH-KOH was then added to each Teflon tube in 10 ml increments, and

vortexed thoroughly after each addition. Samples were then placed in a 37° C water bath for one hour, and mixed every 15 minutes during this hour. Upon removal from the water bath, 2 ml of acetic acid were added to each sample to restore solution neutrality. Next, 5 ml of hexane was added to each sample. Samples were thoroughly vortexed and then centrifuged for 10 minutes at 6000 rpm. The resulting hexane layer (with extracted FAMEs in solution) was transferred via pipette to a 15 ml Pyrex tube. The extraction procedure (hexane addition, centrifugation, and transfer) was repeated one time, and each sample was then filtered through a PTFE 0.2 µm syringe filter into a Pyrex tube. The filtered solvent was then evaporated under N₂ gas to a small volume. Several drops of benzene were added to each sample, mixed, and again evaporated under N₂ gas until visibly dry. The remaining sample was then redissolved in 1 ml of hexane and transferred to a 2 ml vial. Samples were then stored at -20° C until preparation for gas chromatography (GC) analysis. In preparation for GC analysis, hexane in each sample was evaporated under N₂ gas until completely dry and then 500 µl of hexane with C19:0 (0.05 mg/ml; as an internal standard) was added to each vial. A 50 µl aliquot of each sample was then transferred to the GC vial and capped for analysis.

Individual FAMEs were separated by capillary gas chromatography on a Hewlett Packard 5890 Series II gas chromatograph (Hewlett-Packard Company, Palo Alto, CA) with helium as the carrier gas. Oven temperature in the GC was held at 100° C for 1 min and then increased at 2.5° C min⁻¹ to a final temperature of 225° C. Injector and flame ionization detector temperatures were 250° C and 280° C, respectively. Determination of FAME identity was accomplished through a comparison of retention times and equivalent chain lengths with known standards (Bacterial Acid Methyl Esters CP Mix,

Supelco USA). FAME identities were then confirmed by gas chromatography mass spectrometry (GC-MS). FAMES were represented and written as the total number of carbon atoms followed by a colon, the number of double bonds followed by the position of those double bonds from the carboxyl end of the molecule, and its *cis* or *trans* configuration in brackets (e.g., C16:1(*cis*11)).

Data Analysis

Consistent with previous studies, individual FAMES were reported as a ratio (% nmol) of total FAMES (Petersen et al., 1997; Reichardt et al., 1997). FAMES with retention times less than C14:0 and greater than C20:0 were deleted from the data matrix. Remaining FAME ratios or quantities in the data set were first analyzed by analysis of variance (Proc MIXED; SAS 9.2, SAS Institute Inc., Cary, NC, USA) to determine differences in total FAMES and individual biomarkers among cover crop mixture, termination, and main crop treatments.

Stepwise discriminant analysis and canonical discriminant analysis (Proc STEPDISC and Proc CANDISC; SAS 9.2) were then performed to characterize changes in overall soil microbial community structure in response to cover crop mixture and termination method treatment combinations. Stepwise discriminant analysis was used to identify individual FAMES contributing most to treatment segregation. The resulting discriminant model was then subjected to a canonical discriminant analysis. Mahalanobis distances and the associated probabilities of significance (*p*-values) were used to detect differences among treatment combinations. The number of significant ($p < 0.05$) canonical discriminant functions (linear combinations of important FAME markers – those identified in stepwise discriminant analysis) determined the number of dimensions

used to segregate among treatment groups. The first canonical discriminant function always explains the most variation among treatment groups, followed by the second function, and so on. Canonical coefficients were used to determine the relative magnitude and directional relationship of FAME variables contributing to the canonical discriminant functions. Discriminant scores were then calculated for each experimental unit across both years ($N = 168$) with each significant discriminant function. Class means for all discriminant scores within treatment combinations were plotted along with the canonical coefficients for FAMEs in the significant discriminant functions.

To aid in the visualization of statistical interactions, FAME abundance and ratios were often plotted as lines with cover crop mixture on the x-axis (Sosnoskie, 2006). The cover crop treatments were arranged in order (left-to-right) of increasing species diversity (from zero in the WD treatment to eight species in the 8CC treatment) along the x-axis similar to the figures presented by Tilman et al. (2001). However, we recognize that these data are not truly continuous as is traditionally expected in line plots.

Results and Discussion

Total FAMEs

While total extracted FAMEs is not a direct measure of microbial biomass, this method has been well correlated with more traditional measures of biomass (Zelles et al., 1992). Total FAMEs were greatest in the 2CC – undercutter treatment combination ($140.8 \pm 3.9 \text{ nmol g}^{-1}$) followed by the 8CC– undercutter treatment combination ($132.4 \pm 3.9 \text{ nmol g}^{-1}$; Figure 3.1). However, there was no difference in total FAMEs among the remaining treatment combinations. In contrast to our hypothesis, increasing carbon inputs

(in the form of cover crop and weed residue) did not consistently increase short-term total FAMEs. However, cover crop termination with the undercutter generally increased total FAMEs. This suggests that tillage with the undercutter resulted in a more favorable microbial habitat early in the growing season. In previous studies, incorporation of plant residue via disking or plowing typically increased bacterial abundance and reduced the ratio of fungi to bacteria (Zelles et al., 1992; Lundquist et al., 1999; Pankhurst et al., 2002; Drenovsky et al., 2004; Elfstrand et al., 2007). However, the results of this study suggest that disk incorporation will reduce total FAMEs regardless of functional group, relative to cover crop termination and surface residue management with a conservation tillage implement like the undercutter. These results may indicate a general reduction in microbial abundance and biomass as tillage intensity increases. This is consistent with previous studies where microbial biomass was greater in surface soils of no-till treatments relative to plowed treatments in a long-term wheat-fallow cropping system (Doran et al., 1987; Drijber et al., 2000).

Individual FAME Ratios

Five FAME biomarkers (iC16:0, i10MeC17:0, i10MeC18:0, C16:1(*cis*11), C18:1(*cis*11)) were influenced by the effect of cover crop treatment. More specifically, abundance of these biomarkers was reduced in response to the WD treatment (unmanaged spring weed communities; Table 3.3). Despite the relationship between plant and microbial communities, there was no difference in individual FAME abundance between the 2CC and 8CC treatments. The effects of increasing aboveground plant diversity on soil microbial diversity and community composition are often subtle and only detected within the root rhizosphere (Kowalchuk et al., 2002). Moreover, individual

FAME abundance was typically not different between cover-cropped treatments (2CC and 8CC) and the NC control (Table 3.3). These results suggest that early-season weed communities (primarily *Chenopodium album*, *Abutilon theophrasti*, *Amaranthus retroflexus*, *Thlaspi arvense*, and *Setaria viridis* in this study) were altering microbial communities by reducing the abundance of several functional groups relative to soil with and without cover crop growth.

The unique influence of weedy and invasive plant species on soil microbial community composition and specific functional groups has been observed previously (Batten et al., 2006). However, the influence of weedy and invasive plants on microbial community composition is not always consistent. Previous studies have found that weedy species (i.e., *Centaurea maculosa*, *Centaurea solstitialis*, and *Aegilops triuncialis*) can alter microbial community composition and increase the abundance of beneficial microbial groups (i.e., AMF species; Marler et al., 1999; Batten et al., 2006). In addition, these changes in microbial community structure have been shown to increase the competitive advantage of the weedy species relative to native competitors (Marler et al., 1999). In contrast, others have reported that *C. maculosa* reduces the abundance and diversity of AMF (Lutgen and Rillig, 2004; Mummey and Rillig, 2006), which is more consistent with the results of this study. The reduction of C16:1(*cis*11) and C18:1(*cis*11) following spring weed growth in this study is especially relevant, as these markers have been cited as FAME biomarkers for AMF (Olsson et al., 1995; Olsson et al., 1999; van Aarle and Olsson, 2003). Mycorrhizal fungi can form mutualistic relationships with many crop species, improving nutrient uptake and subsequent crop yield (Mosse, 1973); thus, it

would seem reduction of AMF abundance could be an effective competitive strategy for weeds.

While invasive plant species (primarily *C. maculosa*) have been extensively studied for effects on soil microbial communities, there have been relatively few studies examining the role of arable system weeds on soil microbial community structure in agroecosystems. Soil microbes have been viewed as a potential weed management tool (e.g., seedbank depletion and plant pathogenic fungi; Schaefer and Kotanen, 2003; Okalebo et al., 2011), but the influence of unmanaged weed communities on soil microbial community dynamics represents a new frontier in weed and soil ecology. Indeed, changes in the soil microbial community may influence competitive outcomes between weed and crop species (Marler et al., 1999); thus, these interactions warrant further investigation.

Plant termination with the undercutter reduced abundance of four actinomycete biomarkers (8MeC16:0, i10MeC17:0, i10MeC18:0, a10MeC18:0) but increased abundance of the actinomycete marker 10MeC18:0 relative to termination with the field disk (Table 3.4). This result contradicts previous findings, where actinomycete abundance was typically unaffected or reduced following cover crop termination with a field disk or plow (Zelles et al., 1992; Lundquist et al., 1999; Drenovsky et al., 2004; Elfstrand et al., 2007). It was hypothesized that termination with the undercutter would be a less intensive termination strategy and more closely mimic soil community response to no-till management observed in previous studies. Termination with the field disk did reduce abundance of the fungal marker C18:1(*cis*11) and the bacterial marker iC16:0 relative to termination with the undercutter (Table 3.4). Reduced abundance of the

C18:1(*cis*11) fungal marker highlights the potentially negative effects of inversion tillage (e.g., field disking) on soil fungi. C18:1(*cis*11) is part of the neutral lipid fraction in arbuscular mycorrhizal fungi (AMF), which is essential to AMF metabolism (Graham et al., 1995). These lipids are thought to be the substrate for extraradical mycelium respiration (Bago et al., 2002). The majority of AMF biomass in the soil exists as extraradical mycelium, so it has been hypothesized that C18:1(*cis*11) originates primarily from AMF biomass (Olsson et al., 1999; van Aarle and Olsson, 2003). The reduction in C18:1(*cis*11) following disk incorporation (relative to undercutting and the NC control) observed here is congruent with many previous studies demonstrating that residue placement on the soil surface leads to greater abundance of fungi and AMF compared to full soil incorporation of residue (Doran, 1980; Holland and Coleman, 1987; Roper and Gupta, 1995; Elfstrand et al., 2007). Reduced fungal abundance in the disk treatment may be due to the complex interaction of factors associated with the soil habitat, including reduced soil moisture and increased soil disturbance (Elfstrand et al., 2007).

Individual FAMES were less influenced by the main crop, but the presence of sunflower reduced the abundance of bacterial biomarkers iC15:0 and aC15:0 relative to soil sampled in the corn crop (Table 3.5). In contrast, sunflower promoted the abundance of the C18:2 (*cis*9,12) fungal biomarker ($6.32 \pm 0.18\%$) relative to soil sampled in the corn ($5.74 \pm 0.18\%$) or soybean ($5.77 \pm 0.18\%$) crops (Table 3.5). The relatively weak influence of individual plant species on soil microbial community composition is consistent with previous studies (Buyer et al., 1999; Kielak et al., 2008). However, changes in soil microbial community composition may be more pronounced as the crop community matures throughout the growing season. Indeed, current crop can be a strong

driver of microbial community composition, often masking alternative influences like tillage (Drijber et al., 2000).

The FAME C16:1(*cis*11) is commonly cited as a biomarker for AMF (Olsson et al., 1995; Drijber et al., 2000), and was influenced by the three way interaction of cover crop treatment, termination method, and year ($F = 13.11$, $df_n = 2$, $df_d = 122$, $p < 0.0001$). Abundance of C16:1(*cis*11) in the 8CC – undercutter treatment combination (3.53 ± 0.28 nmol g⁻¹) was greater than the 8CC – disk treatment (2.77 ± 0.28 nmol g⁻¹) and both the WD – disk and WD – undercutter treatment combinations (2.93 ± 0.28 nmol g⁻¹ and 2.71 ± 0.28 nmol g⁻¹, respectively) in 2009 (Figure 3.2). C16:1(*cis*11) abundance was also elevated in the 2CC – disk and 2CC – undercutter treatment combinations (3.56 ± 0.28 nmol g⁻¹ and 3.61 ± 0.28 nmol g⁻¹, respectively), but none of the treatment combinations in 2009 was different from the NC control (3.15 ± 0.28 nmol g⁻¹).

Generally, C16:1(*cis*11) abundance was greater in cover-cropped treatments compared to both WD treatments and the NC control in 2010 (Figure 3.2). The response to termination method was inconsistent across cover crop treatments as the undercutter increased C16:1(*cis*11) abundance in the 2CC cover crop mixture (4.18 ± 0.28 nmol g⁻¹) but reduced abundance in the 8CC cover crop mixture (3.54 ± 0.28 nmol g⁻¹) relative to termination with the disk. We hypothesized that cover crop termination with the disk would reduce AMF abundance as intensive tillage has been shown to reduce ratios of fungi to bacteria and AMF hyphal length and abundance (Frey et al., 1999; Drijber et al., 2000; Pankhurst et al., 2002). While the effect of termination method was inconsistent, the presence of cover crops often led to increased abundance of C16:1(*cis*11). This result is consistent with Drijber et al. (2000) who found that abundance of C16:1(*cis*11)

decreased in the absence of carbon substrates (fallow period). However, it is unique that the type of plant residue (weeds vs. cover crops) affected the abundance of C16:1(*cis*11). Despite the addition of fresh carbon substrates in the WD treatments, C16:1(*cis*11) was often lower (though not always significantly so) than levels in the cover-cropped treatments (Figure 3.2). This reduction in AMF abundance following growth of weedy species is consistent with previous studies (Lutgen and Rillig, 2004; Mummey and Rillig, 2006). However, information on the effects of agricultural weeds remains scarce.

Microbial Community Composition

Of the 42 FAMES identified among all soil samples, 9 were included in the discriminant function after stepwise discriminant analysis. Canonical discriminant analysis then identified two significant discriminant functions (DA1 and DA2; $p < 0.05$), which explained 65.2 and 14.3% of the variance, respectively, for a total explained variance of 79.5%. The p -values associated with pairwise squared Mahalanobis distances indicated that a majority of cover crop – termination method treatment groups segregate from one another when using a rejection level of $\alpha = 0.10$ (Table 3.6). However, when using the more traditional $\alpha = 0.05$ rejection level, segregation among treatments required broader classifications. The most obvious segregation occurred between the WD treatments (both disk and undercutter termination methods) and all other treatment groups. This finding is consistent with univariate analyses indicating that the WD treatments reduced five FAME ratios relative to both cover-cropped treatments and the NC control (Table 3.3).

Termination method was effective in treatment segregation within the 2CC mixture, but not within the 8CC or WD treatments. Cover-cropped treatments only

segregated from the NC control when combined with the undercutter for termination. In contrast to our hypothesis, there were relatively minor differences in microbial community composition among the 2CC and 8CC treatments. Instead, the early-season weed communities were driving the most substantial changes in microbial community composition (Table 3.6; Figure 3.3a). Indeed, the effect of increasing plant diversity on soil microbial community composition is often limited to the soil rhizosphere or is not detectable (Kowalchuk et al., 2002; Kielak et al., 2008). Few studies have addressed the role of increasing plant diversity on soil microbial diversity and community composition (Waldrop et al., 2006), but the results of this study in combination with others would suggest that the proposed relationship is relatively weak.

FAME marker ratios positively correlated to DA1 (as indicated by positive canonical coefficients) included i10MeC18:0, C18:1(*cis*11), C16:1(*cis*5), C17:1(*cis*9), and C20:n (in order of highest to lowest canonical coefficients; Figure 3.3b). FAME ratios for these biomarkers were generally greatest in cover-cropped treatment groups and lowest in the WD treatments. The i10MeC18:0 marker has been cited as a FAME biomarker for actinomycetes while C17:1(*cis*9) has been cited as a biomarker for bacteria (Wortmann et al., 2008). Increased ratios of actinomycetes and fungi (C18:1(*cis*11)) in the cover-cropped soils are congruent with previous studies (Schutter et al., 2001; Carrera et al., 2007; Buyer et al., 2010). In contrast, negative canonical coefficients were found for a10MeC18:0, C18:0, C18:1(*cis*13), and cyC19(9,10) (in order of most to least negative canonical coefficients; Figure 3.3b). However, these are less common FAME biomarkers that are not typically associated with major soil microbial function groups.

DA2 was positively correlated with FAME ratios for C17:1(*cis*9), C20:n, cyC19(9,10), and C18:1(*cis*13), and negatively correlated with FAME ratios for C18:1(*cis*11), i10MeC18:0, and C18:0 (Figure 3.3b). The largest segregation among treatment groups by DA2 was between the NC control treatment and the undercutter treatments (Figure 3.3a). This segregation suggests that ratios of C17:1(*cis*9) (a bacterial biomarker), C20:n, cyC19(9,10), and C18:1(*cis*13) were greater in treatments with plant residue managed on the soil surface (cover crops or weeds), whereas ratios of C18:1(*cis*11) (a fungal biomarker), i10MeC18:0 (an actinomycete marker), and C18:0 were greatest in the NC treatment without any plant growth or subsequent residue cover. The negative relationship between the cover crop – undercutter treatment combinations and i10MeC18:0 is consistent with univariate analyses (Table 3.4) and suggests that the soil environment following an undercutting operation is not conducive to actinomycete growth. Previous studies have found that intensive tillage and full soil incorporation of cover crop residue reduces actinomycete abundance (Zelles et al., 1992; Lundquist et al., 1999; Drenovsky et al., 2004; Elfstrand et al., 2007), but this is the first evidence that the sweep plow undercutter for cover crop termination negatively affects actinomycetes. Another unexpected result was the negative relationship observed between C18:1(*cis*11) and the undercutter – cover crop treatment combinations in the second discriminant function. This was not consistent with univariate analysis (Table 3.4) and may not represent a predictable shift in microbial community composition given that DA2 explained only 14.3% of the total variation in the data.

Conclusions

While the results for individual FAMEs and overall community composition were sometimes inconsistent with previous studies, it is clear from this work that the type of residue (cover crops vs. weeds) and the method of plant termination and residue management resulted in unique changes to microbial community structure. While tillage is often a strong driver of soil microbial community structure in managed ecosystems (Drijber et al., 2000), the results of this study highlight the unique influence of weed communities on specific soil microbial function groups and community structure as a whole. Previous studies have found that plant species, community composition, and diversity are relatively weak drivers of microbial community composition (Kielak et al., 2008), but these results demonstrate the potential influence of plants when comparing different plants classifications (weedy species vs. cultivated crops). Future studies should be directed toward understanding the prominent role of agricultural weed communities in driving microbial community composition and also toward determining the functions of these unique communities and functional groups (Torsvik et al., 2002).

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Tables and Figures

Table 3.1. Cover crop species and seeding rates (kg ha^{-1}) used in individual cover crop mixtures for the years 2009 and 2010 (2CC = 2 species mixture; 8CC = 8 species mixture).

Common name	Scientific name	Seeding rate	
		2CC	8CC
		– kg ha^{-1} –	
Hairy Vetch	<i>Vicia villosa</i>	22.4	5.6
Buckwheat (2009)	<i>Fagopyrum sagittatum</i>	28.0	7.0
Idagold Mustard (2010)	<i>Sinapis alba</i>	6.7	1.7
Field Pea	<i>Pisum sativum</i>		14.0
Pacific Gold Mustard	<i>Brassica juncea</i>		1.1
Oilseed Radish	<i>Raphanus sativus</i>		2.1
Crimson Clover	<i>Trifolium incarnatum</i>		3.5
Dwarf Essex Rape	<i>Brassica napus</i>		1.7
Chickling Vetch	<i>Lathyrus sativus</i>		8.4

Table 3.2. Timing of field operations and data collection for each year of the study.

	Date	
	2009	2010
Operation		
Cover crop planting	20 March	30 March
Cover crop termination	22 May	28 May
Main crop planting	28 May	1-3 June
1st inter-row cultivation	1 July	28 June
2nd inter-row cultivation		1 July
Soil sampling	6-7 July	29-30 June

Table 3.3. LS means and standard errors of ratios of FAME peak area to total FAMES (nmol %) as influenced by cover crop mixture.

	Cover crop mixture			
	NC	WD	2CC	8CC
<i>Bacteria</i>				
iC16:0	4.53 (0.08)	4.38 (0.06)	4.56 (0.06)	4.55 (0.06)
<i>Actinomycetes</i>				
i10MeC17:0	1.31 (0.08)	1.15 (0.06)	1.36 (0.06)	1.28 (0.06)
i10MeC18:0	3.65 (0.28)	3.13 (0.20)	3.79 (0.20)	3.60 (0.20)
<i>AMF</i>				
C16:1 (<i>cis</i> 11)	2.59 (0.13)	2.41 (0.10)	2.85 (0.10)	2.69 (0.10)
C18:1 (<i>cis</i> 11)	4.70 (0.10)	4.42 (0.07)	4.74 (0.07)	4.77 (0.07)

Table 3.4. LS means and standard errors of ratios of FAME peak area to total FAMES (nmol %) as influenced by cover crop termination method.

	Cover crop termination method		
	No cover	Disk	Undercutter
<i>Bacteria</i>			
C17:0	0.736 (0.011)	0.718 (0.006)	0.739 (0.006)
<i>Actinomycetes</i>			
8MeC16:0	1.85 (0.08)	1.88 (0.04)	1.71 (0.04)
i10MeC17:0	1.31 (0.08)	1.33 (0.05)	1.20 (0.05)
i10MeC18:0	3.65 (0.28)	3.71 (0.16)	3.30 (0.16)
a10MeC18:0	0.453 (0.020)	0.467 (0.012)	0.441 (0.012)
10MeC18:0	1.438 (0.028)	1.430 (0.016)	1.468 (0.016)
<i>AMF</i>			
C18:1 (<i>cis</i> 11)	4.70 (0.10)	4.56 (0.06)	4.72 (0.06)

Table 3.5. LS means and standard errors of ratios of FAME peak area to total FAMES (nmol %) as influenced by current crop.

	Current crop		
	Corn	Soybean	Sunflower
<i>Bacteria</i>			
iC15:0	5.46 (0.06)	5.37 (0.06)	5.30 (0.06)
aC15:0	3.71 (0.06)	3.69 (0.06)	3.58 (0.06)
<i>Fungi</i>			
C18:2 (<i>cis</i> 9,12)	5.74 (0.18)	5.77 (0.18)	6.32 (0.18)

Table 3.6. Pairwise squared Mahalanobis distance for FAMEs between cover crop mixture by termination method treatment groups pooled across main crops and years.

Treatment	2CC/D	2CC/U	8CC/D	8CC/U	WD/D	WD/U	NC
	Squared Mahalanobis distance/(probability>Mahalanobis distance)						
2CC/D ^a	0.000 (1.000)	2.186 (0.005)	1.492 (0.057)	1.939 (0.012)	1.774 (0.022)	2.328 (0.004)	1.385 (0.082)
2CC/U	2.186 (0.005)	0.000 (1.000)	1.525 (0.051)	0.696 (0.543)	4.270 (0.0001)	5.135 (0.0001)	2.618 (0.001)
8CC/D	1.492 (0.057)	1.525 (0.051)	0.000 (1.000)	0.483 (0.786)	3.171 (0.000)	4.264 (0.0001)	1.377 (0.084)
8CC/U	1.939 (0.012)	0.696 (0.543)	0.483 (0.786)	0.000 (1.000)	3.700 (0.0001)	4.917 (0.0001)	2.265 (0.004)
WD/D	1.774 (0.022)	4.270 (0.0001)	3.171 (0.0001)	3.700 (0.0001)	0.000 (1.000)	0.408 (0.869)	1.903 (0.014)
WD/U	2.328 (0.004)	5.135 (0.0001)	4.264 (0.0001)	4.917 (0.0001)	0.408 (0.869)	0.000 (1.000)	2.482 (0.002)
NC	1.385 (0.082)	2.618 (0.001)	1.377 (0.084)	2.265 (0.004)	1.903 (0.014)	2.482 (0.002)	0.000 (1.000)

^a 2CC/D = 2 species mix + disk; 2CC/U = 2 species mix + undercutter; 8CC/D = 8 species mix + disk; 8CC/U = 8 species mix + undercutter; WD/D = weedy control + disk; WD/U = weedy control + undercutter; NC = no cover crop (or weeds) control.

Figure 3.1. Effects of cover crop mixture and termination method on total FAMES (nmol g⁻¹) at 45 and 32 days after cover crop termination in 2009 and 2010, respectively. Error bars represent the standard error of the LS means. NC = no cover control; WD = weedy mixture; 2- and 8CC = 2 and 8 cover crop species mixtures, respectively (Table 3.1).

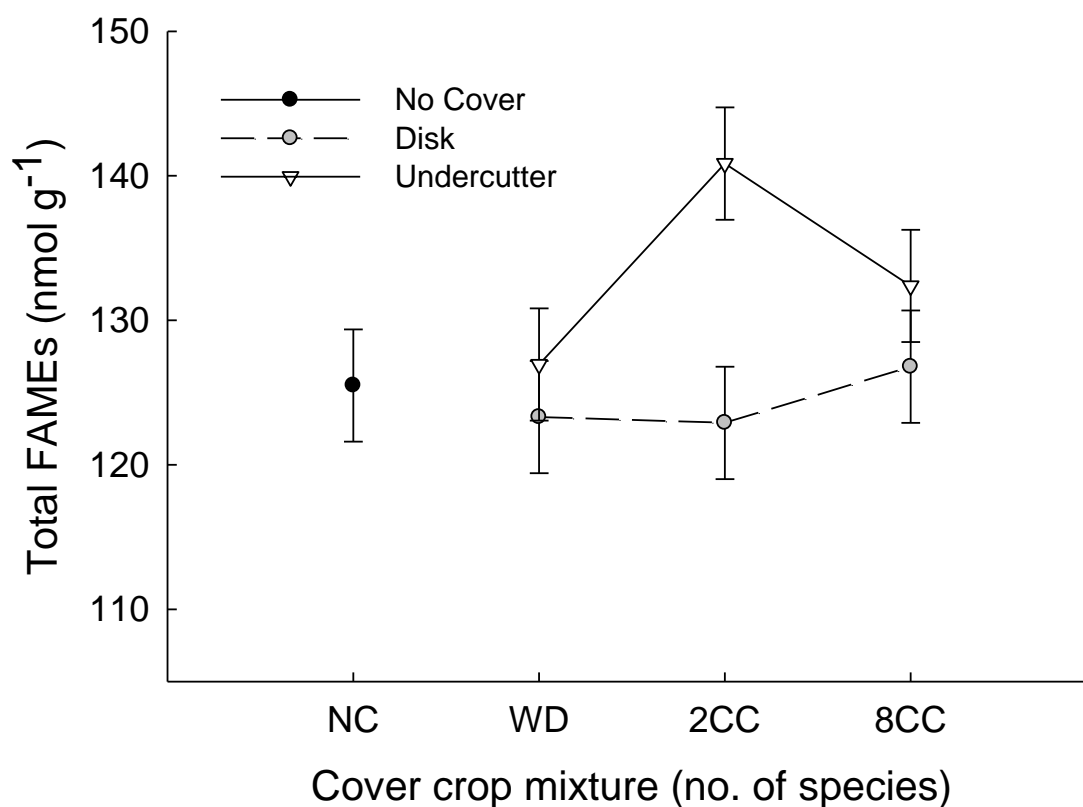


Figure 3.2. Effects of cover crop mixture and termination method on the AMF biomarker C16:1(*cis*11) (nmol g⁻¹) at 45 and 32 days after cover crop termination in 2009 and 2010, respectively. Error bars represent the standard error of the LS means. NC = no cover control; WD = weedy mixture; 2- and 8CC = 2 and 8 cover crop species mixtures, respectively (Table 3.1).

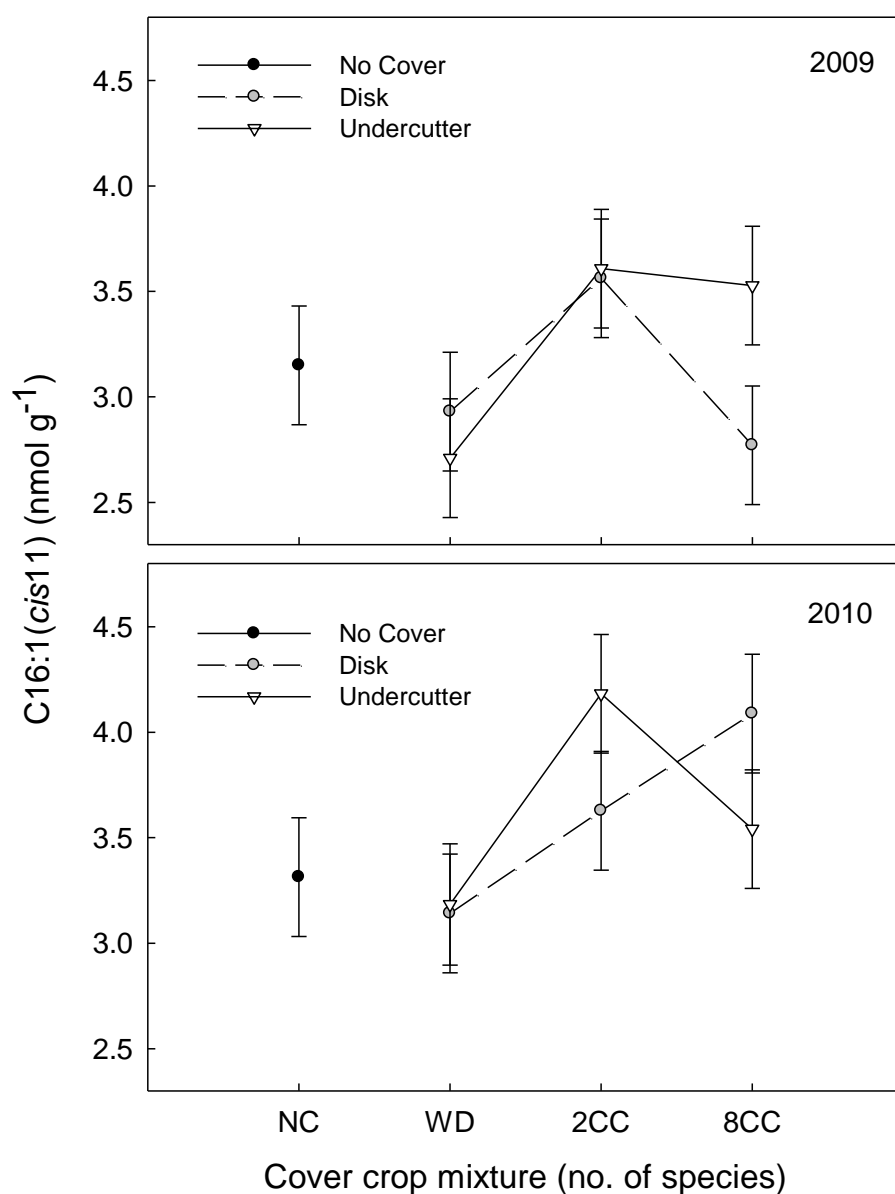
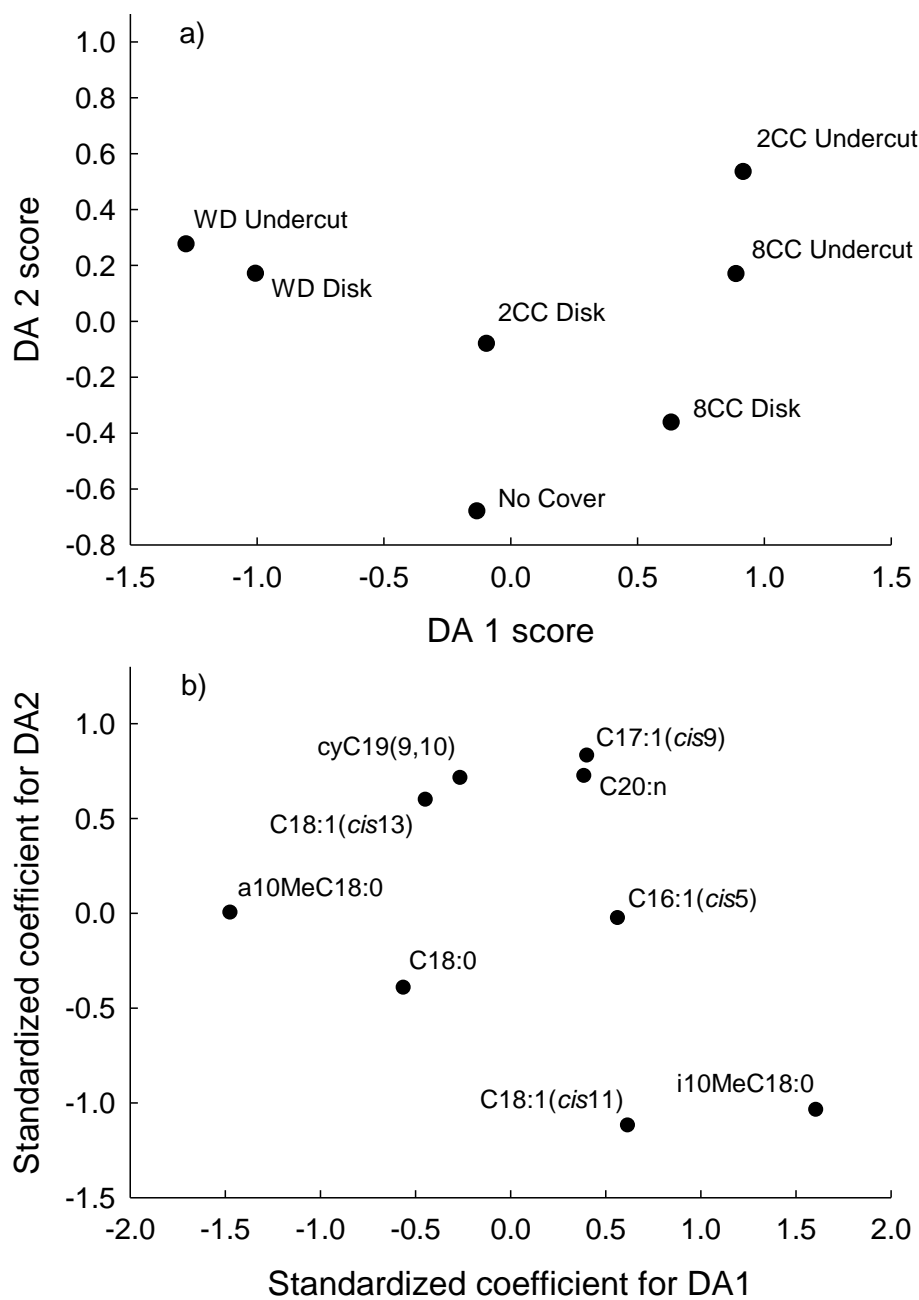


Figure 3.3. Discriminant score means for all cover crop mixture by termination method treatment groups (a), and standardized canonical coefficients for FAMEs (b) contributing to the two significant discriminant functions DA1 and DA2.



Chapter 4

Cover crop mixtures and an alternative termination method for organic grain cropping systems: Influence on soil moisture and nitrogen, crop yield, and profitability

Abstract

Many studies have demonstrated soil quality benefits and fertility contributions of individual cover crop species, but the value of diverse cover crop mixtures has received less attention. Moreover, there is increasing interest in conservation tillage strategies for cover crop termination. The objectives of this research were to determine the effects of spring-sown cover crop mixture diversity and mechanical cover crop termination method on cover crop productivity, soil moisture, soil nitrogen, crop yield and profitability in an organic cropping system. A field experiment was conducted between 2009 and 2011 near Mead, NE where mixtures of 2, 4, 6, and 8 cover crop species, or a summer annual weed mixture were included in a sunflower – soybean – corn crop rotation. Cover crops were planted in late-March, terminated in late-May using a field disk or sweep plow undercutter and main crops were planted within one week of termination. Aboveground biomass of cover crops and weeds was consistently greater in cover crop mixtures (307.3 g m^{-2}) compared to the weed mixture (87.6 g m^{-2}), and in two of three years biomass increased with diversity of the cover crop mixture. Undercutting cover crops increased soil $\text{NO}_3\text{-N}$ (0 to 20 cm) by 1.0 and $1.8 \mu\text{g NO}_3\text{-N g}^{-1}$ relative to disk incorporation at 32 days after termination (DAT) in 2010 and at 55 DAT in 2011, respectively. Cover crop mixtures reduced soil moisture content (0 to 8 cm) by $0.15 \text{ cm}^3 \text{ cm}^{-3}$ prior to main crop planting during an abnormally dry 2009 spring, while cover crop termination with the

undercutter increased soil moisture content by as much as $0.024 \text{ cm}^3 \text{ cm}^{-3}$ compared to termination with the disk during early main crop growth. Crop yields were not influenced by cover crop mixture, but termination with the undercutter (relative to disk incorporation) increased corn and soybean yield by as much as 1.40 and 0.88 Mg ha^{-1} , respectively. Despite differences in productivity between spring cover crop mixtures and weed communities, crop yield was not different among these treatments; thus, profitability of the weed mixture – undercutter treatment combination was greatest due to reduced input costs (i.e., no cover crop seed or planting costs). Short-term yield and economic benefits of using weed communities as cover crops may be offset by reduced environmental benefits of a less productive and more spatially heterogeneous spring plant community.

Introduction

Cover crops have been shown to provide many environmental and agronomic services within agroecosystems. These include reduced soil erosion, increased biological diversity (e.g., microbes, insects, and birds), increased nutrient cycling and biological nitrogen fixation, increased soil organic matter, improved weed control, and increased crop yield (Pimentel et al., 1992; Pimentel et al., 1995; Sainju and Singh, 1997; Williams II et al., 1998; Altieri, 1999; Reddy et al., 2003; Teasdale et al., 2007). While cover crops have traditionally been used as a soil conservation tool (Pimentel et al., 1995), there is increasing interest in using cover crops to enhance agronomic crop performance. However, maximizing agronomic benefits associated with cover crops will depend on appropriate cover crop choice and residue management (Ashford and Reeves, 2003; Wortman et al., 2012). Single species cover crops are often popular among farmers due to the ease of planting, and uniform development and predictable termination efficacy of the cover crop (Creamer et al., 1995; Mirsky et al., 2009). However, multi-species cover crop mixtures may increase productivity, stability, resilience, and resource-use efficiency of the cover crop community (Tilman, 1996; Tilman et al., 1997; Trenbath, 1999; Tilman et al., 2001; Wortman et al., 2012).

Despite the demonstrated benefits of cover crops, on-farm adoption remains limited due to farmer concerns about the cost and management implications of planting cover crops. One of the top concerns among farmers is the amount of soil water used by cover crops, potentially reducing available soil moisture for the cash crop (Corn and Soybean Digest, 2010). During seasons with average and above-average rainfall conditions, differences in available soil moisture among cover crop species and mixtures are often undetectable (Daniel et al., 1999). However, when cover crop productivity is

high and precipitation becomes limiting, cover crop species can differ greatly in their effects on soil moisture (Unger and Vigil, 1998; Daniel et al., 1999). While transpiration demands will undoubtedly vary among species, the method of cover crop termination and residue management may have a greater impact on available soil moisture during main crop growth. Daniel et al. (1999) found that volumetric soil moisture (%) was increased by as much as 2.4% to a depth of 61 cm when cover crops were terminated with herbicides in a no-till system compared to conventional termination with a field disk. Soil water savings associated with no-till practices have been well documented (Blevins et al., 1983; De Vita et al., 2007), but the additional benefits of cover crop residue in a conservation tillage system are not as clear. Liebl et al. (1992) found that cover crop transpiration reduced available soil moisture during dry periods, but following no-till termination cover crop residue conserved soil moisture relative to a no-till system without cover crops. Given that the driest portion of the growing season in eastern Nebraska typically occurs after cover crop growth (i.e., June – August), potential soil moisture savings offered by cover crop residues (post-termination) throughout the growing season may negate moisture deficits observed during cover crop growth.

Despite concerns about water use, many farmers are interested in cover crops because of the potential for improved nutrient cycling and biological nitrogen fixation (Corn and Soybean Digest, 2010). As a result, species in the Fabaceae (legume) family are among the most popular and expensive cover crops. Legume cover crops (e.g., green manures) have been shown to reduce synthetic N input demands by 50 to 100% depending on species and the duration of cover crop growth (Biederbeck et al., 1996; Burket et al., 1997). While legume species have the potential to biologically fix nitrogen,

faster growing cover crop species (e.g., grass and mustard spp.) may be more useful in scavenging nitrates and nutrient cycling (Dabney et al., 2001). A mixture of legume and non-legume cover crop species may maximize the benefits of biological nitrogen fixation and nutrient cycling, as legumes can increase N availability to other species in mixture leading to increased productivity (Kuo and Sainju, 1998; Mulder et al., 2002). Consistent with impacts on soil moisture, termination method and residue management can influence nitrogen mineralization, soil availability, and crop uptake (Sainju and Singh, 2001). Incorporation of cover crop residue via field disk or plow often results in rapid nitrogen mineralization and plant availability, but management of residue on the soil surface has been shown to result in greater crop N uptake and yield (Sainju and Singh, 2001). Therefore, residue management on the soil surface with conservation tillage methods may be effective in syncing nitrogen mineralization and availability with crop demand and uptake (Parr et al., 2011).

Overall, the agronomic objective for cover crop management is to minimize soil water loss and increase the quantity and availability of soil nitrogen to promote increases in crop yield. However, improper management of cover crops can lead to substantial yield loss. The timing and method of cover crop termination have both been shown to affect yield influencing factors including: soil moisture availability, weed communities, cover crop and soil nitrogen content, and crop nitrogen uptake (Daniel et al., 1999; Mirsky et al., 2009; Parr et al., 2011; Wortman, 2012). Yield loss associated with cover crop use is typically attributed to incomplete cover crop control, soil moisture deficit, or nutrient immobilization and deficiency (Waggoner, 1989; Unger and Vigil, 1998; Mischler et al., 2010); thus, management of cover crop residue should be focused toward

termination efficacy, moisture conservation, and optimum soil nitrogen availability during peak crop growth. To this end, conservation tillage implements like the sweep plow undercutter may have great potential (Creamer et al., 1995). In contrast to conventional tillage systems, the undercutter leaves intact cover crop residue on the soil surface, minimizes soil inversion, and presumably reduces evaporative loss from the soil. Moreover, the undercutter may be an improvement upon conservation implements like the roller-crimper, which is often inconsistent in termination efficacy (Mischler et al., 2010). Despite these production challenges, many cover crop systems have been shown to maintain or increase crop yield (e.g., Clark et al., 1994; Davis, 2010; Mischler et al., 2010). Indeed, demonstrating predictable yield and economic benefits associated with cover crop use will be necessary in increasing on-farm adoption (Corn and Soybean Digest, 2010).

The objectives of this research were to determine the effects of spring-sown cover crop mixture diversity and mechanical cover crop termination method on cover crop productivity, soil moisture, soil nitrogen, crop yield and profitability. We hypothesized that increasing cover crop diversity will increase total cover crop biomass, and subsequent grain yield, while soil moisture content will not differ among mixtures (despite differences in productivity). With regard to cover crop termination, we hypothesized that mulching cover crops with the sweep plow undercutter will increase soil moisture content, soil nitrate availability, crop yield, and profitability.

Materials and Methods

Experimental Site and Design

A field experiment was conducted in 2009, 2010 and 2011 at the University of Nebraska-Lincoln Agricultural Research and Development Center (ARDC) near Mead, Nebraska. Dominant soil type at the site is a Sharpsburg silty clay loam (fine, smectitic, mesic typic Argiudoll) with 0 to 5% slopes. The experiment was conducted in a 2.8 ha field that is certified for organic production (OCIA International, Lincoln, NE), and is managed without irrigation. This field was in organic alfalfa hay production between 2004 and 2008. In the fall of 2008 the experimental area was amended with 50 Mg ha⁻¹ of liquid beef feedlot manure and incorporated via field disk. In the spring of 2009, the entire field (excluding the weed-free control) was seeded with 8.1 kg ha⁻¹ of velvetleaf (*Abutilon theophrasti*) seed, 2.6 kg ha⁻¹ of common lambsquarters (*Chenopodium album*) seed, 1.2 kg ha⁻¹ of redroot pigweed (*Amaranthus retroflexus*) seed, and 3.7 kg ha⁻¹ of green foxtail (*Setaria viridis*) seed to establish a common weed seedbank throughout the field for a concurrent weed management study.

The experiment was designed as a split-plot randomized complete block design within a 3-year crop rotation with 4 replications. The rotation sequence consisted of confectionery sunflower (*Helianthus annuus* L. ‘Seeds 2000 Jaguar’) – soybean (*Glycine max* L. Merr. ‘Blue River Hybrids 2A71’) – corn (*Zea mays* L. var. ‘Blue River Hybrids 57H36’). Within each crop species, whole-plots (9.1 x 21.3 m; 12 crop rows spaced 0.76 m apart) were defined by cover crop mixture, while split-plots (4.6 x 21.3 m; 6 crop rows spaced 0.76 m apart) were defined by cover crop termination method. Each “crop x cover crop mixture x termination method” treatment combination was replicated within each

block so that each phase of the 3-year crop sequence was present each year within each block. There were six whole-plot cover crop treatments: 1) two-species cover crop mixture (2CC), 2) four-species cover crop mixture (4CC), 3) six-species cover crop mixture (6CC), 4) eight-species cover crop mixture (8CC), 5) weedy mixture and cover crop-free (prior to main crop planting) (WD), and 6) weed-free and cover crop-free (prior to main crop planting) control (NC). The NC whole-plots were field disked and hand-hoed twice prior to main crop planting, while the WD whole-plots were left unmanaged until cover crop termination. The goal for the WD treatment was to manage existing weed populations as a cover crop. Details on the individual species and seeding rates included in each cover crop mixture whole-plot are included in Table 4.1.

Split-plot cover crop termination methods included either disking or undercutting. Termination method was randomized within the first replication (southernmost) and duplicated in the remaining three replications (north of the first replication) to facilitate adequate speed for effective tillage operations driving north-south through the field. Disking was conducted with a 4.6 m wide Sunflower 3300 (Sunflower Mfg., Beloit, KS, USA) disk to an approximate depth of 15 cm. Undercutting was conducted with either a Buffalo 6000 (Buffalo Equipment, Columbus, NE, USA) cultivator (modified for undercutting) with seven overlapping 0.75 m wide sweep blades (2009) or a Miller Flex-Blade sweep plow undercutter (2010 and 2011) with three overlapping 1.5 m sweep blades. The undercutter sweeps are designed to cut a level plane through the soil at an approximate depth of 10 cm, severing plant roots and minimizing soil inversion, resulting in a layer of intact surface residue. Details on the design of the undercutter can be found in Creamer et al. (1995).

Cover crop mixtures were planted via hand-crank broadcast seeding followed by light incorporation with a John Deere 950 cultipacker (Deere and Company, Moline, IL, USA). Generally, cover crops were planted in late-March, terminated in late-May, and the main crop was planted within one week of termination. Specific dates for field operations across all years are detailed in Table 4.2. Seeding rates for confectionery sunflower, soybean, and corn were 62,000, 556,000, and 86,000 seeds ha⁻¹, respectively. All crops were inter-row cultivated once (2009) or twice (2010 and 2011) approximately 30 days after planting the main crop. Seeds of all legume cover crop and crop species were inoculated with appropriate rhizobia bacterial species prior to planting in 2009 and 2010.

Data Collection

Monthly precipitation (mm) and temperature (°C) for April to September was determined for each growing season by summing daily precipitation and temperature measurements from the High Plains Regional Climate Center station located on the University of Nebraska Turf Farm near Mead, NE (41°10'12"N lat, 96°28'12"W long, elevation= 366 m), located 1 km northwest of the experimental site (Table 4.3). Climate data for the 30-year mean was obtained from a different climate center near Mead, NE (41°8'24"N and 96°28'48"W) between 1971 and 2000 (long-term data from the University of Nebraska Turf farm was unavailable).

Three (2009) or four (2010 and 2011) aboveground biomass samples were taken from each whole plot experimental unit prior to cover crop termination to determine productivity of the cover crop mixtures and weed communities. Samples were combined within each experimental unit, dried at 60°C to constant mass and weighed. The biomass

harvest area included three 0.3 x 0.3 m samples per experimental unit in 2009, and was increased to four 0.3 x 0.6 m samples per experimental unit in 2010 and 2011.

Surface soil moisture (0 to 8 cm) was measured weekly from cover crop planting through the vegetative growth of the main crop. Measurements were taken at three random points within each whole plot (prior to cover crop termination) or split-plot (after cover crop termination) experimental unit using a Theta Probe soil water sensor (SM 200 Soil Moisture Sensor, Delta-T Devices Ltd, Cambridge, UK). Accuracy of the soil water sensor was verified against 21 gravimetric soil samples in 2010 and the ratio between method outputs was approximately 1:1. Indeed, linear regression analysis indicated a positive relationship between outputs from the two methods ($p=0.003$, $F=11.68$, $df_n=1$, $df_d=19$, $R_2=0.38$; water sensor reading = 1.10 (gravimetric soil moisture) – 2.6).

Soil samples were collected three times during the growing season: 1) prior to cover crop planting, 2) approximately 30 DAP, and 3) approximately 60 DAP. A composite soil sample of three (2009) or four (2010 and 2011) soil cores (3.18 cm diameter x 20 cm) per whole plot (prior to cover crop planting) or split-plot (30 DAP and 60 DAP) experimental unit were taken. Composite soil samples were then air-dried and sent to Ward Laboratories (Ward Laboratories Inc., Kearney, NE, USA) for analysis of soil NO₃-N. Soil extraction and analyses were conducted according to routine laboratory procedures at Ward Laboratories, Inc (Ward, 2011).

Crop yield was determined for each main crop by harvesting seed or grain from the middle four rows of each split-plot experimental unit. Contents were weighed using a combine scale (Model 400, Weigh-Tronix, Fairmont, MN) and adjusted for moisture

content in the lab. Corn grain yields were adjusted to 0.155, soybean to 0.130, and sunflower to 0.10 g kg⁻¹ moisture.

Data Analysis

Values for cover crop biomass, soil moisture, soil NO₃-N, and crop yield were analyzed with a linear mixed model analysis of variance using the GLIMMIX procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Fixed effects in the model included main crop, cover crop mixture, termination method, and all possible interactions of these effects. The random effects were block and the interaction of block by current crop by cover crop mixture. The model for data taken prior to cover crop termination (i.e., cover crop biomass and soil moisture) excluded fixed effects for main crop and termination method. In addition, models for soil NO₃-N and soil moisture analysis included a fixed effect for day of year. Effects were often tested within individual years due to experimental changes in the cover crop mixture (buckwheat was replaced in all mixtures with Idagold mustard after 2009) and interactions with year when initially included as a fixed effect (data not shown). Least square means and standard errors were calculated for all significant fixed effects at an alpha level of 0.05. To aid in the visualization of statistical interactions, cover crop biomass data were plotted as lines with cover crop mixture on the x-axis (Sosnoskie, 2006). The cover crop treatments were arranged in order (left-to-right) of increasing cover crop species diversity (from zero in the WD treatment to eight species in the 8CC treatment) along the x-axis similar to the figures presented by Tilman et al. (2001). However, we recognize that these data are not truly continuous as is traditionally expected in line plots.

Results and Discussion

Climate

The average daily air temperature during the growing season for cover crops and summer annual cash crop crops (1 Apr. – 30 Sept.) was 17.8, 19.5, and 19.0°C in the years 2009, 2010, and 2011, respectively (Table 4.3). The 30-year mean (1971 to 2000) air temperature for the growing season near Mead, NE was 19.0°C. The 2009 growing season was exceptionally cool, especially during early cover crop growth (April) and vegetative crop growth (June through August; Table 4.3). Average total precipitation during the growing season for cover crops and summer annual cash crops was 432, 717, and 547 mm in 2009, 2010, and 2011, respectively. The 30-year mean total precipitation was 519 mm (Table 4.3). In addition to abnormally cool temperatures, the 2009 growing season was also relatively dry, especially during cover crop growth and early cash crop establishment. As a result, plant water stress (e.g., curling and cupping of leaves) was observed in all crops during June of 2009.

Cover Crop Productivity

Total cover crop mixture and/or weed biomass was greatest in the 6CC treatment ($328.2 \pm 21.0 \text{ g m}^{-2}$), followed by the 4CC ($287.6 \pm 20.1 \text{ g m}^{-2}$), 8CC ($260.6 \pm 20.1 \text{ g m}^{-2}$), 2CC ($155.0 \pm 20.1 \text{ g m}^{-2}$) and WD ($73.7 \text{ g m}^{-2} \pm 20.1 \text{ g m}^{-2}$) treatments (LS mean \pm standard error) when harvested 60 days after cover crop planting in 2009 (Figure 4.1). Cover crop productivity was not different among cover crop mixtures (ranging from 367.2 to $409.3 \pm 16.7 \text{ g m}^{-2}$), but was lowest in the WD treatment ($68.8 \pm 16.7 \text{ g m}^{-2}$) when harvested 55 days after cover crop planting in 2010 (Figure 4.1). Consistent with trends in 2009, cover crop productivity in 2011 was greatest in the 6CC, 8CC, and 4CC

treatments (309.6 , 307.2 , and $276.2 \pm 14.3 \text{ g m}^{-2}$, respectively), followed by the 2CC treatment ($205.2 \pm 14.3 \text{ g m}^{-2}$), and lowest in the WD treatment ($120.4 \pm 14.3 \text{ g m}^{-2}$). Biomass of weeds in the WD treatment was lower than biomass of cover crop mixtures primarily due to spatial heterogeneity of weeds growing in this treatment and variable emergence and growth of various species in the weed community. Though the productivity of cover crop mixtures was similar in 2009 and 2011, the cause for this response was different between years.

Differences in cover crop productivity in 2009 were likely due to the presence or absence of a Brassicaceae (mustard) spp. in the mixture. Cover crop biomass was lowest in the 2CC mixture as it only included hairy vetch and buckwheat. Both of these species were slow-growing throughout the relatively cool and dry early growing season in 2009 (Table 4.3), and buckwheat was moderately susceptible to early frost. Buckwheat is often used as a summer cover crop or later planted main crop due to its susceptibility to frost, especially during seedling growth (Kalinova and Moudry, 2003); thus, buckwheat may not be a suitable species for use as a spring-sown cover crop in the western Corn Belt. Given these results, buckwheat was replaced in all mixtures with Idagold mustard in 2010 and 2011. Idagold mustard, a mustard spp., was selected as the replacement due to the high level of productivity of the three other mustard spp. used in the 4CC, 6CC, and 8CC mixtures in 2009. Mustard spp., including Idagold mustard, are well adapted to the cool climate of the northern Great Plains, and productivity is often maximized when planting between mid-March and mid-April (Chen et al., 2005). Given the productivity of the mustard spp. used in this study, it is not surprising that biomass was not different among

cover crop mixtures in 2010 when all mixtures contained a 1:1 ratio of mustard and legume spp.

While cover crop productivity responded positively to the mixture adjustments in 2010, it was a May 12, 2011 hail storm that led to 2011 treatment differences. The hail storm damaged all cover crop species within mixtures, but Idagold mustard was most susceptible to hail damage and did not recover well from this extreme disturbance (Wortman et al., 2012). Idagold mustard was a component of all four cover crop mixtures; thus, as the diversity of the cover crop mixture increased, the proportion of Idagold mustard in the mixture decreased. Therefore, we hypothesize that productivity of the mixtures increased with diversity due to decreased proportions of Idagold mustard. These results, in combination with the 2009 results, highlight an important benefit of diverse cover crop mixtures. By reducing the proportion of each species in a diverse cover crop mixture, we observed increased resilience and productivity of the cover crop community despite a management error (2009) and extreme weather disturbance (2011). Similar to a diversified investment portfolio, diverse cover crop mixtures seem poised for stable productivity and resilience despite potential management errors and an increasingly unstable climate (Doak et al., 1998).

Surface Soil Moisture

Surface soil moisture (0 to 8 cm) prior to cover crop termination was unaffected by cover crop mixture, but by day of year (DOY) 141 soil moisture content was greatest in the NC control ($0.310 \pm 0.007 \text{ cm}^3 \text{ H}_2\text{O cm}^{-3} \text{ soil}$), followed by the WD treatment ($0.20 \pm 0.007 \text{ cm}^3 \text{ cm}^{-3}$) in 2009 (Figure 4.2). Soil moisture was lowest in the cover crop mixtures ($0.161 \text{ cm}^3 \text{ cm}^{-3}$ averaged across the four mixtures). The reduction in soil

moisture in cover-cropped and weedy treatments by DOY 141 was related to an exceptionally dry early spring in 2009. Between DOY 110 and 145 there were only two rainfall events totaling more than 10 mm in precipitation, and total precipitation during April and May was 62 mm. The 30-year mean for precipitation in April and May was 176 mm (Table 4.3). These results highlight the risk associated with planting cover crops in non-irrigated grain-based production systems (Ewing et al., 1991). While average annual precipitation is typically sufficient for growth of both a cover crop and cash crop, exceptionally dry years may cause significant production challenges and potential yield loss. Following cover crop termination, surface soil moisture was affected by termination method in 2009. Surface soil moisture was greatest in the NC control ($0.249 \text{ cm}^3 \text{ cm}^{-3} \pm 0.005$), followed by the undercutter treatment ($0.160 \text{ cm}^3 \text{ cm}^{-3} \pm 0.002$), and lowest in the disk treatment ($0.153 \text{ cm}^3 \text{ cm}^{-3} \pm 0.002$) one week following termination (DOY 149; Figure 4.2). However, by DOY 183 surface moisture was greatest in the undercutter treatment ($0.112 \text{ cm}^3 \text{ cm}^{-3} \pm 0.002$), followed by the NC control ($0.103 \text{ cm}^3 \text{ cm}^{-3} \pm 0.005$), and lowest in the disk treatment ($0.095 \text{ cm}^3 \text{ cm}^{-3} \pm 0.002$). At this point in the growing season, all crops were showing severe water stress. While soil moisture was exceptionally low among all treatments, it is interesting that soil moisture was greatest in the undercutter treatment at DOY 183 despite 56% less available moisture than the NC control at DOY 149.

Similar to 2009 results, surface soil moisture was unaffected by cover crop mixture prior to cover crop termination in 2010. However, surface soil moisture was greatest in both the NC control ($0.259 \text{ cm}^3 \text{ cm}^{-3} \pm 0.006$) and the WD treatment ($0.255 \text{ cm}^3 \text{ cm}^{-3} \pm 0.006$) at DOY 126 (Figure 4.3). Variable soil moisture in the cover-cropped

treatments throughout cover crop growth was related to rainfall patterns in early 2010. While soil moisture content was reduced in cover-cropped treatments at DOY 126, four rainfall events totaling 33.8 mm in precipitation over the next six days was sufficient to eliminate soil moisture differences between cover-cropped and non-cover-cropped treatments by DOY 137 (Figure 4.3). Following cover crop termination, surface soil moisture was affected by termination method in 2010. Averaged across the first three sampling dates (DOY 158, 166, and 169), surface moisture was greatest in the undercut treatment ($0.330 \pm 0.003 \text{ cm}^3 \text{ cm}^{-3}$) compared to both the NC and disk treatments ($0.314 \pm 0.006 \text{ cm}^3 \text{ cm}^{-3}$ and $0.306 \pm 0.002 \text{ cm}^3 \text{ cm}^{-3}$, respectively; Figure 4.3). We hypothesize that greater soil moisture following termination with the undercutter in 2009 and 2010 was due to the layer of cover crop mulch present on the soil surface for 14-21 days following termination with the undercutter. This is consistent with previous studies where management of cover crop residue on the soil surface led to increased soil moisture availability (Teasdale and Mohler, 1993; Kornecki et al., 2009; Davis, 2010). While soil moisture savings associated with the undercutter for fallow tillage have been discussed (Zaikin et al., 2007), to our knowledge this is the first report of increased soil moisture availability following cover crop termination with an undercutter.

Soil moisture content varied by cover crop treatment and day of year (DOY) prior to termination in 2011. During early cover crop growth, soil moisture was greatest in the WD and NC treatments (0.161 and $0.156 \pm 0.006 \text{ cm}^3 \text{ cm}^{-3}$, respectively), followed by the cover-crop mixtures (average of $0.127 \pm 0.006 \text{ cm}^3 \text{ cm}^{-3}$; Figure 4.4). However, by the end of cover-crop growth (DOY 153) soil moisture content was greatest in the 4CC, 6CC, and 8CC mixtures (average of $0.288 \pm 0.006 \text{ cm}^3 \text{ cm}^{-3}$), followed by the 2CC

mixture ($0.257 \pm 0.006 \text{ cm}^3 \text{ cm}^{-3}$), and the WD and NC treatments (0.243 and $0.235 \pm 0.006 \text{ cm}^3 \text{ cm}^{-3}$, respectively; Figure 4.4). May 2011 was exceptionally wet (164 mm precipitation) compared to the 30-year mean for May (106 mm), leading to greater surface soil moisture content beneath cover crop canopies (Table 4.3). When there was sufficient soil moisture to meet cover crop transpiration demands, the dense cover crop canopy may have conserved soil moisture by reducing evaporative loss from the soil surface occurring in the relatively bare NC and WD treatments. Indeed, soil evaporation can be reduced through early crop canopy closure (Luening et al., 1994). Following cover crop termination, surface soil moisture was not influenced by termination method or DOY in 2011. Instead, soil moisture was influenced by cover crop treatment, where values were greatest in the 8CC mixture ($0.275 \pm 0.004 \text{ cm}^3 \text{ cm}^{-3}$) and lowest in the NC and WD treatments ($0.262 \pm 0.006 \text{ cm}^3 \text{ cm}^{-3}$ and $0.254 \pm 0.004 \text{ cm}^3 \text{ cm}^{-3}$, respectively) when pooled across the three post-termination sampling intervals (DOY 159 to 186; data not shown). Increased soil moisture in the cover-cropped treatments in the third year of this study may be related to improvements in soil physical structure. Cover-cropping in organic systems has been shown to increase soil water infiltration and soil water holding capacity (Colla et al., 2000; Lotter et al., 2003).

Soil Nitrogen

Soil $\text{NO}_3\text{-N}$ at 45 and 81 days after termination (DAT) was affected by the interaction of cover crop mixture and termination method in 2009. Soil $\text{NO}_3\text{-N}$ was greatest in the WD – undercut treatment combination ($50.2 \pm 6.1 \mu\text{g NO}_3\text{-N g}^{-1}$), but differences among the remaining cover crop and termination treatments were inconsistent at 45 DAT (data not shown). At 81 DAT soil $\text{NO}_3\text{-N}$ was greatest in the NC control (30.0

$\pm 2.4 \mu\text{g NO}_3\text{-N g}^{-1}$), followed by the WD – undercut treatment combination ($22.5 \pm 2.4 \mu\text{g NO}_3\text{-N g}^{-1}$). Similar to the results at 45 DAT, differences among remaining treatments were inconsistent (data not shown). Increased soil $\text{NO}_3\text{-N}$ in the WD and NC treatments at 45 and 81 DAT in 2009 was likely the result of N-immobilization and delayed $\text{NO}_3\text{-N}$ mineralization following cover crop growth, termination, and decomposition. Previous studies have demonstrated delayed soil $\text{NO}_3\text{-N}$ release from cover crop residue especially following late termination (Wagger, 1989; Quemada and Cabrera, 1995; Kuo and Sainju, 1998). Moreover, nitrogen immobilization is most pronounced when cover crop residue is comprised of over 60% non-leguminous residue (Kuo and Sainju, 1998). In this study, mustard spp. dominated the mixtures and typically accounted for over 60% of total mixture biomass (Wortman et al., 2012).

Following the 2008 growing season, the experimental site was amended with 50 Mg ha^{-1} beef feedlot liquid manure. While available soil $\text{NO}_3\text{-N}$ was greatest in the NC and WD treatments throughout the 2009 growing season, the immobilization of soil $\text{NO}_3\text{-N}$ in cover crop residue likely reduced $\text{NO}_3\text{-N}$ leaching and surface runoff from the manure early in the growing season (Staver and Brinsfield, 1998). Moreover, lower levels of available soil $\text{NO}_3\text{-N}$ in the cover-cropped treatments early in the growing season may have aided in the suppression of weeds. High levels of available soil nitrogen have been shown to shift the competitive advantage to weed species especially following manure application (Barker et al., 2006; Wortman et al., 2010).

Soil $\text{NO}_3\text{-N}$ at 32 DAT was affected by cover crop termination method in 2010, as soil $\text{NO}_3\text{-N}$ was greatest in the undercutter treatment ($3.2 \pm 0.2 \mu\text{g NO}_3\text{-N g}^{-1}$), followed by both the disk ($2.2 \pm 0.2 \mu\text{g NO}_3\text{-N g}^{-1}$) and NC treatments ($2.2 \pm 0.4 \mu\text{g NO}_3\text{-N g}^{-1}$).

By 60 DAT, soil NO₃-N levels were only influenced by main crop. Soil NO₃-N was greatest in soybean ($5.0 \pm 0.2 \mu\text{g NO}_3\text{-N g}^{-1}$), followed by corn ($4.4 \pm 0.2 \mu\text{g NO}_3\text{-N g}^{-1}$), and sunflower ($4.0 \pm 0.2 \mu\text{g NO}_3\text{-N g}^{-1}$). Results for soil NO₃-N in 2011 were similar to 2010, except that treatment differences were not observed until later in the growing season. Soil NO₃-N was influenced by cover crop termination with the greatest levels observed in the undercutter treatment ($11.4 \pm 0.5 \mu\text{g NO}_3\text{-N g}^{-1}$), followed by the disk and NC treatments ($9.6 \pm 0.5 \mu\text{g NO}_3\text{-N g}^{-1}$ and $8.4 \pm 1.3 \mu\text{g NO}_3\text{-N g}^{-1}$, respectively) at 55 DAT in 2011. Also consistent with 2010 results, soil NO₃-N was greatest in soybean ($12.4 \pm 0.6 \mu\text{g NO}_3\text{-N g}^{-1}$), followed by corn ($11.3 \pm 0.6 \mu\text{g NO}_3\text{-N g}^{-1}$), and sunflower ($7.1 \pm 0.6 \mu\text{g NO}_3\text{-N g}^{-1}$). As expected, soil NO₃-N levels were generally lower in 2010 and 2011 compared to 2009, presumably the result of grain N removal. As soil N becomes limiting with time, management focus should shift from minimizing NO₃-N leaching and runoff towards maximizing availability. The lower soil NO₃-N observed in the disk treatment compared to the undercut treatment at 29 DAP in 2010 was likely the result of strong N immobilization that is common following soil incorporation of cover crops (Wyland et al., 1995). In contrast, cover crop surface residue mulch achieved with the undercutter may result in lower immobilization and a more gradual release of soil NO₃-N throughout the growing season (Groffman et al., 1987; Parr et al., 2011).

Crop Yield

Crop yield for corn, sunflower, and soybean were affected by cover crop termination method but not cover crop mixture in 2009. Corn grain yield was greater in the undercutter treatment ($8.78 \pm 0.36 \text{ Mg ha}^{-1}$) compared to the disk treatment ($7.37 \pm 0.36 \text{ Mg ha}^{-1}$), while yield in the NC control was not different from either termination

treatment ($8.40 \pm 0.80 \text{ Mg ha}^{-1}$; Table 4.4). Similarly, sunflower seed yield was greater in the undercutter treatment ($2.11 \pm 0.09 \text{ Mg ha}^{-1}$) compared to the disk treatment ($1.91 \pm 0.09 \text{ Mg ha}^{-1}$), while yield in the NC control was not different from either termination treatment ($2.18 \pm 0.20 \text{ Mg ha}^{-1}$). Soybean seed yield was greater in the undercutter and NC treatments ($2.43 \pm 0.09 \text{ Mg ha}^{-1}$ and $2.59 \pm 0.21 \text{ Mg ha}^{-1}$, respectively) compared to the disk treatment ($1.50 \pm 0.09 \text{ Mg ha}^{-1}$; Table 4.4).

Similarly, crop yield in 2010 was affected by cover crop termination method, not cover crop mixture. Corn yield was greatest in the undercutter treatment ($7.75 \pm 0.25 \text{ Mg ha}^{-1}$), followed by the disk treatment ($6.45 \pm 0.25 \text{ Mg ha}^{-1}$), and lowest in the NC control ($5.29 \pm 0.60 \text{ Mg ha}^{-1}$; Table 4.4). Soybean yield was also greatest in the undercutter treatment ($1.11 \pm 0.09 \text{ Mg ha}^{-1}$), but was not different between the disk and NC treatments ($0.82 \pm 0.09 \text{ Mg ha}^{-1}$ and $0.72 \pm 0.21 \text{ Mg ha}^{-1}$, respectively). Sunflower yield was not affected by termination method in 2010 (Table 4.4). Yield trends in 2011 were similar to previous years, except that yield for corn was substantially higher than in 2009 and 2010. Again influenced by the effect of cover crop termination, corn grain yield was greatest in the NC and undercutter treatments ($11.12 \pm 0.64 \text{ Mg ha}^{-1}$ and $10.97 \pm 0.28 \text{ Mg ha}^{-1}$, respectively) and lowest in the disk treatment ($10.16 \pm 0.28 \text{ Mg ha}^{-1}$). Soybean yield was greatest in the undercutter treatment ($2.96 \pm 0.08 \text{ Mg ha}^{-1}$), followed by the NC control ($2.51 \pm 0.18 \text{ Mg ha}^{-1}$), and lowest in the disk treatment ($2.11 \pm 0.08 \text{ Mg ha}^{-1}$; Table 4.4). Consistent with 2010, there were not treatment effects on sunflower yield in 2011.

Difference in yield among years was the result of unique weather and pest incidence in each year of the study. The sharp decline in crop yield from 2009 to 2010

was the result of crop damage from a severe hail storm on 13 September 2010 at the experimental site. During this storm, all plants were completely defoliated (95 to 100%) and severely lodged (>50%) prior to physiological maturity. The timing of the hail damage was especially detrimental to soybean, as soybean yield can be reduced by as much as 57% after full defoliation in late reproductive stages (Caviness and Thomas, 1980). Yield loss in corn and sunflower was more related to plant lodging and ear/head dropping (data not shown). Despite overall yield reduction, damage throughout the field was relatively uniform and comparisons among treatments were still informative. Corn yield loss in 2009 relative to 2011 was likely due to a reduction in grain quality in 2009. The test weight for corn grain was $650 \pm 2 \text{ kg m}^{-3}$ in 2009 compared to $724 \pm 2 \text{ kg m}^{-3}$ in 2011. Lower test weight values in 2009 were the result of an early frost on 4 October 2009 (low temperature of -1.7°C), which occurred prior to physiological maturity of the corn crop. When planting a spring-seeded cover crop in the western Corn Belt, it will often be necessary to delay traditional planting dates of corn and soybean. However, the yield loss observed in 2009 highlights the importance of selecting appropriate early-maturing hybrids and crop cultivars to avoid further reductions in crop yield and quality associated with a later planting date.

Sunflower yield loss in 2010 and 2011, relative to 2009 was primarily due to high incidence of banded sunflower moth (*Cochylis hospes* Walsingham) damage in 2010 and 2011. The banded sunflower moth larvae feed on florets and seeds of sunflower, and are relatively common pests in the northern Great Plains (Charlet and Miller, 1993). Damage from the banded sunflower moth has been shown to affect up to 46.5% of sunflower seeds in a given sunflower head (Charlet et al., 2009). Yield loss in 2011 relative to 2009

ranged from 27 to 33% across termination treatments, which we hypothesize was related to banded sunflower head moth damage. Yield loss in 2010 relative to 2009 was far more severe (61 to 75%), presumably due to the additive effects of banded sunflower moth damage and the severe hail storm prior to harvest. High populations and damage from the banded sunflower moth in two of three years of this study indicate a major pitfall of growing sunflower in the western Corn Belt. This crop will be especially difficult to manage in organic cropping systems, where reactive chemical control options will be limited for the banded sunflower moth.

Soil conservation, quality, and fertility benefits associated with cover crops have been well documented, but increases in crop yield are less commonly reported (Unger and Vigil, 1998; Kuo and Jellum, 2002; Reddy et al., 2003). The lack of yield benefits typically realized following cover crop plantings may be related to previous knowledge gaps regarding the most effective cover crop termination and residue management strategies. However, novel cover crop management systems, like the winter rye – soybean no-till cropping system, have created opportunities for increased crop yield and profitability (Mischler et al., 2010; Davis, 2010). Though unique from the roller-crimper system, results from this study provide support for another effective cover crop management strategy for organic cropping systems. Indeed, termination with the undercutter consistently maintained or increased crop yield relative to disk termination and the more traditional no cover crop organic cropping system. While the utility of the undercutter for cover crop termination and weed management has been previously documented (Creamer et al., 1995; Creamer et al., 2002), this is the first evidence of yield benefits associated with a “cover crop – undercutter” organic management system.

Cropping System Profitability

Throughout the study crop yield was consistently greatest following termination with the undercutter, although cover crop treatment did not influence yield in any crop or year of the study. This was a surprising result given that one of the “cover crop” treatments included a mixture of weeds (WD treatment) managed like a cover crop; thus, results from this study indicate that mixtures of common weed species may provide equivalent cropping system benefits relative to species commonly recognized as cover crops. This result is consistent with at least one previous study, where corn yield following winter annual weed “cover crop” was equal to or greater than yield following a crimson clover cover crop (Sainju and Singh, 2001). Similar to the results of this study, crop yield increase following weed growth occurred despite less than 50% biomass productivity of the weed community relative to cover crop communities (Sainju and Singh, 2001).

The potential utility of weed communities as cover crops becomes increasingly evident after profitability analysis of each cover crop – termination method treatment combination. Indeed, the WD – undercutter treatment combination resulted in the highest net profit for all crops and the entire rotation ($\$1,212 \text{ ha}^{-1} \text{ yr}^{-1}$; Table 4.5). The “traditional” cover crop mixture – undercutter treatment combinations were also profitable ($\$1,035$, $\$1,031$, $\$991$, and $\$986 \text{ ha}^{-1} \text{ yr}^{-1}$ for the 2CC – , 4CC – , 6CC – , and 8CC – undercutter treatment combinations, respectively; Table 4.5), but less so than the WD treatment because of the added annual costs of cover crop seed, seedbed preparation, and planting (Table 4.6). Termination with a disk, regardless of cover crop mixtures or

weeds, was always less profitable than the traditional no cover crop organic cropping system (Table 4.5).

Large differences in the profitability of each crop in the rotation are also informative (Table 4.5). Corn was by far the most profitable crop in all experimental treatments ranging from \$2,225 ha⁻¹ yr⁻¹ in the 8CC – disk treatment combination to \$2,962 ha⁻¹ yr⁻¹ in the WD – undercutter treatment combination. Large economic returns on organic corn are not uncommon (Pimentel et al., 2005), but were especially lucrative in this cropping system due to relatively low input costs (e.g., fewer tillage passes and fertility inputs) and high grain prices (Table 4.6). Soybean production was only profitable in the undercutter management systems ranging from \$361 to \$587 ha⁻¹ yr⁻¹ in the 8CC – and WD – undercutter treatment combinations, respectively. Average annual profitability of soybean production was limited in this study due to the input costs associated with animal manure in the first year of the study (Table 4.6). While manure application can improve soil quality and fertility, yield response is typically less consistent in soybean due to the capacity for biological nitrogen fixation (Schmidt et al., 2001). Sunflower production in this study was only profitable in the WD – undercutter treatment combination (\$87 ha⁻¹ yr⁻¹), but profits were modest compared to those for corn and soybean (Table 4.5). Sunflower profitability was limited by incidence of the banded sunflower moth in 2010 and 2011 and also by a relatively low market value for sunflower seed (Table 4.6). While price premiums for organic sunflower seed may exist in the market, it is often difficult to identify a consistent market value for organic specialty crops (USDA Market News Service, 2012). A guarantee of substantial price premiums would be necessary to make organic sunflower production profitable in eastern Nebraska.

Conclusions

Increasing diversity of the cover crop mixture generally increased biomass productivity in two of three years, highlighting the resilience of diverse cover crop mixtures following management error and severe weather disturbance. Despite differences in productivity, cover crop mixture composition and diversity did not influence soil moisture, soil nitrogen, or crop yield. Instead, differences within these factors were driven by termination method. Cover crop mixtures paired with the undercutter for termination did increase yield and profitability compared to a traditional no cover crop organic cropping system (NC control), but undercutter termination of weed mixtures (WD – undercutter treatment combination) proved to be the most profitable cropping system in this study. Although weeds are consistently a top management concern (Walz, 1999; MNDA, 2007), dense weed communities are a common characteristic of organic cropping systems; thus, it may be useful to identify and develop potential uses for these weed communities (Wortman et al., 2010).

Results of this study demonstrate the potential for weeds to provide crop yield benefits and farm profitability in excess of that achieved with traditional cover crop species. Despite the short-term yield and economic benefits of the WD – undercutter treatment combination, there are potential pitfalls associated with using weeds as cover crops. For example, if using weeds as a cover crop farmers should take extra caution to prevent weed seed production and replenishment of the seedbank (Davis, 2006). Moreover, many weed species can harbor pests between cropping seasons (Venkatesh et al., 2000). While yield and economic benefits were observed, substantially lower biomass

productivity and spatial heterogeneity of weeds relative to cover crop mixtures will potentially limit the soil conservation benefits typically expected of cover crops.

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Tables and Figures

Table 4.1. Cover crop species and seeding rates used in individual cover crop mixtures for 2009 and 2010-11 (2CC = 2 species mixture; 4CC = 4 species mixture; 6CC = 6 species mixture; 8CC = 8 species mixture).

Common Name	Scientific Name	Cover Crop Seeding Rate			
		2CC	4CC	6CC	8CC
		————— kg ha ⁻¹ —————			
Hairy Vetch	<i>Vicia villosa</i>	22.4	11.2	7.5	5.6
Buckwheat (2009)	<i>Fagopyrum sagittatum</i>	28.0	14.0	9.3	7.0
Idagold Mustard (2010-11)	<i>Sinapis alba</i>	6.7	3.4	2.2	1.7
Field Pea	<i>Pisum sativum</i>		28.0	18.7	14.0
Pacific Gold Mustard	<i>Brassica juncea</i>		2.2	1.7	1.1
Oilseed Radish	<i>Raphanus sativus</i>			2.8	2.1
Crimson Clover	<i>Trifolium incarnatum</i>			4.7	3.5
Dwarf Essex Rape	<i>Brassica napus</i>				1.7
Chickling Vetch	<i>Lathyrus sativus</i>				8.4

Table 4.2. Timing of field operations and data collection for each year of the study.

	Year		
	2009	2010	2011
Operation			
Cover crop planting	20 March	30 March	21 March
Cover crop sampling	19-21 May	24 May	1 June
Cover crop termination	22 May	28 May	3 June
Main crop planting	28 May	1-3 June	6 June
1st interrow cultivation	1 July	28 June	30 June
2nd interrow cultivation		1 July	8 July
1st soil sampling	6-7 July	29-30 June	28 June
2nd soil sampling	11-12 August	26-27 July	27-28 July

Table 4.3. Monthly precipitation total (mm) and average air temperature (°C) for April to September in 2009, 2010, and 2011, and the 30-year mean from the University of Nebraska Turf Farm near Mead, NE (41°10'12"N lat, 96°28'12"W long, elevation= 366 m).

Month	2009		2010		2011		30-year mean	
	Temp.	Precip.	Temp.	Precip.	Temp.	Precip.	Temp.	Precip.
April	9.0	28	12.8	85	9.9	76	10.1	70
May	16.9	34	15.6	53	16.2	164	16.3	106
June	21.4	135	22.5	217	22.3	139	22.0	101
July	21.1	68	24.4	156	26.5	80	24.3	84
August	20.9	135	24.3	71	23.2	78	22.9	85
September	17.2	31	17.4	134	15.7	9	18.2	73
Total	17.8	432	19.5	717	19.0	547	19.0	519

Table 4.4. Crop yield (Mg ha^{-1}) \pm 1 standard error for corn, soybean, and sunflower as influenced by termination method in the years 2009, 2010, and 2011. Different letters within a particular year and crop indicate differences among termination methods.

Crop	2009	2010	2011
Corn	————— Mg ha^{-1} —————		
No cover	8.41 \pm 0.64 a	5.29 \pm 0.64 c	11.12 \pm 0.64 a
Disk	7.37 \pm 0.28 b	6.45 \pm 0.28 b	10.16 \pm 0.28 b
Undercutter	8.78 \pm 0.28 a	7.75 \pm 0.28 a	10.97 \pm 0.28 a
Soybean			
No cover	2.59 \pm 0.18 a	0.72 \pm 0.18 b	2.51 \pm 0.18 b
Disk	1.58 \pm 0.08 b	0.82 \pm 0.08 b	2.11 \pm 0.08 c
Undercutter	2.46 \pm 0.08 a	1.11 \pm 0.08 a	2.96 \pm 0.08 a
Sunflower			
No cover	2.18 \pm 0.15 a	0.55 \pm 0.15 a	1.46 \pm 0.15 a
Disk	1.91 \pm 0.07 b	0.74 \pm 0.07 a	1.40 \pm 0.07 a
Undercutter	2.11 \pm 0.07 a	0.74 \pm 0.07 a	1.52 \pm 0.07 a

Table 4.5. Economic costs, returns, and average annual profit (US dollars (\$) ha⁻¹) for the 11 different cover crop mixture by termination method treatment combinations in corn, soybean, and sunflower for the years 2009, 2010, 2011, and for the entire rotation. NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 4.1); D = disk termination; U = undercutter termination.

	Cover crop mixture and termination method										
	NC	WD		2CC		4CC		6CC		8CC	
		D	U	D	U	D	U	D	U	D	U
Costs	US dollars (\$) ha ⁻¹										
2009	1,514	1,472	1,470	1,667	1,665	1,656	1,653	1,690	1,688	1,690	1,688
2010	771	731	729	900	897	912	909	954	951	961	959
2011	771	731	729	899	897	912	909	954	951	961	959
Returns											
Corn											
2009	3,884	3,404	4,055	3,404	4,055	3,404	4,055	3,404	4,055	3,404	4,055
2010	2,193	2,674	3,212	2,674	3,212	2,674	3,212	2,674	3,212	2,674	3,212
2011	4,609	4,211	4,547	4,211	4,547	4,211	4,547	4,211	4,547	4,211	4,547
Soybean											
2009	1,933	1,179	1,836	1,179	1,836	1,179	1,836	1,179	1,836	1,179	1,836
2010	508	578	783	578	783	578	783	578	783	578	783
2011	1,755	1,475	2,069	1,475	2,069	1,475	2,069	1,475	2,069	1,475	2,069
Sunflower											
2009	1,591	1,394	1,540	1,394	1,540	1,394	1,540	1,394	1,540	1,394	1,540
2010	401	540	540	540	540	540	540	540	540	540	540
2011	1,065	1,021	1,109	1,021	1,109	1,021	1,109	1,021	1,109	1,021	1,109
Avg. annual profit											
Corn	2,543	2,451	2,962	2,274	2,785	2,270	2,781	2,230	2,741	2,225	2,736
Soybean	380	99	587	-78	410	-82	406	-122	366	-127	361
Sunflower	1	7	87	-171	-90	-175	-94	-214	-134	-219	-139
3-crop rotation	975	853	1,212	675	1,035	671	1,031	631	991	626	986

Table 4.6. Price estimates and information source for costs and returns associated with each experimental management system.

Costs and returns	US dollars ha⁻¹	Source
Costs		
Cover crop seed		
Idagold mustard	83	L.A. Hearne ^b
Buckwheat ^a	178	Johnny's Selected Seeds ^c
Hairy vetch ^a	118	L.A. Hearne ^b
Pacific gold mustard	51	L.A. Hearne ^b
Field pea ^a	195	L.A. Hearne ^b
Oilseed radish	115	Johnny's Selected Seeds ^c
Crimson clover ^a	363	Johnny's Selected Seeds ^c
Dwarf essex rape	52	Johnny's Selected Seeds ^c
Chickling vetch ^a	298	Johnny's Selected Seeds ^c
Land rent	445	UNL Extension (2011)
Cover crop planting		
Seedbed preparation	17	Jose and Janousek (2010)
Drill planting	30	Jose and Janousek (2010)
Cover crop termination		
Disking	25	Jose and Janousek (2010)
Undercutting	22	Jose and Janousek (2010)
Main crop planting		
Seedbed preparation	17	Jose and Janousek (2010)
Organic crop seed	74	Delate et al. (2003)
Planting	34	Jose and Janousek (2010)
Weed management		
Interrow cultivation	22	Jose and Janousek (2010)
Combine harvest	69	Jose and Janousek (2010)
Fall tillage		
Moldboard plow	22	Jose and Janousek (2010)
Feedlot manure	741	Delate et al. (2003)
Certification costs	40	NCSU Extension (2008)
Returns		
Corn ^a	\$433 Mg ⁻¹	USDA Market News Service (2012)
Soybean ^a	\$698 Mg ⁻¹	USDA Market News Service (2012)
Sunflower	\$295 Mg ⁻¹	National Sunflower Association (2012)

^a Certified organic

^b L.A. Hearne Co., Monterey County, CA, USA

^c Johnny's Selected Seeds, Fairfield, ME, USA

Figure 4.1. Cover crop and/or weed biomass combined (g m^{-2}) for each cover crop mixture treatment in years 2009, 2010, and 2011 of the study. Mixtures are arranged on the x-axis in order of increasing cover crop community diversity. WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 4.1).

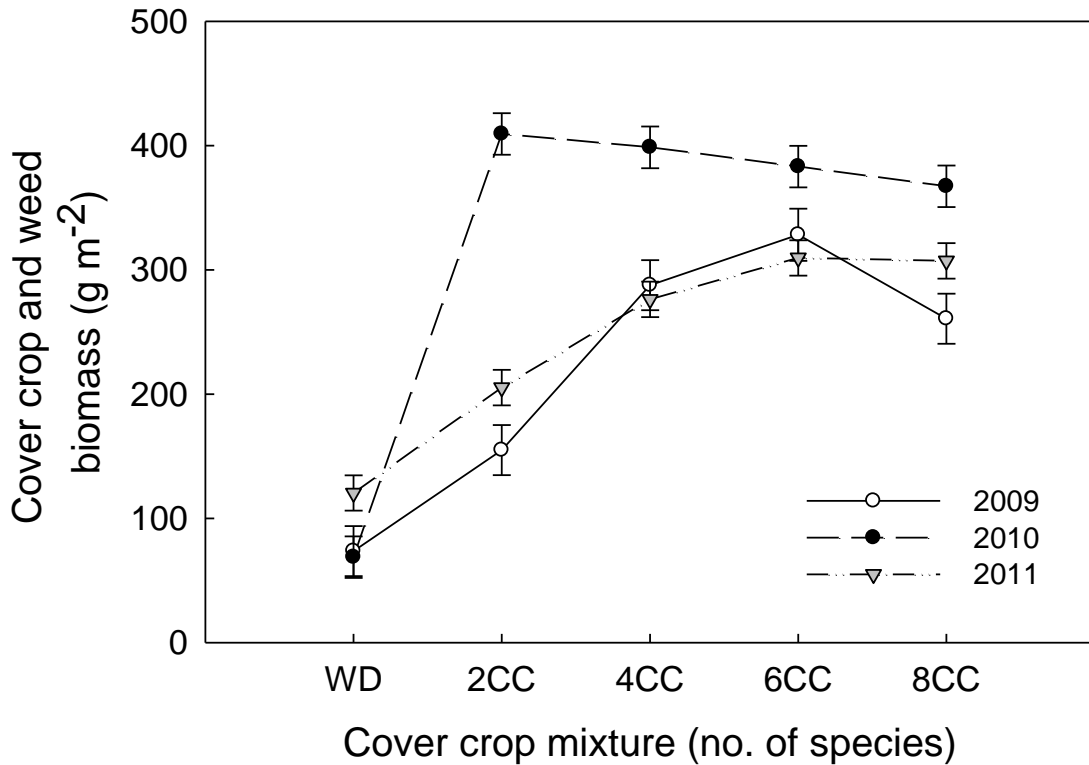
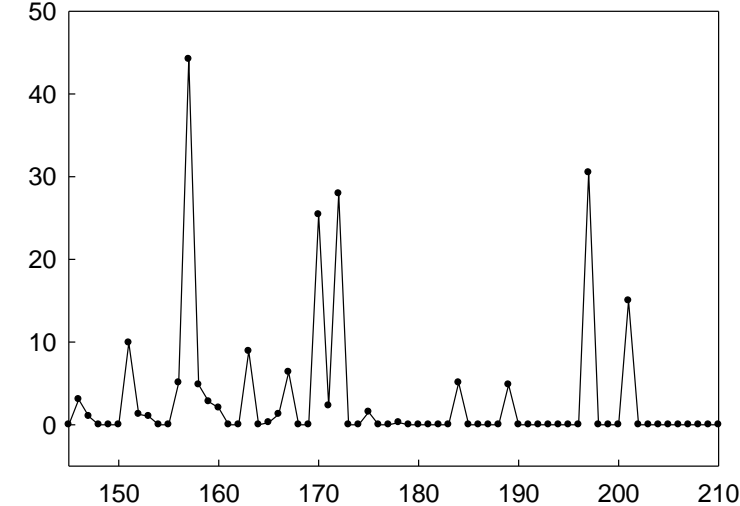
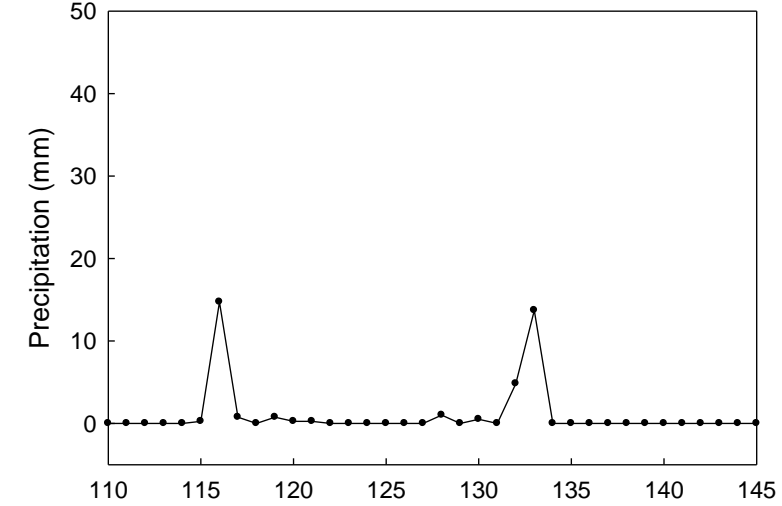
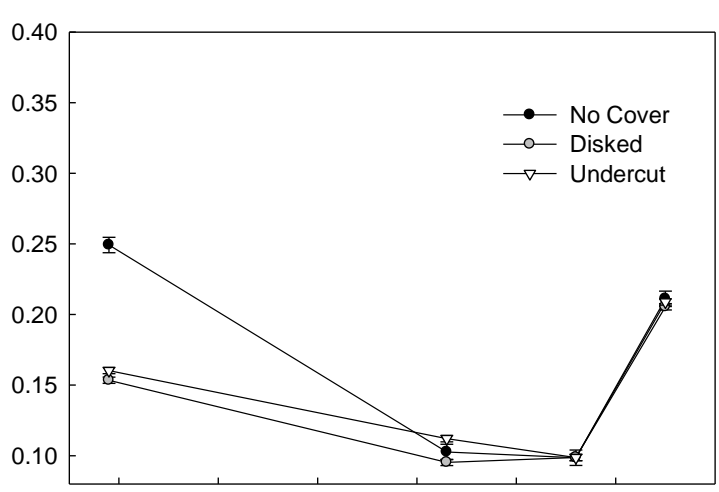
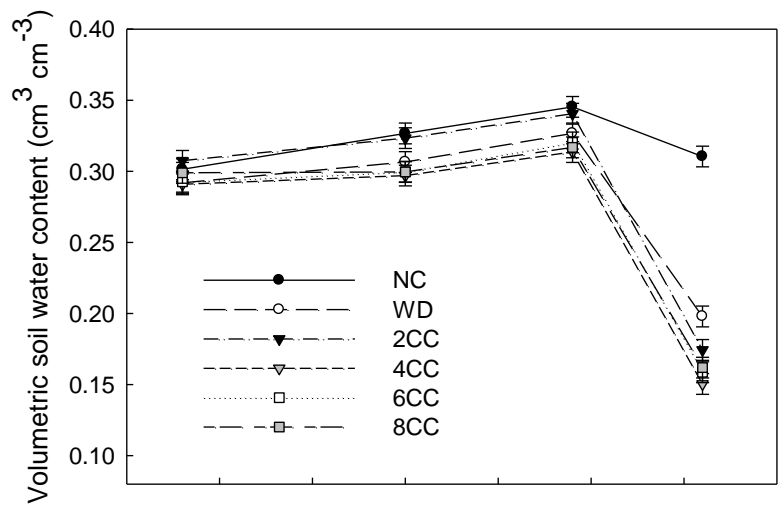
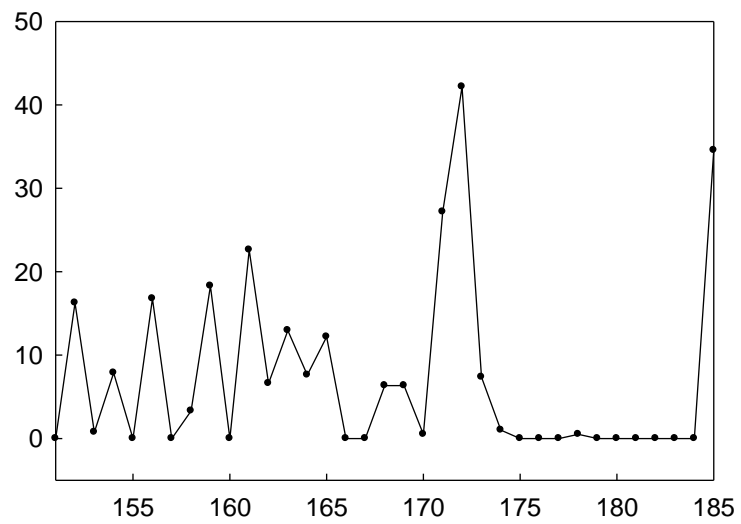
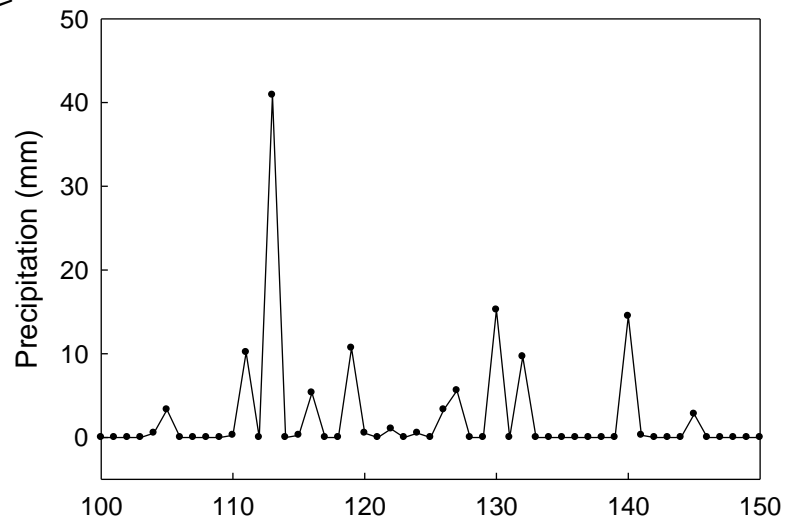
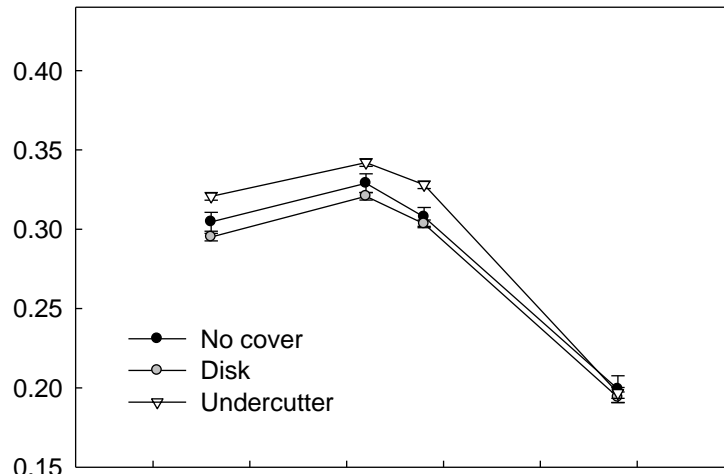
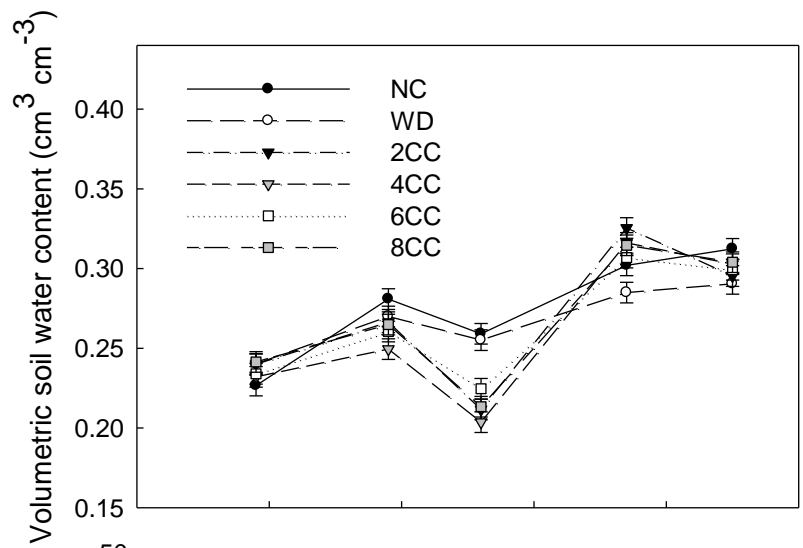


Figure 4.2. Volumetric soil water content ($\text{cm}^3 \text{H}_2\text{O cm}^{-3} \text{soil}$) during cover crop growth (top left) and following cover crop termination (top right) in 2009. Daily precipitation totals (mm) for DOY 110 to 210 are included (bottom left and right). NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 4.1).



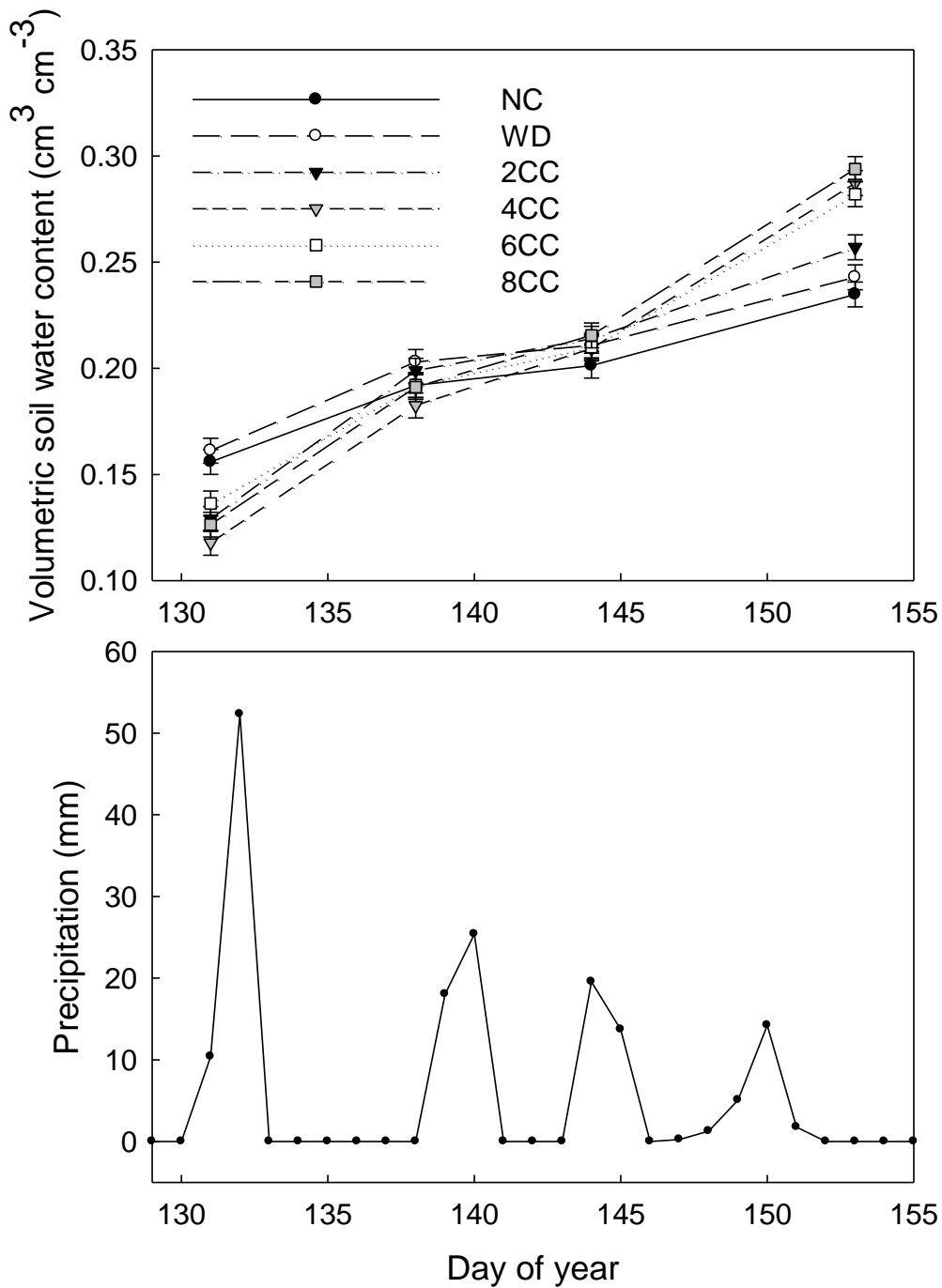
Day of year

Figure 4.3. Volumetric soil water content ($\text{cm}^3 \text{H}_2\text{O cm}^{-3} \text{soil}$) during cover crop growth (top left) and following cover crop termination (top right) in 2010. Daily precipitation totals (mm) for DOY 100 to 185 are included (bottom left and right). NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 4.1).



Day of year

Figure 4.4. Volumetric soil water content ($\text{cm}^3 \text{H}_2\text{O cm}^{-3} \text{soil}$) during cover crop growth (top) and daily precipitation totals (mm) for DOY 129 to 155 (bottom). NC = no cover control; WD = weedy mixture; 2-, 4-, 6-, and 8CC = 2, 4, 6, and 8 cover crop species mixtures, respectively (Table 4.1).



EPILOGUE

Often the most important findings in a research project are the new research questions that arise, and this project is no exception. Overall, we were successful in accomplishing our initial objectives and testing our central hypotheses, but it is hard to feel satisfied when so many interesting and important questions remain. Throughout this epilogue I will address some of the questions I believe this research helped to answer, and other questions this research has spawned. Developing sustainable cropping systems founded in ecological principles is a work in progress, and hopefully the results of this study will serve to advance the science of sustainable agriculture.

Are spring-sown cover crops a feasible option for corn – soybean farmers in the western Corn Belt?

This study has certainly demonstrated that spring-sown cover crops are a realistic option for corn – soybean farmers in the western Corn Belt. However, our results also demonstrate the potential pitfalls of this cover cropping strategy. The most obvious shortcoming of this strategy is the lack of soil coverage achieved from late-fall through the winter months typically achieved with a more traditional winter annual cover crop species. However, this study demonstrated two additional production pitfalls. First, a primary objective for planting the cover crops was to increase soil nitrogen through biological nitrogen fixation. While nodules were observed on most legume roots prior to termination of cover crops in all years (data not shown), the amount of soil N derived from biological nitrogen fixation was probably negligible as soil nitrate did not differ between cover crop mixtures and the WD treatment (ambient weed communities managed as cover crops) in any year of the study or at any sampling interval. While soil nitrate is not a direct measure of biological nitrogen fixation, if “new” nitrogen was being

added to the soil system via fixation one could expect greater levels of mineralized soil N upon decomposition of the leguminous cover crop biomass (which was not observed here). Combined with the reduced potential for biomass productivity (Chapter 1), legumes are likely not an appropriate cover crop choice for spring planting in the western Corn Belt.

The second major pitfall of the spring-sown cover crop option is the potential for yield loss associated with delayed planting of corn and soybean. For a spring-planted cover crop to achieve a substantial level of productivity, it will need to be grown until at least mid-May in most years. A late-May planting date for soybean, and especially corn, can lead to a substantial loss in yield (Lauer et al., 1999). This was observed in 2009 when an early-fall frost terminated the corn crop prior to physiological maturity, reducing grain test weight and yield. The corn hybrid used in this study (BRH 57H36) is a 107 day hybrid, which is shorter than the typical 111-116 day hybrids used in this region of the Corn Belt. This early-maturing hybrid was selected to compensate for the planting dates, but the 2009 yield loss highlights the risk associated with delayed planting even when adjusting crop maturities. Regardless of planting in the fall or spring, if cover crops are to be planted there will need to be some deviation from the traditional corn – soybean rotation and the full season crop varieties often found in the field from April until November. The spring-seeded option tested here is possible, but like all options is accompanied by several challenges and potential pitfalls. Ultimately, the farmer must weigh these challenges against the array of potential benefits offered by cover crops and cover crop mixtures demonstrated here and elsewhere.

Are cover crop mixtures better than a single species cover crop?

The answer to this question, as is the case for many research questions, is “it depends”. In this study we demonstrated the potential for increased productivity, stability, and resiliency in cover crop mixtures compared to the respective monoculture species (Chapter 1). However, the difference in productivity observed among cover crop mixtures rarely led to biologically significant changes in the cropping system. For example, cover crop biomass had no effect on weed suppression, soil nitrogen, or crop yield. While cover crop mixtures may be the most productive and stable option, our results also suggest that cover crop species and community composition may have unique impacts on cropping system properties. This was evident where early-season weed suppression varied among cover crop mixtures, despite no relationship between cover crop biomass and weed biomass. Thus, a single species cover crop may be appropriate when a specific management objective, unrelated to cover crop productivity, is desired. However, farmers should use caution when choosing a single species cover crop as this increases risk of establishment failure associated with management errors and extreme perturbations (Chapter 1; Chapter 3).

Is the undercutter the best tool for mechanical termination of cover crop mixtures?

This study demonstrated that cover crop termination with the undercutter was far superior to termination with the disk. Termination with the undercutter consistently reduced weed biomass throughout the growing season, increased early season soil moisture content, increased soil nitrate availability, and increased crop yield and profitability, relative to termination with the disk. However, many other mechanical termination methods exist and have been tested elsewhere (Creamer et al., 2002).

Currently, the most popular of the mechanical termination methods is the roller-crimper. Development of the roller-crimper and its performance has received a great deal of recent research attention (e.g., Kornecki et al., 2009; Davis, 2010). The roller-crimper is advantageous because it eliminates soil disturbance, but the undercutter is beneficial because of its potential to terminate a wide range of species at different growth stages. The biggest pitfall of the roller-crimper, especially when planting a spring-sown cover crop, is that most species need to reach full-bloom reproductive stages before termination can be achieved. While the undercutter was not always effective against small weed seedlings (Chapter 2), it was generally effective in killing all cover crop species regardless of cover crop growth stage. Thus, the undercutter may be a more appropriate tool for termination of cover crop mixtures, whereas the roller-crimper may be most appropriate for termination of single species cover crops planted in the fall (e.g., winter rye before soybean; Davis, 2010). The merits and short-comings of both implements have now been demonstrated, and the “best” tool will ultimately depend on the management objective, cover crop species/mixture, and cropping system.

How can this experimental cover crop system be improved?

Given the low productivity of legume spp. grown alone and in mixture (Chapter 1), combined with the lack of soil nitrogen contributions from the cover crop mixtures (Chapter 4), it would seem legume spp. could be removed from spring-sown cover crop mixtures in the western Corn Belt. Moreover, legume spp. are typically among the most expensive cover crop options, so removal from the mixture will likely increase the profitability of this cover crop strategy. In contrast, the mustard spp. tested in this experiment were all quite productive and well adapted for early-spring growth in eastern

Nebraska. To replace the legume spp., it would be interesting to include several grass species in the mixture – especially those that have demonstrated allelopathic potential. Potential grass species might include oat (*Avena sativa* L.), cereal rye (*Secale cereale* L.), or wheat (*Triticum aestivum* L.), all of which have demonstrated allelopathic potential (Einhellig and Leather, 1988). The results of this study demonstrated the potential importance of plant and mixture biochemical composition on early-season weed suppression (Chapter 3), but the addition of grass species to a spring-seeded mixture will also increase the C:N ratio of the mixture and delay decomposition of surface residues. Thus, grasses may contribute physical mechanisms of weed suppression to the existing allelopathic mechanisms observed in this study.

What are the long-term consequences of using ambient weeds as cover crops?

Given the results of these studies, especially with regard to cropping system profitability, it would be tempting to suggest that farmers should adopt the use of ambient weeds as cover crops. There may be some merit to this practice, but there are several biological and social implications that should be considered. When weeds were managed as cover crops in this study, there were substantial effects on soil microbial community structure. Some aspects of this community shift have management implications. For example, the WD treatment reduced ratios of FAME biomarkers commonly associated with AM fungi (Chapter 3). Reduced AMF populations may not have affected crop yield in this relatively short-term study, but a long-term reduction in AMF root colonization in crops like corn or wheat, may cause significant yield loss. There were other detectable shifts in community structure in the WD treatment, but unfortunately less is known about the agronomic function and relative value of many microbial groups (e.g.,

actinomycetes). Further studies regarding soil microbial group and ecosystem function will provide insight about the potential agronomic effects of using these weedy species as cover crops. I believe the link between soil microbial community structure and ecosystem functions is fascinating, and remains one of the “great frontiers” in agricultural science.

Perhaps more important than the biological implications, are the social implications of using weeds as cover crops. While some have proposed a paradigm shift in our thinking about weeds (Marshall et al., 2003; Wortman et al., 2010), a large negative stigma will always be attached to weeds and their role in agroecosystems. Even if a farmer were to change their perspective on the role of weeds, it is unlikely that their neighbors would experience a similar conversion. Farmers are undoubtedly driven by social forces at work on the community level, and allowing ambient weed communities to reach reproductive stages would likely draw negative attention in many rural communities. However, this negative attention is not entirely unfounded as the persistence of mature weed communities could have serious biological implications on a landscape level.

One of the most serious weed management issues facing farmers in the Corn Belt right now is the increased incidence and spread of herbicide resistant weed populations. Managing ambient weed communities as a cover crop may not provide additional selection pressures that lead to the incidence of resistant populations, but it may contribute to the spread of resistant populations as pollen and seed dispersal are not confined to individual fields. It is possible that cross pollination would occur with herbicide resistant weeds of neighboring fields; thus, if weedy cover crops were inadvertently allowed to reach full maturity and set seed this practice could potentially

accelerate an already rapidly-growing problem. A similar example, more pertinent perhaps to organic farmers, is the potential for ambient weed communities to promote the incidence of soil pathogens like soybean cyst nematode. Many winter annual weeds like henbit, field pennycress, and purple deadnettle, are alternative hosts for soybean cyst nematode (Venkatesh et al., 2000), which is a difficult soil pathogen to control, especially in organic cropping systems where reactive control options will be limited. These issues (e.g., herbicide resistance and soil pathogens) are often localized to individual farmer fields, but can quickly spread across landscapes if “best management practices” are not used. With regard to these management issues, the persistence of ambient weed communities as a cover crop would not be considered a best management practice by most standards (e.g., social and scientific).

Are the results of this project only applicable to organic farmers?

There are aspects of this project and the results that will be of most use to organic farmers in the western Corn Belt. For example, differences in mechanical termination strategies may not be relevant to conventional farmers in this region who primarily practice no-till agriculture. However, some of the more general findings in this study should be relevant to both organic and conventional farmers. First, evidence for increased productivity, stability, and resiliency with increasing diversity observed in Chapter 1, has implications beyond cover crop mixtures. Increasing the diversity of any aspect of cropping systems or farming enterprises, should provide opportunities to realize the same benefits observed here in our diverse cover crop mixtures. For example, temporal or spatial diversification of the traditional corn – soybean rotation by adding a small grain

like winter wheat to the rotation may increase the long-term productivity and yield stability of the system (Smith and Gross, 2006).

While the specific mechanical termination methods tested in this study may not be adopted by conventional farmers, the results should be relevant to conventional farmers looking to use cover crops. Conventional farmers are often curious about when they should spray their cover crops relative to corn or soybean planting. The best answer to this question will depend on a variety of factors (e.g., herbicide chemistry, soil type, climate, planting dates, and cover crop species), but the agronomic response to the undercutter may provide insight about this question. The undercutter leaves cover crop residue fully intact on the soil surface, whereas chemical termination will result in fully intact residue still standing in the soil. The soil surface to plant contact surface area will be greater following undercutter termination, presumably increasing decomposition rates, but these two methods are likely similar in their agronomic response. Thus, if an appropriate herbicide is used (one that does not have residual planting restrictions) it would seem cash crops can be planted as early as three days after chemical termination of similar cover crop mixtures without adverse agronomic effects (e.g., mustards and legumes; relatively low C:N ratio). Corn, soybean, and sunflower were all planted within 3 to 7 days of undercutter termination without adverse agronomic effects often associated with cover crop decomposition (e.g., N immobilization and reduced crop stands). This is contrary to many anecdotal recommendations that exist within the cover crop community, where 7 to 14 days prior to planting is the usual suggestion for termination. However, this recommendation is based on the assumption that corn will be planted in early-May, not late-May as practiced in this study. If a cover crop is terminated in late-April, soil and

weather conditions may require a longer planting interval due to slower herbicide activity and decomposition rates. While this study contributes new information regarding cover crop termination timing, determination of the most appropriate timing method remains a complex issue that requires consideration of many management factors.

What is the future of cover crop mixture research?

The science of cover crop mixtures is still in its infancy. While multi-species plant community dynamics have been well-studied in the field of ecology, the design of multi-species plant mixtures to meet specific management objectives is unique in many ways. In a natural ecosystem the objective for multi-species plant communities is to maximize net primary productivity and fecundity. However, in a managed agroecosystem farmers may seek to maximize plant characteristics like biological nitrogen fixation or allelopathic chemical synthesis. Indeed, two areas of future research opportunity include the design of cover crop mixtures to maximize biological nitrogen fixation of legumes and also the design of mixtures that will stimulate accumulation of effective phytotoxic compounds. Plant stress has been shown to increase the production of allelopathic compounds in several plant species (Hall et al., 1983; Williamson et al., 1992), and appropriate interspecific competitive interactions in a cover crop mixture may help to maximize allelopathic mechanisms of weed control.

If the objective for the cover crop is to maximize productivity, there are research questions that remain regarding appropriate species composition. Specifically, how do root and canopy architecture contribute to mixture productivity? Is a mixture of species with similar morphology best (i.e., simulated intraspecific competition), or should a range of species with very different morphologies be used in mixture? In this study we assumed

that a mixture of low growing legumes and upright mustards with variable root architectures (e.g., branched, fibrous, and tap) would maximize productivity, but this hypothesis was not directly tested. A great deal of interest and uncertainty remains regarding the most appropriate mixture composition and selection.

Studying the theories of ecological resilience with regard to cover crop mixtures is another area of potential future research. We observed anecdotal evidence of increased resilience in cover crop mixtures relative to monocultures following a 2011 hail storm, but no formal hypotheses were tested regarding the concept of ecological resilience (Chapter 1). The concept of maximizing ecological resilience for stable cover crop productivity is interesting because strong resilience may become problematic when one seeks to terminate the cover crop mixture. This distinction highlights the difference between natural multi-species plant communities and those managed in agroecosystems. The challenge for researchers is to develop cover crop mixtures that are resilient to extreme natural factors like wind, hail, and drought, but are then susceptible (not resilient) to management factors like undercutting, roller-crimping, or broad-spectrum herbicide application.

Though still a management practice used by a minority of farmers, cover crops and cover crop mixtures are increasing in popularity especially among organic farmers. This study provides science-based information and practical solutions for more informed cover crop management decisions, but many knowledge gaps remain. Given the growing enthusiasm and funding opportunities for sustainable agricultural practices at the local, regional, and federal level, it is my hope that we can continue to answer some of these remaining cover crop research questions. Furthering the science of cover crop

management will be essential for increased adoption on organic and conventional farms throughout the western Corn Belt.

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