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Reducing Herbicide Use Through Cropping System Diversification: A Case Study at the Iowa State University Marsden Farm, and Some Recommendations for the Mekong Delta of Vietnam

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Reducing herbicide use through cropping system diversification: A case study at the Iowa State University Marsden Farm, and some recommendations for the Mekong Delta of Vietnam

by

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CHAPTER 1 INTRODUCTION

1.1 Weeds as a key challenge in grain production

Weed management has been a common practice since humans first domesticated crops. However weed management in earlier ages was sometimes so laborious that the gain in crop yield from hand-weeding did not meet a person's need for food (Monaco et al., 2002). Weed management has been recognized as a major challenge in farming because weeds can decrease crop productivity, product quality, and resource management efficiency (Ennis et al., 1963). Affholder et al. (2013) documented that weed infestation and poor soil fertility remain as major yield constraints in grain production in developing countries including Vietnam. Herbicides have been widely adopted in industrialized agriculture settings, and their use dominates all other pesticides in U.S. agriculture (Nehring, 2012). Herbicide use in Vietnam is also increasingly common among all pesticide uses (Khanh et al., 2006).

In the context of intensive agriculture, which is widespread in developed countries, continuous monocropping or short crop rotation systems, synthetic fertilizers and pesticides, and high-efficiency farming machinery are favored for weed control for their convenience, effectiveness and efficiency (Connor et al., 2011). In less intensive agriculture systems, which are often seen in developing countries, yield loss to weeds is about two or three times greater than the loss in developed countries (Labrada, 1996). Weeds are the most serious yield constraint to direct-seeded rice production in the Mekong Delta (Azmi et al., 2005). Chin and Mortimer (2002) estimated that in Vietnam, direct-seeded rice yield loss to weed infestation was about 46 percent.

1.2 Cropping systems in the Midwestern U.S. and Vietnam's Mekong delta region, with special reference to weed control

Cropping systems in both the U.S. Midwest and Vietnam's Mekong Delta region have been dominated by the same crops for a long period of time: corn and soybean in the Midwest and rice in the Mekong Delta. The U.S. Midwest has been planted to corn since the Industrial Revolution and is now dominated by corn and soybean production because the geographic conditions and transportation infrastructure made these crops the most economically beneficial (Hudson, 1994). Corn and soybean are planted on approximately 50% of farmland in the U.S. (Census of Agriculture, 2014), and occupy 50 – 60% of arable land in the U.S. Midwest (Roser, 2016). Rice is planted on 82% of arable land in Vietnam with 52% of rice produced in the Mekong River Delta (International Rice Research Institute, 2016). Three rice crops are produced annually in the Mekong Delta under the Vietnamese government's "rice first" policy (Smith, 2013), which emphasizes rice production for export and therefore focuses on production and productivity rather than domestic consumption and farmers' needs (Yakub et al., 2012). The Mekong Delta region has recently changed its agricultural land use distribution from scattered and fragmented fields to "large field" production, in which neighboring farmers work as members of cooperatives and are encouraged to establish long-term relationships with input suppliers and output traders, for better accommodation of mechanization (Smith, 2013).

Weeds that bear herbicide resistant genes occur at low frequency in nature (Cousens and Mortimer, 1995), but weed populations evolve resistance through a chain of mutation and selection events in which herbicides play key roles as selection pressures (Gressel, 2009;

Gressel and Levy, 2006). Monocropping or short rotation sequences coupled with repeated use of one or a few herbicides, and neglect of other weed management tactics, expose weeds to the same set of selection pressures over a long period and enhance resistance to herbicides via an evolutionary adaptation process (Heap, 2014; Heckel 2012; Shaner, 2014), which ultimately further complicates weed management (Mortensen et al., 2012).

Herbicide resistance in weed populations is now a major challenge for U.S. agriculture (Owen, 2008). Particularly problematic weed species include common lambsquarters (*Chenopodium album* L.), velvetleaf (*Abutilon theophrasti* Medik.), and several *Amaranthus* species. Similarly, in Asian direct-seeded rice fields where herbicides have been applied intensively, weed community composition has shifted from broadleaf and sedge species to noxious graminoids (Azmi and Baki, 1996; Itoh, 1991).

In Vietnam's Mekong Delta region, direct seeding is dominant in rice production because it is more cost-effective than transplanting (Chin and Mortimer, 2002). However, direct seeding techniques applied in the region provide a better environment for weed growth than do transplanting techniques (Dung and Dung, 1999). The most commonly applied weed control approach in the Mekong Delta is herbicides and the next most popular means is hand-weeding (Chauhan et al., 2015). Even though weed resistance to herbicides in rice in Vietnam has not been well documented (Azmi et al., 2005; Khanh et al., 2006), the current rice farming intensity and associated management practices are likely to result in a similar profile of weed resistance to herbicides in the Mekong Delta as has been noted in other rice-based regions in Asia. Some of the most problematic weed species with regard to herbicide resistance in rice-based regions are barnyardgrass (*Echinochloa crus-galli* var. *crus.galli* (L.) P. Beauv.), which has resistance to multiple herbicides in direct-seeded rice in the Philippines (Juliano et al., 2010), and weedy rice (*Oryza sativa* L.), which is thriving under popular chemical control regimes throughout Asia (Singh Chauhan, 2013).

Herbicides have become increasingly common in the U.S. and Canada since the 1950s (Timmons, 1970). Since the early days of herbicide use, academic scientists have discussed conditions enhancing herbicide resistance in weeds and the means to avoid resistance (Appleby, 2005; Gressel and Segel, 1978; Krimsky and Wrubel, 1996; Shaw, 1964). About twenty years after the widespread adoption of herbicides, weed resistance was reported to triazine herbicides in populations of annual grasses in the genera *Digitaria*, *Panicum*, *Setaria* and *Sorghum*, and certain broadleaf species including *Senecio vulgaris* L., *Amaranthus retroflexus* L.*, Chenopodium album* L., and *Ambrosia artemisiifolia* L. (Radosevich and Devilliers, 1976; Ryan, 1970). Later, weeds that express resistance to multiple herbicides were reported in Australia and other nations (Christopher et al., 1992; Powles, 1994; Preston et al., 1996). During 1980 – 1995, the number of weed resistance cases increased from 41 to 191 around the world (Shaner, 2014). Herbicide resistance problems in weeds are exacerbated by the fact that there are sophisticated technical and financial difficulties in herbicide discovery and testing, and thus the current agriculture industry has relied on the same mechanisms of action (MOA) for two decades (Duke, 2012).

Glyphosate is a non-selective herbicide that is applied to plant leaves, and is the most commonly used herbicide in the world. At first, glyphosate was used for non-agricultural purposes or in fallow fields only because it has broad spectrum activity range (Nandula, 2010). However, it became widely applied for weed management on crop fields when glyphosate resistant (GR) crops became available (Padgette et al., 1996). Transgenic approaches were used to create GR crops after a glyphosate-insensitive enzyme (enolpyruvyl shikimate-3-phosphate (EPSP) synthase) (Amrhein et al., 1980), that was similar to plants', was found in a soil bacterium and transferred to crops (Franz, 1997). Glyphosate is the most popular among commercial herbicides because it is a broad-spectrum, nonselective chemical and is considered by a number of analysts to benefit both crop producers and the environment (Brookes and Barfoot, 2013; Green and Castle, 2010). However, use of glyphosate and glyphosate resistant crops decreased the rotation of herbicides with different mechanisms of action (Frisvold et al., 2009b).

The widespread adoption of glyphosate resistant (GR) crops and glyphosate as a technical package produced significant success in weed control for the first few years since introduction in 1996 and glyphosate use in recent years has increased (Benbrook, 2012). However, a closer look at the techniques revealed some consequences of over-reliance on transgenic herbicide-resistant crops such as increased weed resistance, decreased glyphosate effectiveness for weed control, and increased herbicide costs (Benbrook, 2012; Brookes and Barfoot, 2013; Duke and Powles, 2009; Heap, 2014; Mortensen et al., 2012). There are also concerns over transfer of glyphosate resistance from crops to weeds (Boudry et al., 1993; Londo et al., 2010; Légère, 2005; Snow and Palma, 1997; Snow et al., 2003; Wolfenbarger and Phifer, 2000). Despite these concerns, glyphosate and transgenic GR crop packages have remained widely adopted by farmers (Fernandez-Cornejo et al., 2014). In fact, resistance to glyphosate in the U.S. was found in 1999 – 2000, soon after glyphosate and GR crop packages were first commercialized, in horseweed (*Conyza canadensis* (L.) Cronq.) (VanGessel, 2001), Palmer amaranth (*Amaranthus palmeri* S. Wats) and common waterhemp (*Amaranthus rudis* J.D.Sauer) (Heap, 2014). The effectiveness of glyphosate coupled with GR crops is decreasing as a consequence of repeated use for an extended period of time (Green and Owen, 2011). In an effort to solve resistance problems, second generation transgenic herbicide resistant crops that tolerate other popular herbicides are being moved toward commercialization (Wright et al., 2010), especially ones bearing acetyl-CoA carboxylase (ACC), acetolactate synthase (ALS), synthetic auxin, and hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (Green, 2014). Those new HR crops are under USDA review for deregulation in the near future (Bell and Cole, 2015) regardless of evidence for herbicide-resistance evolution in weeds, including cases of multiple resistance that were previously reported (Heap, 2014; Mortensen et al., 2012).

Adoption and diffusion of herbicide resistant seeds in the U.S. were recognized to be dependent on farm profits as well as the simplicity and availability of required technologies (Ervin et al., 2010; Frisvold et al., 2009b). For example, adoption of herbicide resistant soybean and cotton helped reduce by 14.5% and 69.5% time per household required for farm work, respectively (Gardner et al., 2009). Convenience could be the most important reason why farmers are less willing to diversify their cropping systems, but instead stick to monoculture or short rotations with GR crops. These practices subvert the herbicide resistance control effect that extended crop rotations can offer.

Given the available weed management methods such as chemical and mechanical tactics and crop rotation (Chauhan et al., 2015), and current socio-economic context (Ives, 2013; Paris et al., 2009), weed control in the Mekong Delta region is likely to become more dependent on herbicides. Hand-weeding is five times more expensive than herbicide spraying in Vietnam (Moody, 1992). As industrial zones encroach on agricultural areas and offer farmers additional sources of income, a large number of farmers would work two jobs or undermine agriculture (Hong Van, 2016). This phenomenon implies a labor shortage situation for agriculture. To save labor, direct seeding is expected to become more popular in rice production regions (Rao and Ladha, 2013).

Overall, exclusive use of one weed management tactic would not be as effective as combined techniques that follow a “many little hammers” concept (Liebman and Gallandt, 1997). Reduced reliance on herbicide application in combination with strategic cultural practices can be a key to more resilient, cost-effective and environmental-friendly weed management approaches (Liebman, 2001; Mortensen et al., 2012; Moss, 2003). The more complex a weed management program is, the more effectively it could control weeds (Bates et al., 2012; Buhler, 1996; Davis et al., 2012; Green and Owen, 2011). The concept of a multi-faceted weed management program suggests not depending solely on one single means of weed control. However, agro-ecologically based weed control practices are often discouraged by policies favoring monoculture, research agendas focusing mainly on herbicides, and inadequate information and technical support to farmers (Gurian-Sherman and Mellon, 2013).

There has been a paucity of research addressing important weed management problems, such as herbicide drift management, social aspects of weed management, weed community shifts, and global change and invasive species. In contrast, research on herbicide efficacy has been more favored, usually funded by private sources, largely to the exclusion of interdisciplinary research focused on ecologically-based control strategies (Davis et al., 2009). Over the long term, problematic weeds that are successful in thriving under anthropogenic pressures could be highly detrimental to crop yield and productivity. Therefore, it is important to develop crop management strategies that are weed suppressive, highly productive, and sustainably profitable. Further investigations of weed dynamics in diversified cropping systems can be expected to be useful to developing communities, where herbicide use has increased in recent years. Adopting a "many little hammers" strategy for weed management in such communities may limit herbicide resistance evolution due to herbicide overuse (Liebman and Gallandt, 1997; Westerman et al., 2005).

Previous studies, in which transgenic corn and soybean were incorporated in the cropping systems, have proven some advantages of diverse cropping systems in ecological weed control (Davis et al., 2012; Gómez et al., 2013; Heggenstaller and Liebman, 2006; Heggenstaller et al., 2006; Liebman et al., 2008; Westerman et al., 2005). However, the long-term effectiveness of glyphosate resistant crops in integrated cropping systems is uncertain and requires more research (Kruger et al., 2009; Riar et al., 2013a; Riar et al., 2013b).

Facing the shortcomings of transgenic crops and herbicide packages as reviewed above, the research presented in this thesis was carried out to see how much herbicide use could be reduced for conventional hybrid corn and transgenic, glyphosate resistant soybean through cropping system diversification. We focused on the technical aspects of agro-ecological weed management in order to provide practical information for farmers. In this research, a conventional cropping system consisting of corn and soybean rotated over a two-year period was treated with chemical fertilizers and herbicides. Given that description, diverse cropping systems would have at least one of the following characteristics: more crops, multiple sources of nutrients, and a wide range of weed control tactics. We hypothesized that diverse cropping systems, with reduced use of chemical herbicides, would provide equal weed control efficacy to conventional approaches. A metric to evaluate efficacy for weed control was crop yield: if crop yields were not significantly different across cropping systems, then the weed control programs in those systems were considered equally effective. Additional metrics evaluated included total weed biomass accumulation and weed seedbank population densities. We evaluated these metrics in conventional and low herbicide input regimes used for three crop rotation systems (i.e., six treatments in total), in a field experiment at Iowa State University's Marsden Farm, in Boone County, Iowa.

A broader impact of this study is some possible recommendations for Vietnam's Mekong Delta rice-based production. The main reason for the large gap between potential and best farmers' yields in developing countries in general and in Vietnam specifically is that farmers cannot afford the newest farming technologies used in high-yielding, high-input systems (Affholder et al., 2013). In terms of weed management, seeing the advantages and disadvantages of conventional and diverse cropping systems could provide more insights to tailor practical approaches in order to close the yield gap with fewer chances to invite irreversible consequences.

1.3 Thesis organization

My thesis is written in four chapters based on one field experiment with data collection from two seasons.

Chapter one includes a review of pertinent literature.

Chapter two describes the effects of crop rotation systems and herbicide use intensity on weed population density, biomass, and seedbank density.

Chapter three provides a statistical analysis based on a Monte Carlo simulation procedure to evaluate soil seedbank sampling procedures.

Chapter four concludes with key findings and proposes some practical recommendations for the Mekong Delta rice production area at the stage of more intense agricultural industrialization.

CHAPTER 2 WEED POPULATION DENSITY, BIOMASS AND SEEDBANK DENSITY AS AFFECTED BY CROP ROTATION SYSTEMS AND HERBICIDE USE INTENSITY

2.1 Introduction

2.1.1 Advantages of cropping system diversification with special reference to weed control

Pingali (2012) found that yield increases of corn, rice and wheat were made possible mainly with increased stress tolerance and pest resistance abilities, intensive pesticide and chemical fertilizer use, and improved irrigation in favorable regions; in regions in which farmers have not had access to those technologies and resources, yields have remained low. Simons (2012) analyzed three farming approaches, namely industrial, traditional and ecological, and concluded that industrial agriculture, which is dominant in developed countries, relied mainly on high-input systems to maintain high yield. According to Simons' study, the industrial agriculture approach was considered to be unsustainable because it consumed natural resources faster, produced more wastes, and invited more socio-economic troubles for small-scale farmers than the other two farming approaches.

Pingali's and Simons' studies suggest that sustainable farming should rely minimally on external inputs. Low external input (LEI) cropping systems minimize the use of purchased fertilizers and herbicides as much as possible in order to reduce production costs, lower negative environmental effects, and reduce chemical residues in food, all of which can work toward increasing a farm's short-term and long-term profitability (Gold, 2007). Most such systems also involve addition of organic material to the soil and crop diversification as key strategies to address economic, environmental, and social problems associated with conventional farming systems (Karlen et al., 1994; Liebman and Davis, 2000). In developing countries, cropping system diversification also helps improve rural community economies through minimization of financial risks to farmers, enrichment of agricultural-based industries, and establishment of new profitable markets (Herrero et al., 2014).

Cropping system diversification through strategic rotation sequences of main crops, cover crops and intercrops, with additional fertilizer resources from livestock and green manures, can impose stress on weeds and increase mortality effects on weed populations, and thus can be useful for maintaining weed control effectiveness and efficiency while lessening herbicide dependence (Garrison et al., 2013; Liebman and Dyck, 1993; Nazarko et al., 2005; Wortman et al., 2013). For example, the assemblage and activity of ground beetle communities (Coleoptera: Carabidae) were significantly greater in a 4-year rotation system than in a 2-year rotation system (O'Rourke et al., 2008). Ground beetles constitute an important family of generalist predators in agro-ecosystems (Kromp, 1999) that consume a significant amount of weed seeds and invertebrate pests (Toft and Bilde, 2002). Unburnt residues on no-till wheat-soybean double crops effectively suppressed weeds without decreasing herbicide efficacy (Amuri et al., 2010). Furthermore, reducing the number of weed plants exposed to herbicides by using herbicides only when cultural and mechanical controls are ineffective (Blackshaw, 2008) could substantially lessen selection for resistance and eventually decelerate the adaptation process (Gressel and Levy, 2006).

Though the availability of agricultural technologies in developing countries is different from developed countries, cropping system diversification is considered the most advantageous means in developing countries to control the most noxious weeds with least environmental consequences (Labrada, 1996). A rice - mung bean (*Vigna radiata* (L.) R. Wilczek) rotation with a fallow period, and increased use of certified seeds was shown to significantly reduce weedy rice (*Oryza rufipogon*) infestation (Watanabe et al., 2000). Rice-duck and rice-fish combinations in which ducks and fish are introduced into paddy fields at critical periods can be effective for removing weeds and insect pests and improving soil organic content. Berg (2001) noted that rice-fish systems helped reduce herbicide use by almost 50 percent in comparison to a conventionally managed rice system in the Mekong Delta. Rice-fish systems could also increase a farmer's net income up to 65 percent compared to rice monoculture (Halwarth, 1998).

2.1.2 Challenges that constrain cropping system diversification for weed management

Cropping system diversification used to reduce external input reliance could bring greater agro-ecological benefits yet its economic results are less certain due to variation of multiple factors, such as crop yield levels, crop prices and input costs. Consequently, it is not surprising that farmers would hesitate to adopt it. Mahoney et al. (2004) studied a corn-soybean-oat–alfalfa cropping system under organic, low and high input regimes and concluded that the organic regime increased net returns but sometimes required additional spending on alfalfa management. Menalled et al. (2005b) studied a chisel plowed corn-soybean-winter wheat rotation and found that swine manure would pose a small risk of increased weed seed abundance in the seedbank. Therefore, swine manure would pose an additional concern when one wants to diversify nutrient supply resources. Blackshaw (2008) studied cover crops' effects on dry bean and found that cover crops exerted some weed suppression benefits, but dry bean still required herbicide spray in order to maintain yield. Delbridge et al. (2011) compared the economic performance of a corn-soybean rotation and a corn-soybean-oat/alfalfa-alfalfa rotation under chemical and organic inputs and concluded that diversification did not increase crop yield while it further complicated management and reduced overall profitability. Cropping system diversification in Vietnam has received little attention and specific benefits of crop rotation, cover crops, intercropping and mulching on weed control in rice are unclear (Khanh et al., 2006). Overall, the abovementioned results of overall cost-benefit balance of low external input systems are inconsistent and thus their profitability requires further investigation.

Uncertainty of farm profit aside, there is a popular thought among farmers that weed resistance to herbicides is natural and thus cannot be managed (Wilson et al., 2008). Moreover, many farmers also believe that rotating herbicides with different mechanisms of action is substantially more complicated but less reliable than using one, e.g. glyphosate, so they tend to abort herbicide rotation (Arbuckle and Lasley, 2013; Frisvold et al., 2009a) and hold high expectations of new herbicide mechanisms of action becoming available (Foresman and Glasgow, 2008; Llewellyn et al., 2002). In developing countries, farmers' low literacy and inadequate knowledge have discouraged cropping system diversification (Food and Agriculture Organization, 1985).

In addition, market availability is not yet supportive of cropping system diversification. The oat and wheat producing area in Iowa has shrunk and been replaced by corn and soybean (Gibson and Benson, 2002; National Agricultural Statistics Service, 2015) because farmers would face more financial risk growing small grains than growing corn and soybean (Sustainable Food Lab and Practical Farmers of Iowa, 2015). Similarly, demand for rice from Vietnam is growing, for both domestic consumption and export (International Rice Research Institute, 2015). Though some of the rice area is projected to be switched to other crops, the substituting crops are common cash crops, i.e. corn and soybean, and the projected area to be switched is only 2% of the current rice area (Tran, 2015). These facts indicate that monocropping and short rotations are likely to continue to dominate crop-intensive regions including the U.S. Midwest and Vietnam's Mekong delta.

Sustainably reducing reliance on herbicides is a sophisticated process because it requires a comprehensive set of tactics that include cautious selection of herbicide active ingredients, application technology and timing, and combinations of biological, mechanical and cultural weed control practices in order to best accommodate crops while best disturbing weeds (Bastiaans et al., 2000; Liebman and Gallandt, 1997). Notably, crop sequence and associated weed management techniques can strongly affect weed growth, reproduction, and weed seed additions to the soil seedbank (Liebman et al., 2004). In certain cases, chemical inputs can be reduced for weed management by creating more favorable conditions for crops and less favorable conditions for weeds through cropping system diversification, incorporation of green and animal manures, and delayed nitrogen fertilizer application (Liebman and Davis, 2000). To elucidate benefits and challenges of cropping system diversification for weed control in U.S. Midwest, a long-term experiment was established in Iowa State University Marsden Farm.

2.1.3 Summary of previous research at the Marsden Farm site

In 2002, an experiment was established in a 9-ha field at the Iowa State University Marsden Farm in Boone County, IA, to examine whether crop yield and weed suppression characteristics of low external input (LEI) cropping systems equaled or surpassed those of conventional systems. Since establishment, the experiment has included a 2-year conventional corn-soybean rotation and two more diversified cropping systems (3-year and 4-year) with small grains and forage crops. Using field data from this experiment, Westerman et al. (2005) modeled the corn-soybean rotation and a 4-year corn-soybean-triticale/alfalfa-alfalfa rotation and concluded that the more diverse system, which received 82% less herbicide, would effectively suppress velvetleaf (*Abutilon theophrasti* Medik.) through the effects of multiple stresses. Heggenstaller and Liebman (2006) studied the experiment's 2-year corn-soybean rotation, its 3-year corn-soybean-triticale/red clover rotation, and its 4-year corn-soybean-triticale/alfalfa-alfalfa rotation and confirmed Westerman et al.'s (2005) finding. The authors also noted that the 4-year rotation, which used 79% less herbicide than the 2-year rotation during the study period, effectively suppressed giant foxtail (*Setaria faberi* Herrm) populations. Liebman et al. (2008) studied the 2-year, 3-year, and 4-year rotations in the experiment and reported that the two more diverse cropping systems required less external inputs such as synthetic fertilizer and herbicides, and provided a reliable agronomic and economic alternative to conventional agriculture. Having further researched the same cropping systems, Davis et al. (2012) reported that, over a nine-year period, soil weed seed densities decreased at an equal rate in the 2-year corn-soybean rotation managed with conventional herbicide inputs as compared with the more diverse 3-year and 4-year systems managed with low herbicide level inputs. Gómez et al. (2013) observed that, over a three-year period, weed biomass was maintained at a low level in each of the rotation systems regardless of herbicide use intensity. The abovementioned findings are useful in detailing ecological and economic benefits of diversified cropping systems, yet several questions regarding the agronomic performance of these cropping systems and their effects on weed population dynamics remain unanswered.

Overall, in previous research projects at the Marsden Farm site, the conventional cropping system was a 2-year corn and soybean rotation (*Zea mays* L.)-soybean (*Glycine max* (L.) Merr.) treated only with inorganic fertilizers and chemical herbicides for fertility maintenance and weed control. The 3-year (corn-soybean- oat (*Avena sativa* L.)/red clover (*Trifolium pretense* L.) and 4-year (corn-soybean-oat/alfalfa-alfalfa (*Medicago sativa* L.)) systems that used additional types of crop management inputs (livestock manure and mechanical weed controls) and included more crops were considered diversified. In 2014 – 2015, the experiment followed the same description of cropping system diversity. Each of the three rotation systems was managed with two herbicide regimes: conventional and low-input.

2.2 Materials and methods

2.2.1 Cultural and mechanical weed control methods

In general, we were interested in how weed dynamics respond to cropping system-herbicide regime combinations, including how weed communities might differ between the conventional and low herbicide regimes that are applied to the same crop phase. We hypothesized that diverse cropping systems, with cultural and mechanical weed controls used to substitute for a portion of herbicide inputs, would be equally effective in weed control compared to conventionally managed cropping systems receiving herbicides at normal rates.

2.2.1.1 Crop sequence and cropping system diversification

There can be more events of weed mortality in diverse cropping systems than in monoculture (Martin and Felton, 1990). The effectiveness of a rotation for weed suppression is based on the strategic crop sequence selection in which crops have different characteristics, such as planting and maturation date, growth habit, relative competitiveness with weeds, and requirements for management (Liebman and Janke, 1990). Crop sequencing for weed control should not promote proliferation of any single weed species by creating frequently changed, disruptive stresses over multiple species and therefore should include a diversity of crop types (Liebman and Dyck, 1993). Intercrop mixtures that include species that use different nutrient sources usually establish complementary relationships (Willey, 1990) and are usually more effective in weed suppression than sole crops (Liebman and Dyck, 1993). Over the long term, longer rotations with crops spanning a diverse range of phenology should reduce the weed seed population densities in the soil seedbank (Teasdale et al., 2004).

We chose oat, alfalfa and red clover to diversify the conventional corn-soybean system because these crops were believed capable of improving weed suppression. Each crop also serves at least other two purposes: cattle feed and live mulch. Oat/alfalfa and alfalfa/red clover companion crops suppressed weeds and increased hay yield relative to the legumes grown alone (Lanini et al., 1992; Wiersma et al., 1999). Oat has been recognized for weed suppression when incorporated in various cropping systems (Liebman and Dyck, 1993; Weston, 1996). Oat and oat residue helped suppress common lambsquarters (*Chenopodium album* L.) and shepherd's purse (*Capsella bursa-pastoris* (L.) Medik) (Grimmer and Masiunas, 2005). Alfalfa was found superior in suppressing common lambsquarters in the Netherlands as compared to other five popular cover crops (Kruidhof et al., 2008). Non-dormant alfalfa was effective in suppressing pigweed (*Amaranthus* spp.) in the western U.S. (Huarte and Arnold, 2003). Manipulation of alfalfa cutting regimes significantly suppressed yellow foxtail (*Setaria glauca*) in California (Norris and Ayres, 1991). Phytotoxins from red clover were recognized as a potential alternative to synthetic herbicide (Ohno et al., 2000). Red clover is chemically suppressive to small-seeded weeds (Liebman and Davis, 2000), and can enhance conditions for granivorous invertebrates that help control weed seedbanks (Gallandt et al., 2005; Heggenstaller et al., 2006). Red clover when undersown into cereals decreased weed emergence (Dyke and Barnard, 1976; Maiksteniene et al., 2009). Red clover was found more effective than herbicide for suppressing common ragweed (*Ambrosia artemissiifolia* L.) growth and seed production in winter wheat (Mutch et al., 2003).

2.2.1.2 Combination of banded herbicide application and mechanical weed control

Weed seedlings in agricultural fields often occur in aggregated patches (Dessaint et al., 1991; Vangroenendael, 1988). With banded pre-emergence herbicide application over crop rows in commercial corn in Nebraska, 71% and 94% intra-row areas were found free of broadleaf weeds and grasses, respectively while those numbers for inter-row areas were 30% and 72% (Johnson et al., 1995). The authors therefore suggested that herbicide use could be reduced if applied in bands instead of broadcast where weeds were present or exceeded a threshold density. According to the threshold density concept, weeds that grow within crop rows would be relatively more competitive to crops than weeds that grow between crop rows. Post-emergence banded herbicide combined with inter-row cultivation in soybean also reduced the amount of herbicide used by 50 to 75 percent (Buhler et al., 1992). We drew on these results for our experiment in which corn and soybean rows under a low herbicide regime were treated with 38 cm-bands of post-emergence herbicides. Inter-row cultivation was used to kill weeds between crop rows. This combination of weed controls, which apply "many little hammer" concept in diversifying weed control means used to manage the present weed community, was intended to reduce the amount of herbicide used and the number of weeds exposed to herbicide and eventually contribute to slowing weed resistance to herbicide evolvement.

2.2.1.3 Composed livestock manure and green manure amendments

Manure is a valuable resource for nutrient supply but may contain viable weed seeds. Dairy manure that was collected from dry pens and liquid manure sedimentation handling facilities was found highly contaminated with weed seeds (Cudney et al., 1992). Compared to swine manure (Menalled et al., 2005a), ruminant manure seems to pose less risk of increasing the number of viable seeds in the soil because ruminant digestion reduces weed seed viability, (Atkeson et al., 1934; Harmon and Keim, 1934). Composting was found to be effective in reducing weed seedbank persistence because of compost phytotoxins and the heat generated during composting (Cook et al., 2007; De Cauwer et al., 2010; Eghball and Lesoing, 2000; Larney and Blackshaw, 2003). Compost also enhances microbiological activity, which may lead to greater mortality of weed seeds in soil (Gallandt et al., 1999; Kremer and Li, 2003). Crops residue is a valuable soil amendment. Upon termination of crops in our experiment, their residues were incorporated into the soil to take advantage of alfalfa and red clover allelopathy for suppressing weed seed and seedlings (Ohno et al., 2000). Biomass of all the crops was used to return and, in the case of the legume species, add nutrients to the soil.

2.2.1.4 Conservation tillage

Effects of tillage on weed population dynamics vary case by case (Mohler, 1993; Roberts and Feast, 1973). For example, zero-tillage could increase the population of some particular native beetles and rodents (Brust and House, 1988) that consume a significant amount of weed seeds in low-input soybean or leave newly shed seeds on soil surface and expose them to desiccation (Anderson, 2005), but it may also limit seed predation in a corn-soybean system due to greater amount of crop residue (van der Laat et al., 2015).

Reduced tillage helps protect seed predators in the soil by limiting unfavorable disturbance, but may require additional stresses like herbicide to control weeds (Landis et al., 2000). Tillage can place buried seeds near the soil surface in conditions more favorable for germination and seedling establishment or expose seeds to fatal germination conditions (Kurstjens, 2007). Tillage with a moldboard or chisel plow exposes seeds to germination stimulants in soil that can be used to introduce mortality factors at one of the most vulnerable stages of weed life history (Mohler, 2001b). Introducing mortality factors during weed germination, such as phytotoxins may increase the number of weeds that germinate but cannot reach maturity.

Exposing germinated weeds to phytotoxins can be done with livestock and green manure incorporation to the soil. Green manure or fresh plant residue increases fungal activities for weed suppression (Davis and Liebman, 2003; Pitty et al., 1987; Toussoun and Patrick, 1963). Green residues of crimson clover (*Trifolium incarnatum* L.) applied during tillage reduced aboveground dry biomass of lambsquarters, but that of sweet corn was only temporarily reduced (Dyck and Liebman, 1994).

In our experiment, chisel plowing was applied between corn and soybean in each of the rotations, and moldboard plowing was used before corn in the 3- and 4-year rotations to incorporate forage crops, and to bury weed vegetation and disrupt weed roots.

2.2.2 Experiment design

The present study was conducted in the same area as the previously mentioned studies (Davis et al., 2012; Gómez et al., 2013; Heggenstaller and Liebman, 2006; Heggenstaller et al., 2006; Liebman et al., 2008; Westerman et al., 2005) at the Iowa State University Marsden Farm (42^{o}01’ North; 93^{o}47’West; 333 meter above sea level).

Soils vary across the site. The detailed description of soil type is provided in Chen et al. (2014).

Weather data was automatically recorded at a weather station approximately 1 km from the site. The two-year average air temperature from April through October was 17.5 ^{o}C (Mesonet, 2016a). The two-year average precipitation total from April through October was 994 mm (Mesonet, 2016b).

The soybean cultivar used in both herbicide regimes was glyphosate-tolerant (Latham L 2758). The corn hybrid used in both herbicide regimes was not glyphosate-tolerant (Viking 07-04 N).

The experiment was arranged in a randomized complete block with four replicates. Each replicate block comprised the three rotation systems (2-, 3- and 4-year), with each crop phase grown in one plot, resulting in nine plots in each block. Plots measured 18 m x 84 m and were managed with standard farm machinery. Each plot was divided into two equal subplots (9 m x 84 m) for low and conventional herbicide regimes. Each subplot was considered one experimental unit.

The low and conventional herbicide treatment designations reflect herbicide regimes for the corn and soybean phases of each rotation. Weed control means and intensity are detailed in Table 2-1. No herbicides were used in the oat/red clover, oat/alfalfa, and alfalfa phases.

Different tillage practices were applied for each cropping systems (Table 2-2).

Table 2-1 Herbicide applications for corn and soybean plots

		Corn		***Soybean***	
Year	Input factor	Low herbicide	Conventional herbicide	Low herbicide	Conventional herbicide
2014	Herbicides applied (kg ai./ha)	tembotrione (0.049)	thiencarbazone methyl (0.037), isoxaflutole (0.092)	imazamox (0.023), lactofen (0.075)	glyphosate as isopropylamine salt (1.326), acifluorfen (0.297)
	Total (kg a.i./ha)	0.049	0.129	0.098	1.623
	Application timing	38-cm banded post-emergence, once	Broadcast pre-emergence, once	38-cm banded post-emergence, once	Broadcast post-mergence, once
	Mechanical control	Interrow cultivation, once	None	Interrow cultivation, twice	None
2015	Herbicides applied (kg ai./ha)	tembotrione (0.049)	thiencarbazone methyl (0.037), isoxaflutole (0.092)	imazamox (0.023), lactofen (0.075)	glyphosate as isopropylamine salt (1.203), acifluorfen (0.263)
	Total (kg a.i./ha)	0.049	0.129	0.098	1.466
	Application timing	38-cm banded post-emergence, once	Broadcast pre-emergence, once	38-cm banded post-emergence, once	Broadcast post-mergence, once
	Mechanical control	Interrow cultivation, twice	None	Interrow cultivation, twice	None

Table 2-2 Tillage regimes used for the three contrasting cropping systems

Rotation	Crop	Tillage regime	Crop	Tillage regime	Crop	Tillage regime	Crop	Tillage regime
2-year	Corn	Spring field cultivation Fall chisel plowing	Soybean	Spring field cultivation				
3-year	Corn	Spring field cultivation Fall chisel plowing	Soybean	Spring field cultivation	Oat with red clover	Spring disking and field cultivation Fall moldboard plowing		
4-year	Corn	Spring field cultivation Fall chisel plowing	Soybean	Spring field cultivation	Oat with alfalfa	Spring disking and field cultivation	Alfalfa	Fall moldboard plowing

2.2.3 Data collection and analysis

2.2.3.1 Weed community composition

We assessed weed community composition for each combination of the two herbicide regimes and every crop phase within each rotation system. Thus with three crop rotations (2-year, 3-year and 4-year) corresponding to two, three and four crop phases and two herbicide regimes, there were 18 treatments in total.

Weeds in this study grew unintentionally (i.e., they were not sown experimentally). Volunteer crops from previous years' seeds or from neighboring plots were not prevalent, and were not of interest. Aboveground weed biomass and population densities were measured in fall 2014 and 2015. In corn and soybean phases, eight 3.05 m x 0.76 m randomly located areas per experimental unit were surveyed during September 2014 and 2015. Four sampled areas were taken approximately 5 m from the plot borders, between rows 6 and 7 and between rows 9 and 10 in the west subplots; four between rows 15 and 16 and between rows 18 and 19 in the east subplots. In oat stubble with red clover, oat stubble with alfalfa, and established alfalfa, eight 0.25 m^2 randomly located quadrats per experimental unit were surveyed during October 2014 and 2015. Four sample areas were taken in a line that was 3 m from the east or west border and another four in a line that was 5 m from the other border of each experimental unit. In both 2014 and 2015, weeds collected in the field were counted and bagged by species and oven dried for at least 48 hours at 70^{o} C. Dried weights were recorded and tallied for each species in each experimental unit.

Seedbanks in this study were assessed to a depth of 20 cm from the soil surface because the most active seedbank dynamics generally occur within that depth (Gomes Jr and Christoffoleti, 2008; Yenish et al., 1992). Seedling recruitment mostly occurs from seeds buried up to 10 cm from the soil surface (Froud-Williams et al., 1984). According to a model by Mohler (1993), seed survival reaches an asymptotic value of 60% starting at 20 cm depth below the soil surface. According to that assumption, assessing the soil seedbank up to 20 cm depth would give representative observations of the viable seedbank. We sampled weed seeds for whole community assessment and assumed that all seeds had the same probability of survival regardless of species and of the time they entered the seedbank (Borgy et al., 2015). This assumption is made in order to disregard the differentiated germination and survivorship patterns of different weed species in response to changing environmental conditions. Accepting that assumption would allow no species to be omitted from the collected seedbank that is used to make predictions of successive emergence cohorts. Otherwise, assuming that weed species with lower occurrence in the soil seedbank would not emerge in the successive season could underestimate weed flora composition. For example, velvetleaf (*Abutilon theophrasti*), which could be present in the soil seedbank at lower density relative to other species, could proliferate under favorable conditions (Cardina and Norquay, 1997).

Weed seed sampling was conducted after seed shed and before germination, in accordance with recommendations from (Forcella et al., 2003). Soil samples were collected using JMC wet tip soil probes (Clements Associates Inc., Newton, IA 50208). In 2014, soil cores were taken on October 20th, October 22nd, and October 27th. Thirty-five cores that were 1.75 cm in diameter were taken to 20 cm depth in each experimental unit at random locations, resulting in 84 cm^2 sampled area and 1,684 cm^3 sampled volume in each experimental unit. Random sampling was found superior with regard to precision in assessing soil seedbanks with a small number of samples (20, Colbach et al., 2000), so we used this sampling pattern for our experiment. In 2015, soil cores were taken on October 20th, October 22nd, October 23rd and October 26th. Thirty-six 1.75-cm-diameter cores were taken to 20 cm depth in each subplot in a three-by-three matrix allocated evenly across the area, with four cores taken at each of the nine grid points. The 2015 sampling area and volume were 87 cm^2 and 1,732 cm^3, respectively. In both years, soil samples containing weed seeds were stored at 4°C until processing began.

Seeds and other plant materials were mechanically separated from soil material using an elutriator and a flotation procedure (Forcella et al., 2003). First, soil samples were weighed and placed in meshed tubes and washed for four hours with an elutriator to remove clay and silt particles. Before washing, soil samples weights were recorded to be used later in converting the number of seeds found in each sample to seed density. Soil material collected from one experimental unit usually occupied seven to nine mesh tubes. The remaining materials, which were sand, plant debris and seeds, were oven dried at 40°C for 24 hours and then bulked to one bag. The dried, bulked remaining material was then treated with a 5 M calcium chloride solution in a process called floatation. The remaining material was put in a beaker with calcium chloride and stirred well until all plant material floated and sand sank. Floating materials were then transferred to strainers and washed with pure water to remove calcium chloride. Seeds were separated from retrieved plant material with magnifying glass (8MC-100, 118 VAC 60 Hz 0.38 Amp, Dazor® Manufacturing Group, St. Louis, MO) and enumerated by species. Selected seeds had to be recognizable in Uva et al.'s (1997) manual and be intact. Seeds, which were separated by species, were then transferred to petri dishes containing germination paper and pure water and incubated in a growth chamber for five days at 28^{o}C with a 16/8 hour light/dark cycle. Non-germinated seeds that were imbibed with water were tested for viability using a forceps crush method (Rothrock et al., 1993; Westerman and Liebman, 2007). Seeds that resisted laboratory forceps crushing were considered dormant but viable (Roberts and Ricketts, 1979). Germinated and dormant seeds were grouped as viable. The percentages of germinated, dormant, and non-viable seeds were recorded.

Shannon's diversity (H') and evenness (E') indices were calculated for each experimental unit. Shannon's indices were selected because they are the least sensitive to sample size (Nkoa et al., 2015). Higher H' values indicate a richer diversity. E' ranges from 0 to 1; the greater E' is, the more even are the abundances of different species within a community. A value of 0 for E' means a community is dominated by one species (Magurran, 1988).

H' for each experimental unit was calculated with $H' = \Sigma[-p_i(\ln p_i)]$, where:

p_i: number of plants of species i divided by the total number of plants found in the experimental unit (proportional abundance), and

$\ln(p_i)$: natural logarithm of p_i.

E' for each experimental unit was calculated with $E' = H'/\ln(S)$, where:

H': Shannon's diversity index for the experimental unit, and

S: number of species found in that experimental unit.

Six rows of corn and soybean 84 m long were harvested with a combine and yield was determined with weigh wagons. Yields were adjusted to moisture concentrations of 155 g H_2O/kg for corn and at 135 g H_2O/kg for soybean.

2.2.3.2 Statistical analysis

We analyzed weed biomass, plant population density, and viable seedbank population density data using linear mixed-effects models with replicate block as a random effect and crop rotation system and weed management regime as fixed effects. 2014 and 2015 data were analyzed separately. Crop phase was treated as a main plot factor and weed management regime was treated as a split plot factor. Comparison was conducted among rotation systems based on averages across each phase of each system.

Weed plant density data and viable seedbank density were analyzed with PROC MIXED procedure by SAS® (Littell, 1996) after square root transformation to satisfy analysis of variance (ANOVA) normality assumptions.

Dried weed biomass data were analyzed with PROC MIXED procedure by SAS® (Littell, 1996) on natural logarithm scale to satisfy ANOVA normality assumptions. 2014 data were transformed using $\ln(x + 0.275)$ because no weeds were found in one experimental unit and 0.3 kg/ha was the smallest amount of dried weed biomass retrieved. 2015 data were transformed with $\ln(x)$ because weeds were found in all experimental units.

Shannon’s diversity and evenness indices were analyzed with the PROC MIXED procedure in SAS® (Littell, 1996) on the original scale because the data satisfied ANOVA normal distribution assumptions.

Crop yield data were analyzed with the PROC MIXED procedure in SAS® (Littell, 1996) using non-transformed data.

2.3. Results

2.3.1. Weed species diversity

Overall, 25 and 29 species or genera were identified in the above ground communities in 2014 and 2015, respectively; 10 and 16 species or genera were identified in seed bank communities in 2014 and 2015, respectively. Among all the identified species, 11 were monocots and 18 were dicots. This pattern was consistent with older studies in which more species were found in the aboveground than in the seedbank community (Numata et al., 1964; Roberts, 1981; Roberts and Hewson, 1971; Wilson et al., 1985).

The 2015 weed plant diversity index was smaller in alfalfa than in corn and in soybean for the 4-year rotation under the low herbicide regime, and greater in oat than in alfalfa for the same herbicide treatment (Figure 2-1). Differences in weed plant diversity in 3-year rotations were observed between oat and corn under low herbicide, in which oat had a smaller diversity index value, and between corn and soybean, in which soybean had a smaller diversity index value (Figure 2-1).

Table 2-3 List of species present in the weed aboveground and seedbank communities

Code	Common name *Latin name*	*2014*		*2015*	
		Aboveground	Seedbank	Aboveground	Seedbank
Monocots					
BROIN	Smooth brome *Bromus inermis*	-	-	+	-
CYPES	Yellow nutsedge *Cyperus esculentus*	+	-	+	-
DIGSA	Large crabgrass *Digitaria sanguinalis*	+	+	-	+
ECHCG	Barnyardgrass *Echinochloa crus-galli*	+	-	+	+
ELYRE	Quackgrass *Elymus repens*	-	-	+	-
ERBVI	Woolly cupgrass *Eriochloa villosa*	+	-	+	-
PANCA	Witchgrass *Panicum capillare*	+	-	+	-
PANDI	Fall panicum *Panicum dichotomiflorum*	-	-	+	-
PANVI	Switchgrass *Panicum virgatum*	-	-	+	-
POAPR	Kentucky bluegrass *Poa pratensis*	-	-	+	-
SETFA	Giant foxtail *Setaria faberi*	+	+	+	+
SETLU	Yellow foxtail *Setaria glauca*	+	+	+	+

Table 2-3 (cont.)

Code	**Common name** ***Latin name***	***2014*** Aboveground	***2014*** Seedbank	***2015*** Aboveground	***2015*** Seedbank
Dicots					
	Maple sp. *Acer sp.*	-	-	+	-
ABUTH	Velvetleaf *Abutilon theophrasti*	+	+	+	+
AMATA	Common waterhemp *Amaranthus rudis*	+	+	+	+
ASCSY	Common milkweed *Asclepias syriaca*	+	-	+	-
CHEAL	Common lambsquarters *Chenopodium album*	+	+	+	+
CIRAR	Canada thistle *Cirsium arvene*	+	-	+	-
CONAR	Field bindweed *Convolvulus arvensis*	-	-	-	+
DATST	Jimsonweed *Datura stramonium*	-	-	-	+
EPHHT	Prostrate spurge *Euphorbia humistrata*	+	-	+	-
MORAL	White mulberry *Morus alba*	+	+	-	+
OXAST	Yellow woodsorrel *Oxalis stricta*	+	-	+	-
PHYSU	Smooth groundcherry *Physalis subglabrata*	+	-	+	-
PLAMA	Broadleaf plantain *Plantago major*	+	-	+	-
POLAV	Prostrate knotweed *Polygonum aviculare*	+	-	+	-
POLCC	Swamp smartweed *Polygonum coccineum*	+	-	+	-
POLCO	Wild buckwheat *Polygonum convolvulus*	-	-	-	+

Table 2-3 (cont.)

Code	Common name *Latin name*	*2014*		*2015*	
		Aboveground	Seedbank	Aboveground	Seedbank
Dicots					
POLLA	Pale smartweed *Polygonum lapathifolium*	+	-	+	-
POLPE	Ladysthumb *Polygonum persicaria*	-	+	-	-
POLPY	Pennsylvania smartweed *Polygonum pennsylvanicum*	+	-	+	-
RUMCR	Curly dock *Rumex crispus*	+	-	+	-
SINAR	Wild mustard *Brassica arvensis*	-	+	-	+
SOLCA	Horse nettle *Solanum carolinense*	-	-	-	+
SOLPT	Eastern black nightshade *Solanum ptycanthum*	+	+	+	+
SONAR	Perennial sowthistle *Sonchus arvensis*	+	-	+	-
SONSP	Sowthistle *sp.* *Sonchus sp.*	-	-	-	+
TAROF	Dandelion *Taraxacum officinale*	+	-	+	-
Total		**25**	**10**	**29**	**16**

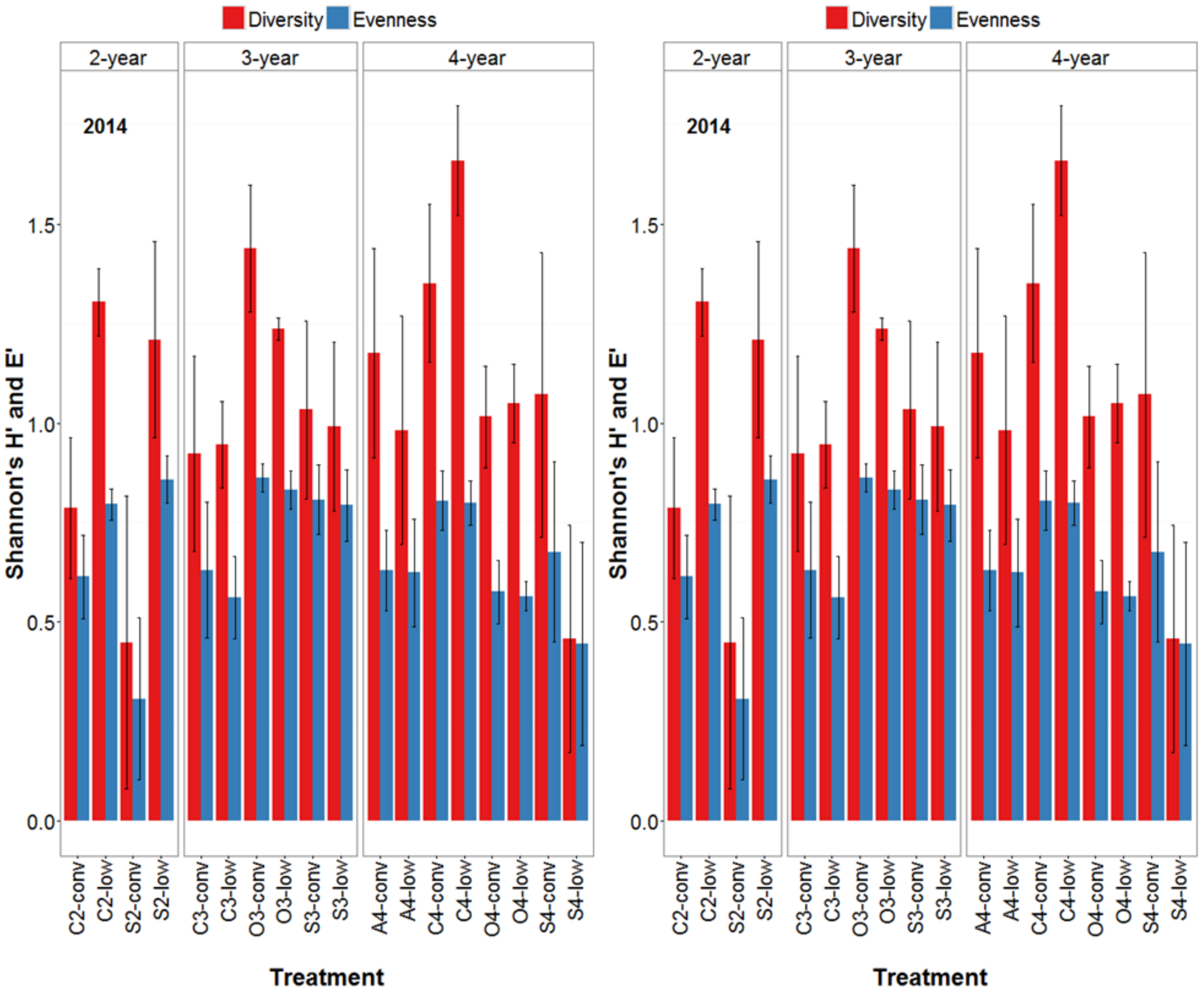

Figure 2-1 Shannon's diversity and evenness indices

H': Shannon's diversity index, E': Shannon's evenness index

Weed community evenness index values in 2015 for the 4-year rotation were smaller in alfalfa than in soybean under the conventional herbicide regime; smaller in alfalfa than in soybean under the low herbicide regime; and smaller in alfalfa than in corn under the low herbicide regime (Figure 2-1).

Table 2-4 Effects of treatment factors and their interaction on weed community diversity

Source of variation	2014		2015	
	H'	E'	H'	E'
	p-value			
Herbicide	0.5053	0.4691	0.0208	0.9253
Rotation	0.0536	0.0689	0.0019	<.0001
Herbicide x Rotation	0.1146	0.2338	0.0323	0.1499

H’: Shannon’s diversity index, E’: Shannon’s evenness index

2.3.2 Weed plant density and accumulated biomass

2.3.2.1 Weed plant density

Weed plant density was comparable in all crop phases in 2014 and 2015 in the 2-year and 3-year rotations and in corn and soybean phases of the 4-year rotation. The main contributor to increased weed density in 2015 was dandelion (*Taraxacum officinale* Weber) (Figure 2-2). Rotation treatments had significant effects on weed plant density, for both the 2014 and 2015 field seasons; herbicide and rotation treatments had interactive effects in 2014, but not in 2015 (Tables 2-5, 2-6 and 2-7).

Weed plant density was higher in the longer rotations. In 2014, there were 1.1 weeds/m^2 in the 2-year rotation, 8.1 weeds/m in the 3-year rotation, and 13.2 weeds/m^2 in the 4-year rotation; in 2015, there were 1.5 weeds/m^2 in the 2-year rotation, 8.2 weeds/m^2 in the 3-year rotation, and 66.6 weeds/m^2 in the 4-year rotation).

Table 2-5 Effects of treatment factors and their interaction on weed plant density

	2014	**2015**
Source of variation	**p-value**	
Herbicide	0.3218	0.3107
Rotation	<0.0001	<0.0001
Herbicide x Rotation	0.0025	0.3897

Weed plant density was comparable across rotation x herbicide treatments for the same crop phase in 2014. In 2015, the same pattern was observed except for 3-year and 4-year soybean under the low herbicide regime (Table 2-6).

The average rotation weed plant density was comparable for the two herbicide regimes (Table 2-7). Weed plant density in the 2-year and 3-year rotations was similar across different crop phases within the same herbicide treatment, except for 3-year soybean and oat in 2014 (Table 2-7). For the 4-year rotation, weed plant density in both years was comparable between corn and soybean, and between oat and alfalfa, but different between the row crops and the forage crops. In both years, the 3-year rotation contained high densities of common lambsquarters, common waterhemp and foxtail species; the 4-year rotation had high densities of dandelion and foxtail species (Figure 2-2).

Table 2-6 Differences between means of weed plant density (weeds/m^2) (sqrt(x)) in the same crop phase across herbicide x rotation treatments.

Herbicide	Crop	Contrast	2014		2015	
			Estimate	p-value (Bonferroni adjusted)	Estimate	p-value (Bonferroni adjusted)
Conventional	Corn	3yr – 2yr	2.2 (0.3; 0.6)	1	1.0 (0.4; 0.3)	1
		3yr – 4yr	2.6 (0.5; 0.6)	1	-1.7 (-0.4; 0.3)	1
		4yr – 2yr	-0.5 (-0.2; 0.6)	1	2.7 (0.9; 0.3)	0.1635
	Soybean	3yr – 2yr	-0.2 (0.0; 0.6)	1	1.0 (0.4; 0.4)	1
		3yr – 4yr	0.2 (0.1; 0.6)	1	1.8 (0.8; 0.3)	0.3020
		4yr – 2yr	-0.4 (-0.2; 0.6)	1	-0.8 (-0.5; 0.3)	1
	Oat	3yr – 4yr	-8.0 (-0.9; 0.7)	1	-78.8 (-6.2; 1.8)	0.1208
	Averaged	3yr – 2yr	8.9 (1.8; 0.7)	0.6550	7.5 (2.0; 0.9)	1
		4yr – 3yr	30.0 (3.4; 1.0)	0.0443	197.7 (12.6; 1.9)	0.0001
		4yr – 2yr	14.5 (2.1; 0.4)	<0.0001	69.7 (5.2; 0.5)	<0.0001
Low	Corn	3yr – 2yr	6.6 (1.3; 0.6)	1	2.2 (0.5; 0.3)	1
		3yr – 4yr	5.5 (0.9; 0.6)	1	-0.6 (-0.1; 0.3)	1
		4yr – 2yr	1.1 (0.4; 0.6)	1	2.8 (0.7; 0.3)	1
	Soybean	3yr – 2yr	2.5 (0.9; 0.6)	1	3.8 (1.2; 0.3)	0.0160
		3yr – 4yr	2.8 (1.1; 0.6)	1	4.2 (1.4; 0.3)	0.0016
		4yr – 2yr	-0.3 (-0.2; 0.6)	1	-0.5(-0.2; 0.3)	1
	Oat	3yr – 4yr	-1.8 (-0.2; 0.7)	1	-88.8 (-5.7; 1.8)	0.2136
	Averaged	3yr – 2yr	19.1 (3.8; 0.7)	0.0005	19.4 (3.5; 0.9)	0.0376
		4yr – 3yr	0.6 (-0.3; 1.0)	1	152.4 (9.4; 1.9)	0.0039
		4yr – 2yr	9.7 (1.8; 0.4)	0.0005	60.5 (4.9; 0.5)	<0.0001

Estimates show differences between least square means for original data; differences between least square means of transformed data and standard errors of those differences are shown in parentheses.

Table 2-7 Differences in mean weed plant density (weeds/m^2) across crop phases under the same herbicide x rotation treatment

Herbicide	Rotation	Contrast	2014		2015	
			Estimate	p-value (Bonferroni adjusted)	Estimate	p-value (Bonferroni adjusted)
Conventional	2-year	Soybean - Corn	-0.8 (-0.5; 0.6)	1	0.0 (0.1; 0.3)	1
Low		Soybean - Corn	-0.6 (-0.3; 0.6)	1	-1.8 (-0.7; 0.3)	0.9316
Conventional	3-year	Soybean - Corn	-3.2 (-0.8; 0.6)	1	0.0 (0.0; 0.3)	1
		Oat - Soybean	12.1 (2.8; 0.6)	0.0040	8.3 (1.8; 1.3)	1
		Oat - Corn	8.9 (2.0; 0.6)	0.1371	8.3 (1.7; 1.3)	1
Low		Soybean - Corn	-4.6 (-0.7; 0.6)	1	-0.2 (0.0; 0.3)	1
		Oat - Soybean	17.2 (2.8; 0.6)	0.0043	20.1 (2.7; 1.3)	1
		Oat - Corn	12.6 (2.1; 0.6)	0.0938	20.0 (2.6; 1.3)	1
Conventional	4-year	Soybean - Corn	-0.7 (-0.4; 0.6)	1	-3.6 (-1.3; 0.3)	0.0057
		Oat - Soybean	20.2 (3.8; 0.6)	<0.0001	88.9 (8.8; 1.3)	0.0001
		Alfalfa - Soybean	40.1 (5.7; 0.6)	<0.0001	189.5 (12.5; 1.3)	<0.0001
		Oat - Corn	19.5 (3.4; 0.6)	0.0003	85.3 (7.5; 1.3)	0.0008
		Alfalfa - Corn	39.4 (5.2; 0.6)	<0.0001	186.0 (11.2; 1.3)	<0.0001
		Alfalfa - Oat	19.9 (1.8; 0.6)	0.2283	100.6 (3.7; 1.8)	1
Low		Soybean - Corn	-1.9 (-1; 0.6)	1	-5.0 (-1.6; 0.3)	0.0004
		Oat - Soybean	21.8 (4.1; 0.6)	<0.0001	113.1 (9.8; 1.3)	<0.0001
		Alfalfa - Soybean	17.5 (3.7; 0.6)	<0.0001	129.1 (10.5; 1.3)	<0.0001
		Oat - Corn	19.9 (3.1; 0.6)	0.0011	108.1 (8.2; 1.3)	0.0003
		Alfalfa - Corn	15.7 (2.7; 0.7)	0.0014	124.1 (8.9; 1.3)	<0.0001
		Oat - Alfalfa	4.3 (0.4; 0.6)	1	-16.0 (-0.8; 1.8)	1

Estimates show differences between least square means for original data; differences between least square means of transformed data and standard errors those differences are shown in parentheses.

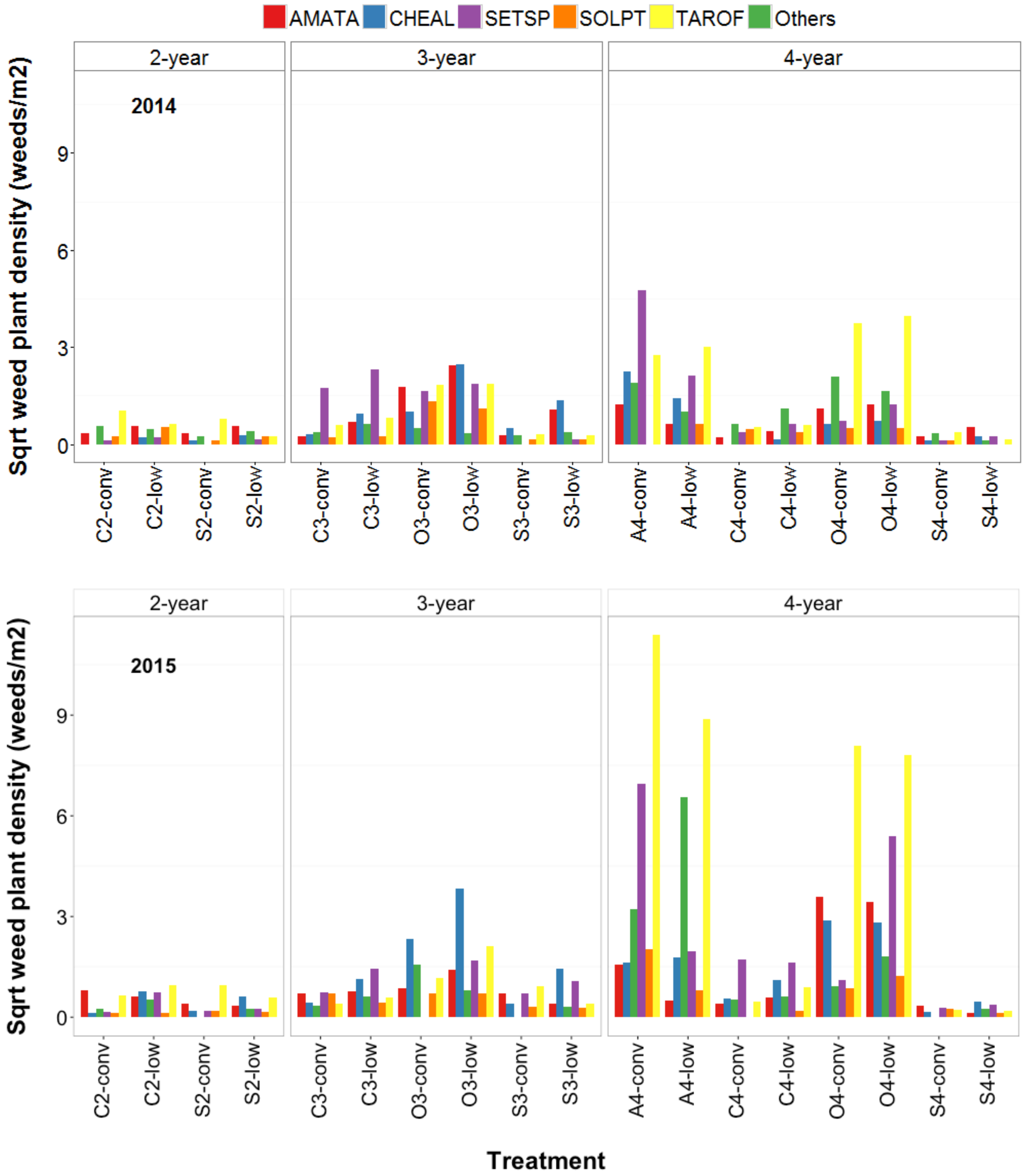

Figure 2-2 Weed plant density (weeds m^{-2}) on square root scale (sqrt) of the five most abundant species

AMATA: *Amaranthus rudis*, CHEAL: *Chenopodium album*, SETSP*: Setaria faberi* and *S. glauca*, SOLPT: *Solanum ptycanthum*, TAROF: *Taraxacum officinale*

2.3.2.2 Weed biomass

Herbicide treatments, rotation treatments and their interaction had significant effects on total weed biomass for both the 2014 and 2015 field seasons (Table 2-8). In general, weed biomass under the low herbicide treatment was greater than under the conventional herbicide treatment. The greatest difference was observed in the 3-year rotation in which the corn-soybean weed biomass average under the low herbicide regime was 543.9 ± 115.7 kg/ha greater than that under the conventional regime. The average rotation weed biomass accumulation was comparable in the 2-year and 4-year rotations for both herbicide regimes. The total weed biomass in oat and alfalfa phases of the 3- and 4-year rotations was similar under the two herbicide regimes.

Table 2-8 Effects of treatment factors and their interaction on weed biomass

Source of variation	**2014**	**2015**
	p-value	
Herbicide	<0.0001	<0.0001
Rotation	<0.0001	<0.0001
Herbicide x Rotation	0.0003	0.001

As described in Section 2.2, herbicide was not applied in oat and alfalfa phases and conventional and low herbicide regimes designated the herbicide regimes on corn and soybean in the corresponding rotation. However, weed biomass composition in oat and alfalfa subplots was different depending on herbicide regimes applied on preceding corn and soybean phases; this pattern was more obvious in the longer 4-year rotation than in the shorter 3-year rotation (Figure 2-3). In the 2-year and 3-year rotations, either common waterhemp (*Amaranthus rudis*) or common lambsquarters (*Chenopodium album*) was the most prevalent weed. In the 4-year rotation, each crop x herbicide treatment had one or two dominant weeds: foxtails (*Setaria* *faberi* and *S. glauca*) in 2014 alfalfa following conventional corn and soybean and in corn under the low herbicide regime; common lambsquarters in 2015 soybean under the low herbicide regime and corn; dandelion (*Taraxacum officinale*) in 2015 alfalfa; and dandelion and common waterhemp in 2015 oat.

Considering corn and soybean phases under the low herbicide regime of the three rotation systems, the 3-year corn and soybean were found to be weediest (Figure 2-3). Considering oat phases of the 3-year and 4-year rotation systems under the low herbicide regime, the 4-year rotation oat crop was found to be weediest. Notably, soybean and alfalfa phases under the low herbicide regime of the 4-year rotation system, and oat and corn under the low herbicide regime of the 3- and 4-year rotations were observed with comparable weed biomass accumulation (Table 2-9).

Significant differences in weed biomass were most evident in 2014 between the 2- and 4-year rotations under the low herbicide regime; weed biomass accumulation in the 4-year rotation was greater than that in the 2-year (Table 2-10). Differences in 2015 were evident between the 2- and 4-year rotations under the conventional herbicide regime; weed biomass accumulation in the 4-year rotation was greater than that in the 2-year (Table 2-9). For oat in the 3- and 4-year rotations in both years, both rotations were dominated by common waterhemp and common lambsquarters (Figure 2-3).

Table 2-9 Differences in mean weed biomass (kg/ha) (log(x+0.275) in 2014 and log (x) in 2015) across crop phases of the same rotation x herbicide treatment

			2014		2015	
Herbicide	**Rotation**	**Contrast**	**Estimate**	**p-value (Bonferroni adjusted)**	**Estimate**	**p-value (Bonferroni adjusted)**
Conventional	2-year	Soybean - Corn	0.1 (0.1; 1.3)	1	22.6 (3.1; 1.0)	0.2130
Low		Soybean - Corn	9.7 (-0.4; 1.2)	1	101.2 (2.3; 1.0)	0.9548
Conventional	3-year	Soybean - Corn	-13.0 (-0.7; 1.2)	1	8.7 (0.7; 1.0)	1
		Oat - Soybean	83.4 (5.3 0.9)	<0.0001	62.4 (1.9; 0.8)	0.6685
		Oat - Corn	70.4 (4.6; 0.9)	<0.0001	71.1 (2.6; 0.8)	0.0893
Low		Soybean - Corn	22.6 (-0.2; 1.2)	1	971.1 (2.6; 1.0)	0.5903
		Oat - Soybean	-1.0 (0.7; 0.9)	1	-936. 5 (-2.2; 0.8)	0.3353
		Oat - Corn	21.5 (0.5; 0.9)	<0.0001	34.7 (0.4; 0.8)	1
Conventional	4-year	Soybean - Corn	-1.4 (-0.4; 1.3)	1	7.1 (-0.6; 1.0)	1
		Oat - Soybean	249.2 (6.0; 1.0)	<0.0001	753.4 (5.0; 0.8)	<0.0001
		Alfalfa - Soybean	127.4 (5.2; 1.3)	0.0008	137.8 (3.5; 0.8)	0.0036
		Oat - Corn	247.8 (5.5; 0.9)	<0.0001	760.5 (4.4; 0.8)	<0.0001
		Alfalfa - Corn	125.9 (4.8; 1.2)	0.0050	181.0 (2.9; 0.8)	0.0317
		Alfalfa - Oat	-121.9 (-0.8; 0.9)	1	-579.6 (-1.5; 0.4)	0.1942
Low		Soybean - Corn	-0.7 (-1.3; 1.3)	1	-52.3 (-1.1; 1.0)	1
		Oat - Soybean	304.4 (4.0; 1.0)	<0.0001	819.3 (3.9; 0.8)	0.0006
		Alfalfa - Soybean	36.6 (2.3; 1.3)	0.5544	78.0 (1.8; 0.8)	0.8181
		Oat - Corn	303.7 (2.6; 0.9)	0.0717	767.0 (2.9; 0.8)	0.0340
		Alfalfa - Corn	35.9 (1; 1.2)	1	25.6 (0.8; 0.8)	1
		Oat - Alfalfa	267.7 (1.6; 0.9)	1	741. 3 (2.1; 0.4)	0.0195

Estimates show differences between least square means for original data; differences between least square means of transformed data and standard errors of those differences are shown in parentheses.

Table 2-10 Differences in mean weed biomass (log(x+0.275) in 2014 and log (x) in 2015) in the same crop phase across herbicide x rotation treatments

Herbicide	Crop	Contrast	2014		2015	
			Estimate	p-value	Estimate	p-value
Conventional	Corn	3yr - 2yr	13.5 (1.3; 1.2)	1	8.0 (2.3; 1.0)	0.9548
		3yr - 4yr	11.9 (-0.1; 1.2)	1	-6.3 (-0.7; 1.0)	1
		4yr - 2yr	1.5 (1.4; 1.2)	1	14.3 (3.0 ; 1.0)	0.2548
	Soybean	3yr - 2yr	0.3 (0.5; 1.3)	1	-5.8 (-0.1; 1.0)	1
		3yr - 4yr	0.3 (-0.4; 1.3)	1	-4.7 (0.6; 1.0)	1
		4yr - 2yr	0.0 (0.8; 1.4)	1	-1.1 (-0.7; 1.0)	1
	Oat	3yr - 4yr	-165.5 (-1.1: 0.2)	0.0331	-695.8 (-2.4; 0.4)	0.0045
	Averaged	3yr - 2yr	65.0 (5.1; 1.6)	0.0619	46.7 (3.7; 1.2)	0.2130
		4yr - 3yr	248.2 (5.1; 2.3)	1	867.5 (5.0; 1.8)	0.3353
		4yr - 2yr	94.5 (3.8; 0.8)	0.4619	240.2 (3.1; 0.6)	0.0003
Low	Corn	3yr - 2yr	68.0 (1.9; 1.2)	1	55.0 (1.8; 1.0)	1
		3yr - 4yr	54.7 (0.9; 1.2)	1	-22.8 (0.2; 1.0)	1
		4yr - 2yr	12.2 (1.0; 1.2	1	77.8 (1.6; 1.0)	1
	Soybean	3yr - 2yr	80.8 (2.2; 1.2)	1	924.9 (2.0; 1.2)	1
		3yr - 4yr	78.0 (2.1; 1.3)	0.8895	1000.7 (3.8; 1.0)	0.0317
		4yr - 2yr	2.8 (0.1; 1.3)	1	-75.8 (-1.8; 1.0)	1
	Oat	3yr - 4yr	-227.4 (-1.2; 0.2)	0.0150	-775.1 (-2.2; 0.4)	0.0105
	Averaged	3yr - 2yr	155.7 (4.5; 1.5)	0.0793	679.3 (3.2; 1.2)	0.5120
		4yr - 3yr	61.2 (-1.9; 2.3)	1	-509.7 (-2.2; 1.8)	1
		4yr - 2yr	93.1 (1.8; 0.7)	0.0006	212.2 (1.1; 0.6)	1

Estimates show differences between least square means for original data; differences between least square means of transformed data and standard errors of those differences are shown in parentheses.

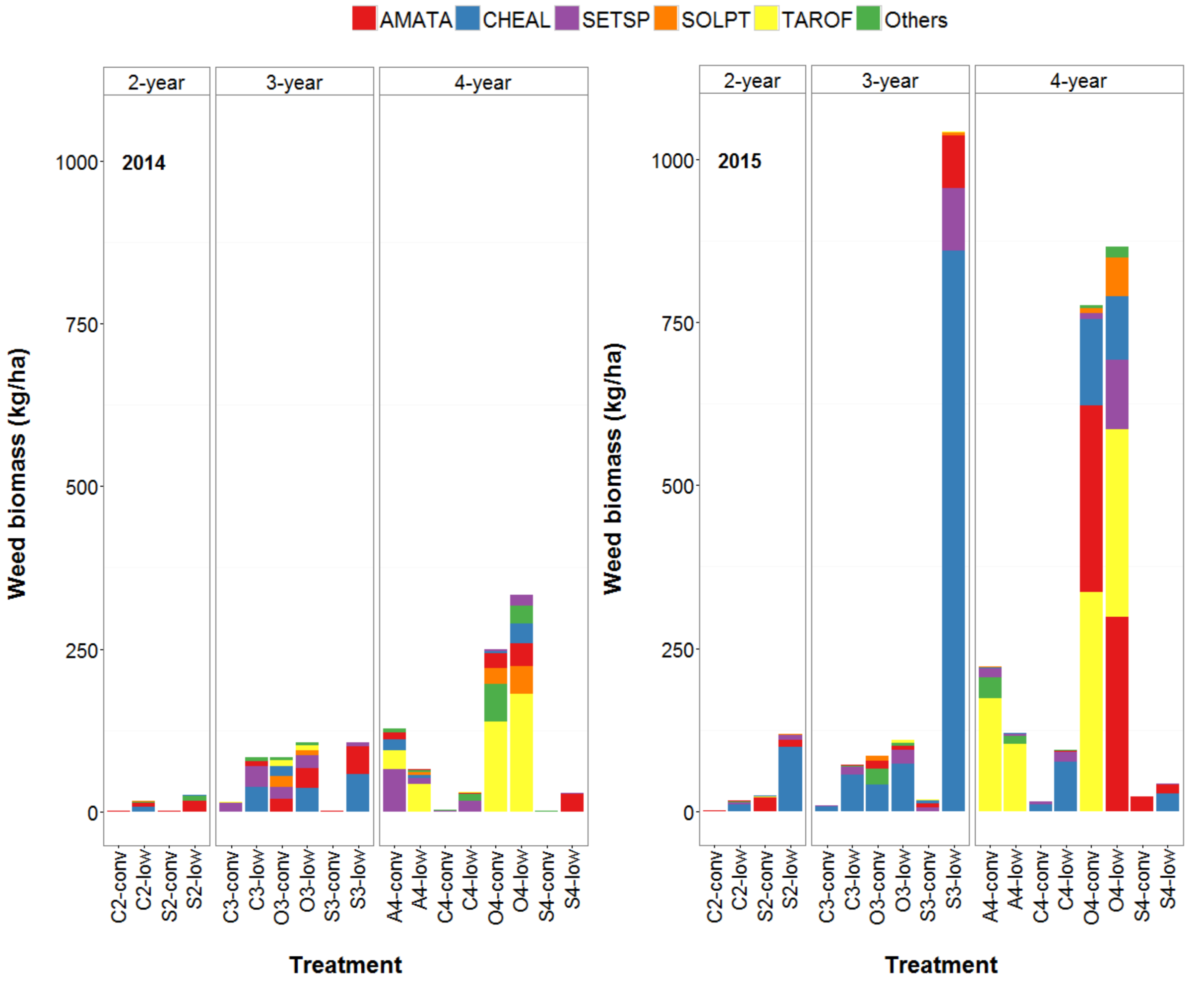

Figure 2-3 Weed biomass contribution (kg/ha) of the five most prevalent weed species

AMATA: *Amaranthus rudis*, CHEAL: *Chenopodium album*, SETSP*: Setaria faberi* and *S. glauca*, SOLPT: *Solanum ptycanthum*, TAROF: *Taraxacum officinale*

2.3.3 Density of viable seeds in the soil seedbank

Herbicide treatments, rotation treatments and their interaction had significant effects on viable seeds in the soil seedbank for the 2015 field season; only rotation treatments had a significant effect in 2014.

Table 2-11 Effects of treatment factors and their interaction on weed seedbank density

	2014	**2015**
Source of variation	**p-value**	
Herbicide	0.4040	0.0046
Rotation	0.0090	<0.0001
Herbicide x Rotation	0.2386	0.0052

Viable seed density in the soil seedbank was not significantly different across crop phases under the same herbicide treatment in the 2- and 3-year rotation systems and under the conventional herbicide regime in the 4-year rotation. Differences among the rotation system in average viable seed density in the soil seedbank were most evident under the low herbicide regime in 2015 (Table 2-13): seedbank density under the 2-year rotation was significantly smaller than that of the 3-year and the 3-year rotation seedbank was much greater than that of 4-year. Seedbank density was not significantly different between 2- and 4-year rotations. Among all crop phases, the most profound change of viable seed density was in 4-year soybean when switching herbicide regime from conventional to low in 2014; this increased the seedbank density by 9,286.1 ± 2,000.5 seeds/m^2 (p = 0.0064). This reflected a dramatic increase in the number of viable seeds of common waterhemp and common lambsquarters (Figure 2-4).

Table 2-12 Differences in viable seed density (seeds/m) (sqrt(x)) in the soil seedbank across crop phases under the same herbicide x rotation treatment

Herbicide	Rotation	Contrast	2014		2015	
			Estimate	p-value	Estimate	p-value
Conventional	2-year	Soybean - Corn	-528.7 (-7.1; 13.0)	1	3664.2 (34.6; 11.7)	0.2681
Low		Soybean - Corn	956.6 (11.6; 13.0)	1	3259.9 (31.3; 11.7)	0.5475
Conventional	3-year	Soybean - Corn	5.0 (0.7; 13.0)	1	-236.9 (-3.7; 11.7)	1
		Oat - Soybean	3490.8 (20.1; 14.9)	1	-869.4 (-5.3; 14.4)	1
		Oat - Corn	3495.8 (20.7; 14.9)	1	-1106.3 (-9.0; 14.4))	1
Low		Soybean - Corn	-16.8 (-3.5; 13.0)	1	8844.5 (42.1; 11.7)	0.0538
		Oat - Soybean	-424.2 (0.8; 14.9)	1	-2929.2 (-11.6; 14.4)	1
		Oat - Corn	-441.0 (-2.6; 14.9)	1	5915.4 (30.5; 14.4)	1
Conventional	4-year	Soybean - Corn	1939.1 (18.1; 13.0)	1	1392.9 (9.8; 11.7)	1
		Oat - Soybean	-1469.1 (-12.2; 14.9)	1	1826.2 (10.8; 14.4)	1
		Alfalfa - Soybean	408.3 (4.8; 14.9)	1	-643.0 (-7.3; 14.4)	1
		Oat - Corn	470.0 (5.8; 14.9)	1	3219.1 (20.6; 14.4)	1
		Alfalfa - Corn	2347.4 (22.9; 14.9)	1	749.9 (2.5; 14.4)	1
		Alfalfa - Oat	1852.9 (18.3; 16.5)	1	-2469.2 (-18.1; 16.6)	1
Low		Soybean - Corn	72.7 (1.3; 13.0)	1	10826.0 (66.5; 11.7)	0.0001
		Oat - Soybean	-1109.0 (-16.0; 14.9)	1	-6784.9 (-36.3; 14.4)	0.7368
		Alfalfa - Soybean	743.9 (2.3; 14.9)	1	-11680.0 (-74.1; 13.3)	<0.0001
		Oat - Corn	-1036.3 (-14.7; 14.9)	1	4041.0 (30.2; 11.4)	1
		Alfalfa - Corn	816.6 (3.7; 14.9)	1	-854.3 (-7.6; 13.3)	1
		Oat - Alfalfa	2894.5 (12.9; 16.5)	1	4895.2 (37.8; 15.8)	1

Estimates show differences between least square means for original data; differences between least square means of transformed data and standard errors those differences are shown in parentheses.

Table 2-13 Differences in viable seed density (seeds/m) (sqrt(x)) in the soil seedbank under the same crop phase across herbicide x rotation treatments

Herbicide	Crop	Contrast	2014		2015	
			Estimate	p-value	Estimate	p-value
Conventional	Corn	3yr - 2yr	-1852.9 (-18.3; 16.5)	1	4888.4 (42.0; 11.7)	0.0538
		3yr - 4yr	1995.7 (18.7; 13.0)	1	3555.8 (26.7; 11.7)	1
		4yr - 2yr	1746.1 (15.0; 13.0)	1	1332.6 (15.3; 11.7)	1
	Soybean	3yr - 2yr	249.6 (3.7; 13.0)	1	987.3 (3.6; 11.7)	1
		3yr - 4yr	2529.5 (26.4; 13.0)	1	1926.0 (13.2; 11.7)	1
		4yr - 2yr	-188.0 (-2.4; 13.0)	1	-938.7 (-9.5; 11.7)	1
	Oat	3yr - 4yr	2717.4 (28.8; 13.0)	1	-769.5 (-2.9; 16.6)	1
	Averaged	2yr - 3yr	6854.0 (58.7; 17.5)	0.1014	5217.1 (40.9; 16.1)	0.6407
		4yr - 3yr	-5171.9 (-31.4; 23.5)	1	-5302.9 (-42.8; 22.5)	1
		4yr - 2yr	1703.1 (18.9; 8.4)	1	840.9 (6.2; 7.7)	1
Low	Corn	3yr - 2yr	5697.2 (48.9; 13.0)	0.0432	4811.6 (41.7; 11.7)	0.0561
		3yr - 4yr	4273.7 (31.4; 13.0)	1	1194.6 (28.7; 11.7)	0.8368
		4yr - 2yr	1423.5 (17.4; 13.0)	1	1194.6 (13.0; 11.7)	1
	Soybean	3yr - 2yr	4723.8 (33.9; 13.0)	0.8490	10396.0 (52.5; 11.7)	0.0045
		3yr - 4yr	4184.3 (26.6; 13.0)	1	1635.7 (4.3; 11.7)	1
		4yr - 2yr	539.6 (7.2; 13.0)	1	8760.6 (48.3; 11.7)	0.0125
	Oat	3yr - 4yr	4869.0 (43.4; 16.5)	0.9822	5491.4 (29.0; 16.6)	1
	Averaged	3yr - 2yr	10133.0 (82.1; 17.5)	0.0017	16203.0 (100.6; 16.1)	<0.0001
		4yr - 3yr	-12474.0 (-95.4; 23.5)	0.0130	-15102.0 (-91.8; 22.2)	0.0088
		4yr - 2yr	908.4 (9.3; 23.5)	1	3067.8 (19.7;7.6)	0.5997

Estimates show differences between least square means for original data; differences between least square means of transformed data and standard errors those differences are shown in parentheses.

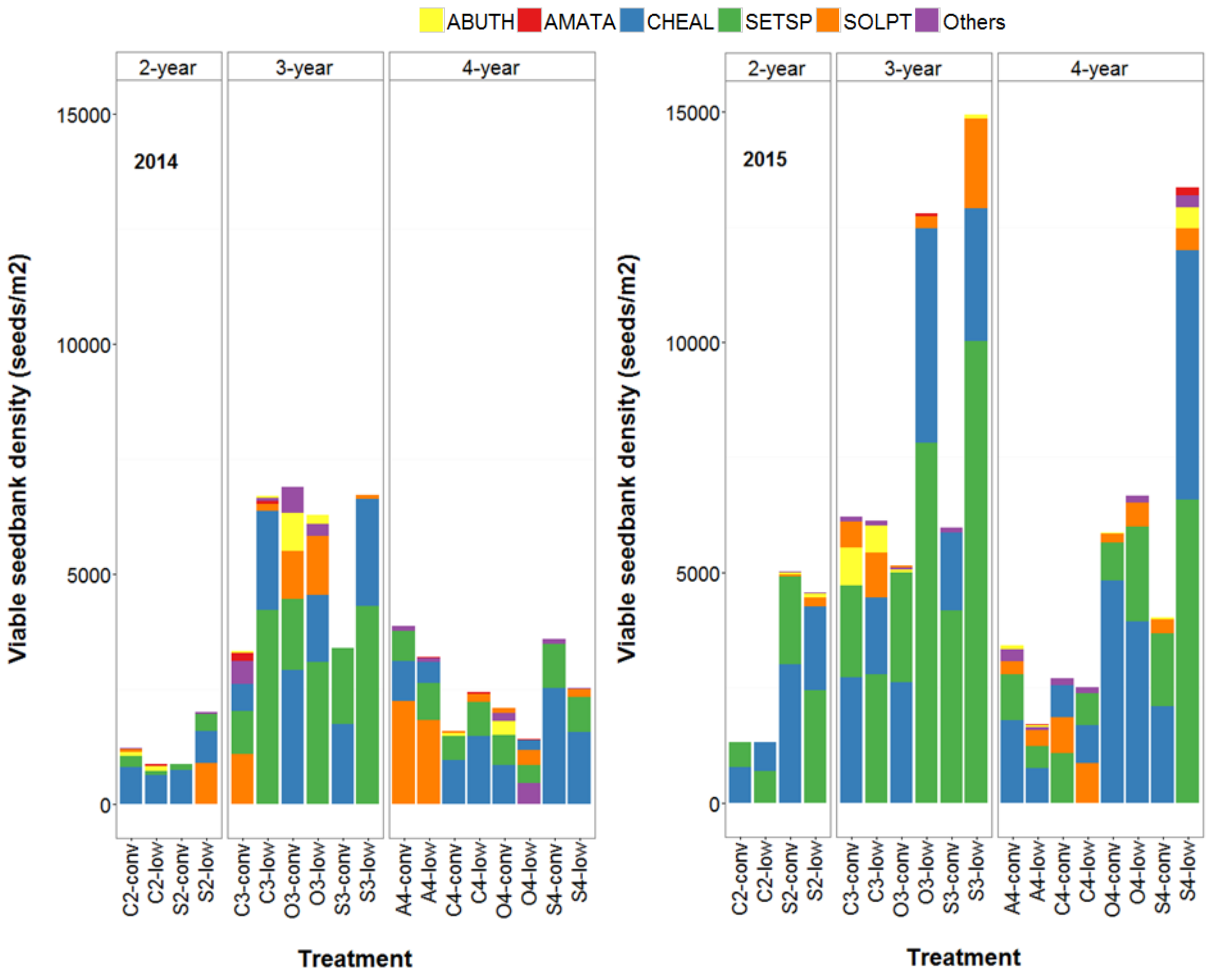

Figure 2-4 Density of viable seeds in the soil for the five most prevalent weed species

ABUTH: *Abutilon theoprasti* AMATA: *Amaranthus rudis*, CHEAL: *Chenopodium album*, SETSP*: Setaria faberi* and *S. glauca*, SOLPT: *Solanum ptycanthum*,

2.3.4 Corn and soybean yields

In both 2014 and 2015, corn yields did not differ among rotations or herbicide regimes.

Table 2-14 a Corn yields 2014 - 2015

	Corn yield (Mg ha^{-1} at 155 g H_2O kg^{-1})			
Rotation system	**2014**		**2015**	
	low	*conv.*	*low*	*conv.*
2-year	14.07	13.80	12.68	12.83
3-year	14.29	14.39	12.61	12.74
4-year	14.48	15.11	13.32	12.75
SE	0.3322		0.1710	
Source of variation	**p-value**			
Rotation	0.0571		0.1188	
Herbicide	0.5788		0.4985	
Rotation x herbicide	0.4155		0.0836	

In 2014 and 2015, soybean yields differed significantly among rotations but were unaffected by herbicide regime. Soybean yielded the highest in the 4-year rotation and the lowest in the 2-year rotation.

Table 2-14 b Soybean yields 2014 -2015

	Soybean yield (Mg ha^{-1} at 130 g H_2O kg^{-1})			
Rotation system	**2014**		**2015**	
	low	*conv.*	*low*	*conv.*
2-year	3.25	3.26	2.97	3.11
3-year	3.59	3.60	3.85	4.09
4-year	4.05	4.07	4.21	4.37
SE	0.1843		0.1390	
Source of variation	**p-value**			
Rotation	0.0014		< 0.0001	
Herbicide	0.9092		0.1209	
Rotation x herbicide	0.9995		0.9271	

2.4 Discussion

2.4.1 Cropping system diversification changed total accumulated weed biomass, weed plant density and seedbank density

Switching herbicide regime from conventional to low within a crop phase did not change weed plant density (Figure 2-2) but caused dramatic increases in the biomass of some weeds, such as *Amarnthus rudis*, *Chenopodium album*, *Solanum ptycanthum* and *Setaria* spp., which eventually contributed to large increases in total weed biomass of a treatment (Figure 2-3). Rotation average weed plant density and biomass differences were most evident when comparing the 2- and 4-year rotations, especially in the oat and alfalfa phases (Figure 2-2 and Table 2-7). Higher weed plant density was observed in the longer rotations due to reduced amount of herbicide application (Table 2-2) and varying effects of tillage regime as detailed in Section 2.1.4. Weed plant density was comparable across crop phases regardless of herbicide regime in the 2- and 3-year rotations, confirming the effectiveness of oat/red clover companion crops in weed emergence control as detailed in Section 2.1.1. The 4-year rotation required less herbicide (Table 2-1) and still maintained comparable weed biomass accumulation as compared with the 3-year (Table 2-10). Weed seedbank density averaged over the various crop phases of each rotation system was similar between the 3- and 4-year rotations. The 4-year rotations therefore better suppressed weed biomass than did the 3-year rotation. The greater size of the seedbanks of the 3- and 4-year rotations as compared with that of 2-year rotation could be attributed to the reduced total amount of herbicide applied, crop sequence and mechanical weed control tactics; and their overall interaction.

As noted in Section 3.2, in the 4-year rotation, weed plant density was highest in alfalfa and oat and lowest in corn and soybean, regardless of herbicide regimes because of the collective effectiveness of weed control applied. Banded herbicide and cultivation (low herbicide input) and hay crops reduced weed in following annual crops (Entz et al., 1995) and hay crop mowing before corn planting resulted in reduced weed density by three times during the corn growing season (Anderson, 2016). Those findings explained how the low herbicide regime provided comparable weed emergence and survival suppression to the conventional herbicide regime in our experiment.

Comparable weed plant density and biomass accumulation in the 4-year oat and alfalfa phases (Tables 2-7 and 2-10) suggested equal effects of oat/alfalfa companion crop and alfalfa sole cropping on weed plant suppression. Having alfalfa in the crop rotation reduced seedbank population densities regardless of herbicide regimes (Gulden et al., 2011). Lower seedbank density in alfalfa also translated into lower weed biomass accumulation. Oat in the 3-year rotations that followed different herbicide treatments in the preceding corn and soybean crops had similar weed plant density and biomass, but differences in viable weed seedbank density were evident between herbicide treatments (Figures 2-2, 2-3 and 2-4). Surviving weeds, especially common lambsquarters, common waterhemp and foxtails, in the low herbicide corn and soybean accumulated greater biomass than the same weeds (Figure 2-3), and thus could have collectively produced more seeds than did the weeds in corn and soybean under the conventional herbicide regime (Figure 2-4 and Table 2-12). However, viable seedbank densities in corresponding oat and alfalfa phases to conventional and low corn and soybean were not closely correlated (Table 2-12). This observation may be due to large coefficients of variation due to limited labor availability for seedbank studies, which will be further explained in details in Chapter 3 Section 3.1.

As documented by Mohler (2001b), seeds of broadleaf weed species tend to survive in the soil longer than those of grasses, annual species and stationery perennials tend to accumulate longer-lasting seedbanks than do wandering and woody species, species with strong dormancy mechanisms survive longer in the soil, and small, round seeds tend to remain in the soil seedbank longer than large or elongated seeds. Our seedbank observations followed these four patterns. Specifically, four out of the six most abundant species in the soil seedbank were broadleaves (Figure 2-4 and Table 2-3). The most abundant species were annuals (Uva, 1997), and common lambsquarters and common waterhemp are small seeded species (Buhler and Hartzler, 2001; Forcella et al., 1997) and have sophisticated dormancy mechanisms (Odum, 1965). Our results were consistent with previous studies in which seedbanks in arable soils were found to be dominated by broadleaf species, and reduced herbicide and strip cultivation management increased the abundance of grasses (Barberi et al., 1998; Froud-Williams et al., 1984; Mohler, 1993).

Viable seedbank density appeared to be stable in all crop phases across different rotations (Figure 2-4 and Table 2-13). Two main possible explanations on highest weed seedbank density in soybean under the low herbicide regime in the 3- and 4-year rotations compared to other crops of the same rotation (Figure 2-4) were disturbed predators due to chisel plow tillage application after corn and increased amount of weeds due to reduced chemical amount applied compared to conventional soybean. This explanation is supported by recent studies of soil granivory (Blubaugh and Kaplan, 2015; van der Laat et al., 2015). No tillage reduced disturbance to predators and thus increased predation on the soil (Davis et al., 2003). No-till favors seed decay on the soil surface (Gomez et al., 2014). Legume-small grain intercropping also enhances predation by providing habitat for predators (Davis and Liebman, 2003). Comparing corn and soybean under the same herbicide x rotation treatment combination, soybean was always observed with higher seedbank density. This could be explained by different tillage as well. Ball (1992) measured irrigated row crop sequences in 2-year rotations and concluded that chisel plowing moved seeds to the soil surface while moldboard plowing buried seeds; consequently, seedbank density was reduced faster after moldboarding than after chisel plowing. In our experiment, soybean received spring cultivation while corn received spring cultivation and fall chisel plowing (Table 2-2). In addition, corn is taller than soybean and thus, corn would be more competitive against weeds for light than soybean. This characteristic also explained why weed infestation in corn was lower than in soybean.

Oat and alfalfa under the 3- and 4-year rotations did not receive herbicide regardless of low/conventional herbicide designation (Table 2-1) and thus, weed biomass accumulation in those treatments was higher (Figure 2-3 and Table 2-9) than the sprayed crops of the same herbicide x rotation designation. However, viable weed seedbank densities in oat and alfalfa were intermediate among the crop phases of the 3- and 4-year rotations. Lower viable seedbank densities in oat and alfalfa could be explained by less disturbed soils and more reliable in-field granivore habitat compared to corn and soybean soils. Less disturbance to granivores was imposed in 3- and 4- year rotations because reduced tillage was applied between soybean and oat and no-till between oat and alfalfa. More favorable habitat for granivores was provided with oat intercropped with red clover in the 3-year rotation and with alfalfa in the 4-year rotation than with corn or soybean sole cropping (Westerman et al., 2006).

No-till also favors seed desiccation (Anderson, 2005) and led to lower seedbank density and weed biomass accumulation than conventional tillage (Davis et al., 2003). System-wise, all the weed control tactics applied in the 4-year rotation would minimize the risks of more weeds emerging and greater weed biomass accumulation in corn and soybean phases after seedbank enrichment during two years of reduced herbicide application in oat and alfalfa phases. Oat was intercropped with red clover in the 3-year rotation, whereas oat was intercropped with alfalfa in the 4-year rotation. Differences in companion crop combination could be one reason why weed biomass composition and total accumulation were different between 3- and 4-year oat (Figure 2-3). Overall, the system-wise risks of more weed emergence in corn and soybean after oat in the 3-year rotation could be higher than that in 4-year corn and soybean after unsprayed oat and alfalfa.

2.4.2 Cropping system diversification increased weed biomass but that increase did not affect yield

Herbicide regime did not have a significant effect on weed plant density (Table 2-5), indicating equal effectiveness of the two set of weed control techniques in limiting weed emergence and establishment. The same result was observed in no-till, banded herbicide application and between row mowing for sorghum in Missouri (Donald, 2007). In the present experiment, weed biomass was higher under the low herbicide regime of a given rotation than under the conventional regime (Tables 2-9 and 2-10), but that did not affect yields of corn and soybean (Tables 2-14 a and b). The observation that the two herbicide regimes had comparable impacts on seedbank size of the same crops in the same rotation (except 3-year soybean under low herbicide in 2015) confirms the effectiveness of our chosen weed seedbank management strategies.

2.4.3 Cropping system diversification did not increase weed community overall competitiveness

The aboveground and seedbank patterns seen in our experiment are consistent with other observations made in the U.S. Midwest (Gibson et al., 2016). Significant variations of Shannon's index were observed across herbicide x rotation treatments (Table 2-4) suggesting that unexpected proliferation of one or a few species that could potentially damage crop yields could be present. Mohler (2001a) documented that the richer weed diversity a community exhibits, the more flexible that community would be with regard to adapting to management pressures that ultimately require additional weed control means in which one or a few species may thrive and dominate the weed community and be very competitive to crops. In our experiment, such domination trend was observed in 2- and 3-year rotations but crop yields were not damaged. Shannon's E' index values were close to 1 in all herbicide x rotation treatments indicating that the present species in each treatment were more or less similarly abundant. In the same vein, the 4-year rotation seemed to promote the most even weed diversity in which each crop x herbicide treatment had one or two prevalent species. Collectively, these results confer a sign of relatively low chance of single species proliferation, within each crop x herbicide treatment in each rotation and rotation-wise. Single weed species proliferation is not desirable because it could confer high competitiveness that would be harmful to crop yield(Mohler, 2001a).

2.4.4 Weed species that would likely to be problematic in diversified cropping systems and proposed means of control

Species that were dominant in both number and accumulated biomass are assumed to be better adapted to agricultural management practices compared to their fellow species under the same management. Using two criteria (number of plant and total accumulated biomass), we observed three groups of weed species: 1) common waterhemp (*Amaranthus rudis*) and common lambsquarters (*Chenopodium album*), which were abundant in both the aboveground and seedbank communities across all crop phases regardless of herbicide and rotation treatments; 2) velvetleaf (*Abutilon theophrasti*), foxtails (*Setaria* spp.) and eastern black nightshade (*Solanum ptychanthum*), which were moderately abundant in both the aboveground and seedbank communities, though sometimes absent in a particular herbicide x rotation combination; and 3) species that were present only in the aboveground community, e.g., dandelion (*Taraxacum officinale*).

2.4.4.1 Common waterhemp (*Amaranthus rudis*) and common lambsquarters (*Chenopodium album*)

Common waterhemp (*Amaranthus rudis*) and common lambsquarters (*Chenopodium album*) are frequently found in U.S. Corn Belt soils (Forcella et al., 1992). These species have smaller seeds and higher fecundity compared to other species. In the present experiment, common waterhemp and common lambsquarters produced more biomass than other weed species in almost all crop phases, so even though there were not many of them, they could become problematic. In fact, they were the biggest contributors to the soil viable seedbank

(Figure 2-4). Common waterhemp could become problematic in soybean and common lambsquarters in corn and soybean as herbicide was reduced. For example, a substantial gain of weed biomass in 3-year soybean under the low herbicide regime of 2015 was due mainly to common lambsquarters biomass gain. Soybean plots in 2015 were planted with corn in 2014, in which common lambsquarters seedbank densities were greater than other species. Common lambsquarters' high fecundity was also noted in previous studies (Norris, 2007; Ward et al., 2013; Webster and Grey, 2015). Another explanation of common lambsquarters proliferation in soybean is that this weed can be adapted to soybean field management (Norris, 1996). Common lambsquarters viable seedbank density was second or third highest in the 4-year rotation under low herbicide regime in both years. Therefore, under reduced stress as such in the low herbicide 3-year soybean, common lambsquarters was able to produce substantial biomass. However, common lambsquarters biomass was lowest in 4-year soybean under low herbicide compared to 2- and 3-year rotations. This could be attributed to common lambsquarters' sensitiveness to longer rotations (Lanini and Wertz, 2016). These results would suggest that the collective weed control techniques in the 4-year rotation, among other rotation systems, were effective in reducing common lambsquarters biomass accumulation. Even though the weed seedbank for 4-year oat under the conventional herbicide regime was mainly common waterhemp and common lambsquarters (Figure 2-2), the biomass of those two weeds that was present in the successive alfalfa crop (2015) was low (Figure 2-3) with moderate plant density relative to other weeds. This result could be attributed to the alfalfa cutting regime in killing common waterhemp and common lambsquarters seedlings.

For the same species, bigger plants that survived until maturity would likely contribute more seeds to the seedbank compared to smaller plants. One prominent example of this trend was common waterhemp. Common waterhemp was found with larger parent plants in soybean than in corn (Figure 2-3) and in fact more common waterhemp seeds were found in soybean than in corn (Figure 2-4).

2.4.4.2 Velvetleaf (*Abutilon theophrasti*)*,* foxtails (*Setaria* spp*.*) and eastern black nightshade (*Solanum ptychanthum*)

Velvetleaf (*Abutilon theophrasti*) was not abundant in the aboveground communities so the collective competition posed by velvetleaf on crops would be less than that of other more abundant weeds. Velvetleaf may produce at least 60-70 seeds per mother plant but seeds that are introduced to the soil seedbank from a subthreshold plant community could surpass the plant threshold in the successive season (Cardina and Norquay, 1997). The weed plant threshold should be considered because above threshold weed biomass is undesirable. Thresholds are difficult to define because they vary in different conditions (Coble and Mortensen, 1992). In our experiment, a threshold is the level of weed presence that causes damage to crop yield. Seed survivorship is the most important mechanism for velvetleaf populations to remain in the soil seedbank (Jordan et al., 1995). Velvetleaf was among the top five contributors to the soil seedbank of 4-year soybean in the present experiment and was negligible in successive oat and alfalfa, between which no tillage was used. This observation suggests that reduced soil disturbance substantially reduced velvetleaf seedbank density (Cardina and Norquay, 1997; Liebman and Gallandt, 1997).

Foxtails, including giant and yellow foxtails (*Setaria faberi* and *S. glauca*), could become problematic in corn and soybean due to herbicide regime switching in the 3-year rotation. Foxtail density was high in alfalfa following conventional corn and soybean in the 4-year rotation, but did not become problematic under current management because biomass accumulation was low (Figure 2-2) and seedbank density was small (Figure 2-4). This observation suggests that alfalfa and the weed management techniques associated with it successfully suppressed foxtails in the aboveground and seedbank communities. Giant foxtail seeds were more preferred by invertebrates in corn and soybean compared to *Amaranthus* spp. (O'Rourke et al., 2006) and seed predation was highest in the more diverse 4-year cropping system relative to the 2-year, especially in the alfalfa phase of the 4-year rotation (O'Rourke et al., 2008). Reduced tillage, which can promote granivory, might be the reason why the number of foxtail seeds was lowered. In fact, a previous study on weed population dynamics in corn found that grasses were more sensitive to management practices than broadleaves so it would take less time to substantially reduce the population size of a grass species than a broadleaf (Hartzler and Roth, 1993). Higher rates of depletion of foxtail seedbanks, compared to other species like common waterhemp, common lambsquarters or eastern black nightshade, have also been attributed to a higher germination rate of this species (Cardina and Sparrow, 1996; Gomez et al., 2014).

Eastern black nighshade (*Solanum ptychanthum*) could become problematic in the 3-year rotation, as indicated by its frequent occurrence in the soil seedbank across crop phases, especially under the low herbicide regime. Eastern black nightshade emerging in May could produce up to 7,000 berries and 825,000 seeds per mother plant at maturity, with fecundity decreasing at later emergence dates; plants emerging in August usually failed to produce berries (Quakenbush and Andersen, 1984) because they are highly sensitive to shade (Myers and Stoller, 1984). A possible explanation of why eastern black nightshade was less prevalent in soybean and alfalfa (Figure 2-3) was that the soybean canopy, to which eastern black nightshade was very sensitive, was well maintained (Quakenbush and Andersen, 1984) and alfalfa was early planted in 2014 and overwintered to 2015, giving alfalfa stronger competiveness over eastern black nightshade (PennState Extension, 2016). Eastern black nightshade plants that were present in some crop phases might have survived our May – July weed control applications (Quakenbush and Andersen, 1984). However, the total contribution of eastern black nightshade to the soil seedbank was considerably lower than common lambsquarters and common waterhemp (Figure 2-4).

Total weed biomass accumulation in alfalfa following oat was considerably decreased relative to oat, especially for common waterhemp, common lambsquarters, foxtails and eastern black nightshade (Figure 2-3). Therefore, weed biomass would be more effectively suppressed by alfalfa than the oat/alfalfa companion crop in the 4-year rotation. In this case, induction of fatal germination and exposure of seedlings to phytotoxins from oat, red clover and alfalfa could contribute to exhaustion of common waterhemp, common lambsquarters, foxtails, and eastern black nightshade in the soil seedbank (Kurstjens, 2007; Mohler, 2001b). Other than fatal germination, habitat maintenance for rodents and insects would help reduce the seedbank as well (O'Rourke et al., 2006; van der Laat et al., 2015; Westerman et al., 2006).

2.4.4.3 Dandelion (*Taraxacum officinale*)

Why dandelion (*Taraxacum officinale*), which was prevalent in the aboveground communities, was absent from the seedbank could be explained by its delicate seed coat, which could have been easily damaged by biotic and abiotic agents. Evidence that supports this explanation was the positive correlation of seed survival rate and seed coat thickness (Borgy et al., 2015). Therefore, if the soil temperature were not effective for germination, dormant seeds could soon be degraded or consumed by granivores (Honek et al., 2011; Lundgren et al., 2013; Menalled et al., 2006; O'Rourke et al., 2006). From the available literature, we believe that dandelion found in our experiment could have germinated soon after the mother plant shed seeds (Luo and Cardina, 2012; Ogawa, 1978). The original mother plants that were present in the experiment site and particularly in the field during sampling periods could have germinated from seeds transported from areas surrounding the experiment site or from other regions by wind (Tackenberg et al., 2003).

Higher dandelion plant density and biomass were observed in 4-year oat and alfalfa compared to corn and soybean of the same rotation because it has adapted to cereal after cereal and alfalfa management (Canevari, 2001; Ominski et al., 1999). Increases in dandelion plant density and biomass could be attributed to thinner stand density of alfalfa through time due to alfalfa production of autotoxins that inhibit germination of new alfalfa seedlings (Undersander, 2011), or to a longer no-till period in the 4-year rotation systems (Derksen et al., 1994).

How to prevent dandelion colonization would therefore be more dependent on the specific weather conditions (when the first cohort of dandelion could germinate and produce seeds in the field) and crop phase (dandelion did not proliferate in corn and soybean or 3-year oat but it did in 4-year oat and alfalfa), than other variables. In case dandelion does not pose significant resource competition with crops, it could be retained in the field for benefits of promoting lady beetle predation on aphid species that attack legumes, such as pea aphids (Harmon et al., 2000).

2.4.5 General summary

Switching from the conventional to low herbicide regime allowed herbicide reduction by 64% in corn and 94% in soybean on the basis of unit mass of active ingredients per unit of land area.

Longer rotation further decreased the amount of herbicide required without damaging corn and soybean yields. Soybean yield was highest in the 4-year rotation system and lowest in the 2-year rotation, while corn yields were similar across rotation systems. Cropping system diversification did not change weed plant density but caused increased total accumulated weed biomass and seedbank density.

The increased weed biomass did not affect yield. Cropping system diversification did not increase weed community overall competitiveness. No species became overly dominant in any of the treatments. Weed community evenness value ranged from 0.6 to 1.0 in most of the herbicide x rotation treatments (Figure 2-1) indicating a fairly even community.

Three groups of weed species were observed in the study: abundant in both aboveground and underground communities, moderately abundant in aboveground and underground communities though sometimes absent from some herbicide x rotation treatment, and present in the aboveground communities only. Seven weed species were found experiment-wise that need special attention when switching herbicide regime from conventional to low were *Abutilon theophrasti*, *Amaranthus rudis*, *Chenopodium album, Setaria faberi, S. glauca, Solanum ptycanthum,* and *Taraxacum officinale.*

CHAPTER 3 SAMPLING INTENSITY FOR ESTIMATING WEED SEED POPULATION DENSITY IN SOIL

3.1 Introduction

In weed population dynamics studies, weed seedbank information is complementary to floristic data. Core sampling, among other available soil sampling methods, is considered the most accurate in estimating population means (Cardina and Sparrow, 1996). However, researching soil seedbanks is laborious with rigorous requirements for sampling strategies and statistics (Benoit, 1986; Benoit et al., 1989; Dessaint et al., 1996) and thus, sampling procedures for a particular field should be customized based on research interest, and time and labor availability (Forcella et al., 2003).

Weeds are direct competitors with crops for resources and have developed eco-physiological traits to be able to cope with stress and mortality factors and maintain their existence. Some of those traits include small seed size, high fecundity, extended seed longevity in soil, and a high frequency of innate seed dormancy (Mohler, 2001b). Weed seeds, upon being produced and dispersed can germinate and grow, germinate and die, remain dormant or lose viability, be eaten, or be transferred to other areas by wind, water, animals, or machinery. The proportions of weed seeds represented in these categories are poorly known and are dependent on multiple field variables (Davis et al., 2005). Previous studies of the effects of cropping system and tillage regime combinations indicate that cropping systems tend to be more important than other factors such as tillage and herbicide use in determining weed seedbank composition (Ball, 1992; Cardina et al., 2002; Sosnoskie et al., 2006). Those findings suggested the importance of weed seedbank composition recorded in each crop phase of one cropping system and in the same crop across different cropping systems in order to develop prescriptive seedbank management strategies.

Because new and recurring annual weeds emerge from soil seedbanks, Davis et al. (2005) recommended limiting the number of seeds of persistent weed species entering the soil. That goal could be supported with a better understanding of seedbank dynamics, especially the seedbank composition (Buhler and Forcella, 1997) and species-specific germination patterns (Cardina and Sparrow, 1996). In the same vein, limiting weed seed input to the soil was recognized as the key to long-term field weed management (Norsworthy et al., 2012). For example, suppressing weed seedbank densities in one year greatly increased the effectiveness of herbicides used in the following years on the plant community and eventually reduced the number of new weed seeds entering the soil seedbank (Forcella et al., 1993; Hartzler and Roth, 1993).

A meta-analysis of seedbanks across the U.S. Corn Belt indicated that at least 15 soil cores (5 cm diameter by 20 cm depth) be taken for stable variance for estimates of total seedbank density across field size units and seedbank density at different locations (Forcella et al., 1992). In a general statistical sense, for a fixed volume of soil material, the more samples of smaller size taken, the more precise estimates of field seedbank densities of prevalent species that can be achieved, compared to fewer samples of larger size (Benoit, 1986; Bigwood and Inouye, 1988; Roberts, 1958). Coefficients of variation (CV) are often greater than 50% for seedbank assessments of all species present in an area, or greater than 150% for individual species (Forcella, 1984). Relatively rare species in an area tend to have greater CVs than more abundant species (Cardina and Sparrow, 1996). Sample sets with higher CV indicate aggregated seeds and in that case a larger sample size is required to precisely assess the population means (Ambrosio et al., 1997). Dessaint et al. (1996) conducted a large-scale research project across five European countries to determine a framework for calculation of the number of 5-cm-diameter soil cores needed for differing levels of precision**.** They concluded that attempting to include rare species in sampling efforts for high precision would require an exponentially higher number of samples to be taken and analyzed. Benoit et al. (1989) conducted an experiment to test precision of sampling for *Chenopodium* spp. using a matrix sampling method as a standard to test others. They suggested taking 60 to 75 cores with 1.9 cm diameter and 15 cm depth, equivalent to approximately 2552 to 3190 cm^3, in random or stratified random arrangement, for estimating seed population density of a single, abundant species.

Results of these studies have been highly referenced among weed researchers, so we adapted the data collection method to our project. Taking together previous seedbank statistics research, we chose to focus on the most abundant species.

Two key factors of soil sampling for seedbank analysis are the total volume of soil and the number of soil cores to be taken (Chauvel et al., 1989). Total soil volume to be taken is dependent on the available labor to process the sample. In case of limited labor, only a finite amount of soil can be processed. The adequacy of soil sampling intensity, based on the available labor and time, could be estimated using Dessaint's equation (1996). In the scope of our research, we were interested in the following issues:

1. The relationship between the total soil volume, represented by number of soil cores taken as samples, and the precision of estimates of weed seedbank densities in soil; and
2. For a particular number of soil cores per experimental unit, the relationship between the number of separately processed samples and the precision of estimates of weed seedbank densities in soil.

Here, we focused on weed seedbank variation in one crop phase of one crop rotation system. Soil seedbank analysis is confounded by patchiness of individual weed species due to irregular distribution of parent plants and different seed shedding patterns (Nkoa et al., 2015; Ritz et al., 2015). However, the collective seedbanks of all species present in a field can be distributed almost uniformly (Forcella, 1984). Consequently, we assessed collective seedbanks of all species recovered. Since the seedbank in our experiment has been dominated by a few abundant species (Figure 2-4), which is also the case in the U.S. Midwest (Forcella et al., 1992), assessing their density precisely could provide sufficient information for seedbank management.

3.2 Materials and methods

3.2.1 General approach

Due to labor constraints, in the past our samples were taken randomly in each experimental unit and then combined and processed to generate one data point. We sought to check if the soil sampling intensity used in this study would be acceptable in estimating the seedbank population density and if increasing the sampling intensity would meaningfully increase precision. The current sampling intensity would be acceptable for seedbank population density estimation if two conditions were met: first, increasing the number of cores to be drawn would not considerably decrease the coefficient of variation of the average soil seedbank density; and second, combining all samples taken within an experimental unit and processing them together to generate one data point would not considerably increase the coefficient of variation of the average soil seedbank density.

3.2.2 Experiment design

Analyses of soil samples drawn in 2014 showed that soybean plots in the 4-year rotation under the low herbicide regime in the Marsden Farm experiment discussed in Chapter 2 had an intermediate size viable seedbank (2,525 seeds/m^2) relative to other treatments in the experiment. Consequently, soybean plots of the 4-year rotation/low herbicide treatment were chosen for the present investigation. Its viable seedbank density also fell into the average range of glyphosate-tolerant cropping systems, 300 to 25,000 viable seeds/m^2 (Heard et al., 2003).

We investigated the precision of our current seedbank sampling method by analyzing clustered sample variation in fall 2015. In field observations, we found a diverse range of plant sizes present randomly in heterogeneous patterns, so one very well established plant with tens of thousands of seeds could be present in one place and a few other seedlings in other places. In addition, as seeds usually are shed closer to mother plants, chances of finding no seeds in one single soil core could be possible if the number of cores taken was low. Pooled samples from spatially close soil cores was recommended for increasing accuracy of data (Benoit et al., 1989; Bigwood and Inouye, 1988; Wiles and Schweizer, 2002). Therefore, we chose to take samples in nine clusters, with each cluster consisting of four closely located cores (Figure 3-1). With that arrangement, soil material in each cluster was combined and processed to generate one data point at each of nine cluster locations per plot (details in Section 3.2.3). The selected experimental units consisting of soybean under a low herbicide regime in a 4-year rotation were sampled in a three-by-three cluster matrix and each cluster was processed individually. Seed extraction and germination tests were done as described in Chapter 2 of this thesis.

X X X X	X X X X	X X X X
X X X X	X X X X	X X X X
X X X X	X X X X	X X X X

Figure 3-1 – Sampling diagram for seedbank density in 4-year soybean under low herbicide

3.2.3 Data collection and analysis

According to a study by Forcella (1984), a sample size that is appropriate for soil seedbank species diversity assessment would be usable for soil seedbank density assessment. An important concern in soil weed seedbank analysis is that elutriation procedure provides higher accuracy in terms of number of seeds but lower accuracy in terms of number of species when compared to direct germination methods (Gross, 1990; Gross and Renner, 1989). Taking the three aforementioned studies together, there are two critical requirements in assessing soil seedbank richness and density, which are sample size and sample processing procedure. An adequate sample size could provide reliable information of weed species richness, but seeds may be lost during sample processing. Considering our seedbanks across rotation x herbicide treatments, which were dominated by a few species, if there were a risk of yield damage due to higher weed population densities, that risk would be attributed more to the most abundant species than the rarer ones, so the former are more important from the standpoint of crop protection. Therefore, we accepted the sampling and sample processing trade-off in order to precisely assess the seedbank of those abundant species.

For data analysis, each of the four experimental units was considered an independent observational field. Each set of four cores from nine locations within each experimental unit was bagged and analyzed as one sample. Since total number of seeds from all species in a field can be distributed almost uniformly (Forcella, 1984), we chose to use Monte Carlo simulation with Poisson distribution to analyze the data.

The Monte Carlo method was used to interpret the probability distribution of expected average number of seeds in soil samples by simulating hypothetical data sets of the same variations with the collected data (Kroese et al., 2014).

For the analysis of field collected data, let Y_{ij} denote the number of seeds in field *i* (i = 1,2,3,4), and cluster *j* (j = 1,2,3,…,9). Y_{ij} is distributed following a Poisson distribution: $Y_{ij} \sim \text{Poisson}(\lambda_{ij})$. The expected number of seeds in a field was calculated with: $\text{Ln}(\lambda_{ij}) = \mu + \alpha_i + \gamma_{ij}$, where

α_i : random field effect $\alpha_i \sim N(0, \sigma_f^2)$

γ_{ij} : random cluster effect $\gamma_{ij} \sim N(0, \sigma_c^2)$

From the field data, the variation of α_i and γ_{ij} were calculated. One thousand data sets of different size that share the same cluster and field effects were generated by the lme4 (Bates et al., 2015) procedure in R®. For those 1,000 hypothetical data sets, the following five steps were taken to simulate their behavior in order to examine the relative change of precision as the number of clusters changed.

Step 1: Simulate $\alpha^*_i \sim N(0, \hat{\sigma}_f^2)$ and $\gamma^*_{ij} \sim N(0, \hat{\sigma}_c^2)$

Step 2: Calculate $\lambda^*_{ij} = e\hat{}(\hat{\mu} + \alpha^*_i + \gamma^*_{ij})$

Step 3: Simulate $Y^*_{ij} \sim \text{Poisson}(\lambda^*_{ij})$

Step 4: Refit model and get estimates

Step 5: Estimate the number of seeds in clusters with $e\hat{}[\hat{\mu} + (\hat{\sigma}_f^2 + \hat{\sigma}_c^2)/2]$

3.3 Results

In 2014, common waterhemp (*Amaranthus rudis* J.D. Sauer) and common lambsquarters (*Chenoposium album* L.) comprised 93% of the total soil weed seedbank for soybean grown in a 4-year rotation under low herbicide treatment. In 2015, common waterhemp and common lambsquarters composed 91% of the viable seedbank of the chosen experimental units. Experiment-wise, the five most abundant species that were found in the chosen experimental units made up over 99% of the viable seedbank in both years (Figure 3-2) so we are confident that the finding presented below is representative of the seedbank density.

The average soil seedbank density in the chosen experimental units was 13,333 seeds/m^2 in 2015. The number of soil samples (35 or 36 soil cores of 1.75 by 20 cm) taken to date in this experiment resulted in coefficients of variation below 20% across experimental units, regardless of whether the material was processed as one or nine samples (Figure 3-3). If field means and cluster means were available, the coefficient of variation of the estimated average number of seeds decreased by 9.2% as the number of clusters increased from seven to nine and by 11.3% from nine to eleven. If only field means were available, the coefficient of variation of estimated average number of seeds decreased by 9.7% as the number of clusters increased from seven to nine and by 11.1% from nine to eleven.

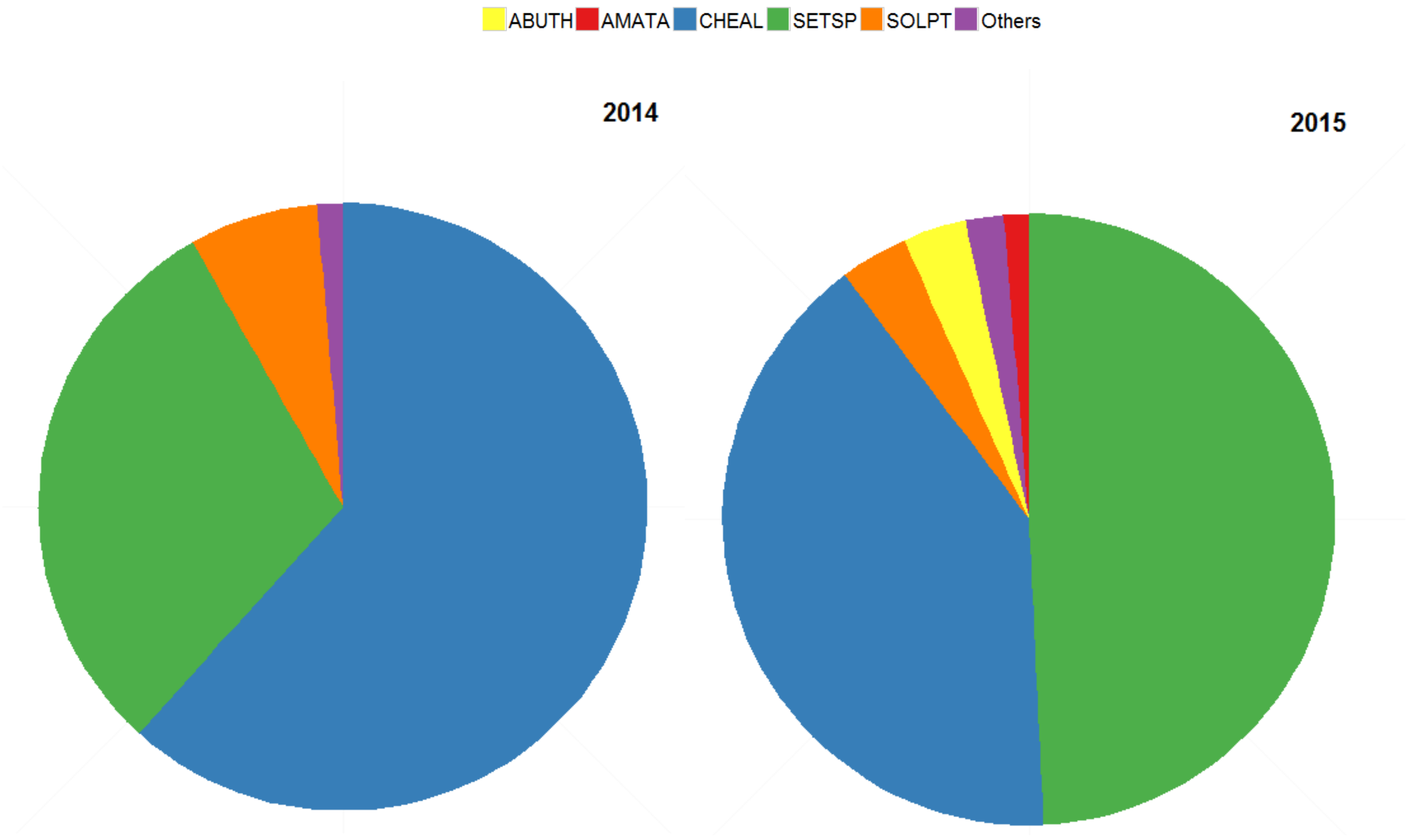

Figure 3-2 Weed seedbank composition in for soybean grown in a 4-year rotation under a low herbicide regime

ABUTH: *Abutilon theoprasti* AMATA: *Amaranthus rudis*, CHEAL: *Chenopodium album*,
SETSP*: Setaria faberi* and *S. glauca*, SOLPT: *Solanum ptycanthum*,

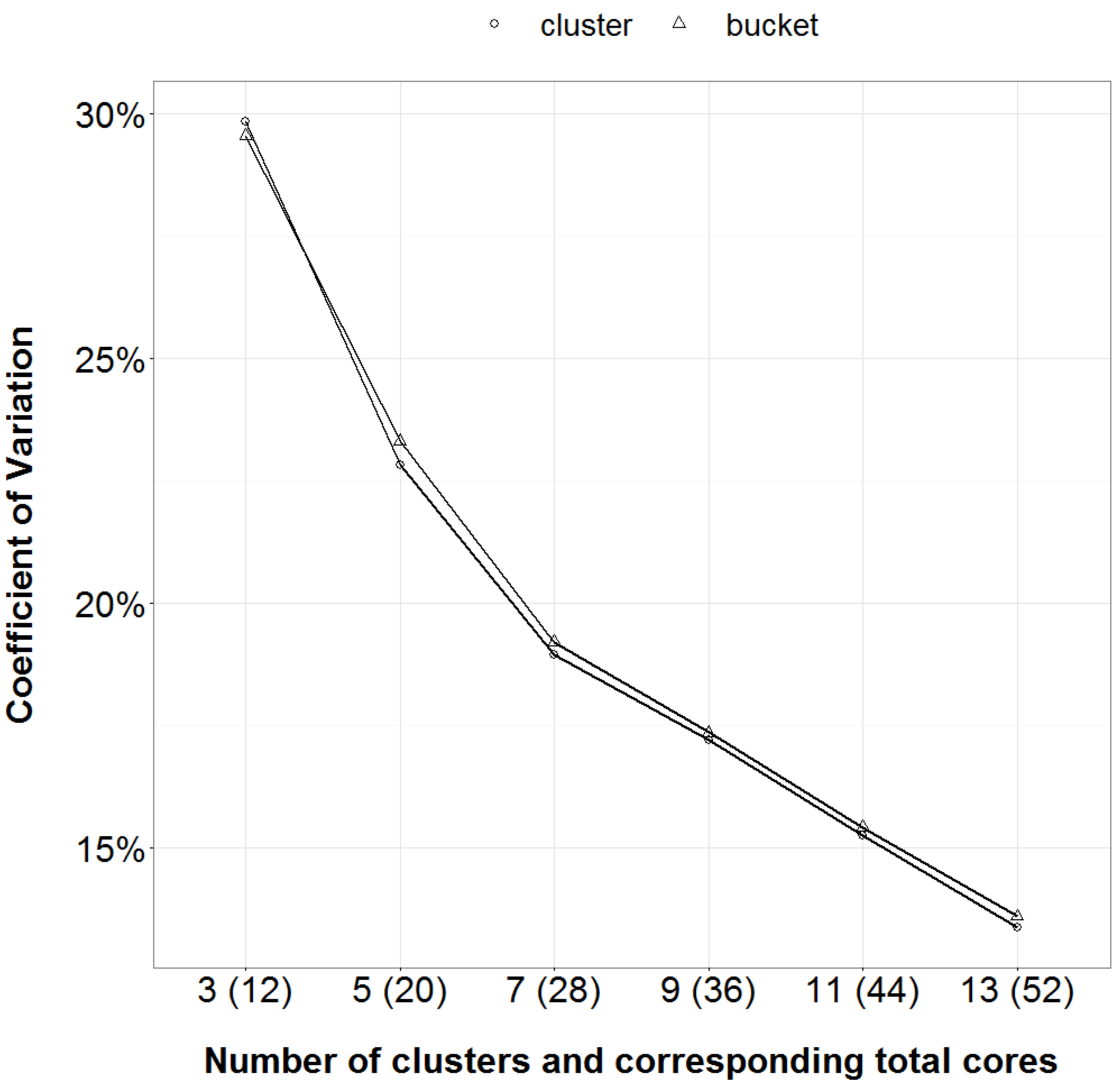

Figure 3-3 Decrease of coefficients of variation as the amount of soil material increases

Cluster: scenarios in which soil materials from each set of 4 cores were processed separately
Bucket: scenarios in which soil materials from all clusters were processed as one sample

3.4 Discussion

Overall, the two criteria for precise sampling as detailed in Section 3.2.1 were met. Our CV of < 30% in all simulated scenarios was consistent with CV behaviors in previous studies (Cardina and Sparrow, 1996; Forcella, 1984). The results of seedbank density from our current sampling intensity agree with average range in the U.S. Midwest (Forcella et al., 1992) and in other glyphosate-resistant crop dominated regions (Barberi et al., 1998; Heard et al., 2003).

The cores were drawn from plots with intermediate level seedbank population density and thus for higher population density seedbanks, it would be possible to take fewer samples without compromising precision. Similarly, it would require more samples in regions of lower seedbank density. Sampling in clusters would not increase precision in a meaningful way. Taking nine clusters of soil cores, comprising 1,732 cm^3 in volume, provided an acceptable estimate of the seedbank population density under labor and time constraints. Increasing the volume of soil taken or the number of clusters per fixed soil volume would not decrease the coefficient of variation in a meaningful way (Figure 3-3). Therefore, combining all soil material from each subplot to one sample is acceptable. Processing all the collected soil material from an experimental unit in one sample would save significant cost of labor. Future soil seedbank sampling in our long-term experiment could be done with the current intensity. Arable soils under similar management with similar weed seed spatial distributions could be sampled with the same intensity.

Our total sampled area per each experimental unit was 84 cm^2 in 2014 and 87 cm^2 in 2015. These values are considerably lower than typical recommendations of 200 cm^2 to 1,000 cm^2 in classical studies (Forcella, 1984) and thus would be encouraging to other researchers who are interested in sampling soil seedbank density.

CHAPTER 4 CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Overall, the main similarities between the dominant cropping systems in the U.S. Midwest and Vietnam's Mekong Delta are the lack of crop diversity and shrinking labor force. With regard to weed control, those similarities have resulted in the same requirement for sustainable and profitable management that could synchronize current available resources and accommodate on-going demands of increasing productivity per unit area of land. Monocropping and short rotation sequences coupled with heavy reliance on herbicides have resulted in herbicide resistance in populations of a number of weed species. Some of the most noxious weeds have evolved resistance to multiple herbicide modes of action (Heap, 2014). The evolution of resistance to herbicides in weeds is attributed to recurrent herbicide applications (Horowitz et al., 1974; Moss, 2003) and dependence upon a limited group of active ingredients over the past two decades in accommodating genetically modified herbicide-tolerant crops has resulted in wide-spread herbicide resistance issues (Heap, 2014). Because no new herbicide mechanism of action has been commercially introduced over the past 20 years (Duke, 2012; Owen, 2014), herbicide-based weed management can be expected to become more complicated and more difficult.

In the context of increased weed resistance to herbicides, weed control strategies that are cost-effective and less reliant on herbicides are essential for maintaining yield while not accelerating the rate of resistance evolution (Liebman and Gallandt, 1997). Among the available options for management strategies, agroecological approaches including cropping system diversification, reduced tillage, and employment of both mechanical and chemical control tactics appear to be the most promising (Gliessman, 1998). However, agroecology requires rigorous planning of crop sequences, management timing, fertilizers, and pesticides, and thorough understanding of current pest problems, and thus seems more sophisticated to apply. One main reason why cropping system diversification has not been popular among farmers is the uncertain cost-benefit balance associated with additional sophistication of cropping system and management tactics design. Our research was put forward to examine how we could smoothly transfer the current corn-soybean cropping system, which is heavily reliant upon herbicides, to less external agrichemical input reliance, with three locally well-known but neglected crops, oat, red clover, and alfalfa, and a combination of chemical and mechanical weed control tactics. The general patterns observed for weed plant density, weed biomass accumulation, weed seedbank density, and crop yield suggested a tolerable level of weed biomass in the field that did not damage yield. We conclude that integration of mechanical and chemical tactics provided effective weed control in corn and soybean. In addition, soybean yielded higher in longer rotations. Overall, the 4-year rotation was more effective than the 3-year rotation in maintaining or boosting crop yields while keeping weed infestations at reasonably low levels and reducing herbicide use.

The first research question addressed was whether weed control efficacy was affected by cropping system diversification. Cropping system diversification with multiple crops, diverse sources of nutrient supply, and multiple weed control tactics was offered as an alternative to a simpler rotation system and a conventional herbicide regime. We addressed the question by testing if the aforementioned changes to the current corn-soybean cropping system would increase weed biomass and density, and if any increase of weed infestation would affect yield.

Data showed that herbicide regime and rotation system both had significant effects on weed biomass. Higher weed biomass was observed in diverse cropping systems, i.e. cropping systems that employed mechanical weed control in addition to herbicide and that involved more crops than the 2-year corn-soybean rotation. Data also showed that even though higher weed biomass was collected in the low herbicide regime, yields of corn and soybean were unaffected relative to yields in the conventional herbicide regime. It is important to continually reduce the weed seedbank, limit weed plant establishment and the number of weeds exposed to herbicide, and induce fatal germination using the current integrated management applied in our 3- and 4-year rotations. Doing so would help keep weed populations at low densities and ultimately reduce weed community competition with crops. Soybean yielded highest under the most diverse cropping system (the 4-year rotation) and corn yield was equivalent across cropping systems, indicating positive effects of cropping system diversification for producer income. Responses measured in the field experiment were within the range of results reported from other experiments conducted in the U.S. Midwest.

The second research question was whether the current soil sampling intensity provided a reliable estimate of weed seedbank population density. This question was divided into two smaller questions: did the volume of soil collected suffice for assessing the weed seedbank density, and was it necessary to have multiple data points per one experimental unit? We approached these questions using experimental units with weed seedbank densities at what we considered intermediate levels, based on previous field work. Data were then analyzed under different scenarios. Results indicated a minimal decrease of coefficients of variation as the number of soil cores increased above 36, and that combining soil materials from clusters within experimental units would not increase coefficients of variation in a meaningful way. We concluded that taking 36 soil cores of 1.75 cm diameter to 20 cm depth was acceptable for estimating total (all-species) weed seed densities and that soil material from one experimental unit could be combined for single processing.

4.2 Recommendations

Overall, strategic cropping system and weed management diversification, which comprise more crop species and incorporation of chemical and mechanical weed control tactics, could keep weeds at acceptable levels that did not incur damage to crop yields. Therefore, we recommend this set of techniques for farming. Our research has offered another example of how glyphosate resistant crops, reduced levels of external farm inputs, and conservation tillage can be knitted into a profitable and practical cropping system following an agroecological philosophy.

Synthetic herbicides are often considered the most cost-effective short-term solution for weed-infested land, saving an enormous amount of labor cost (Gianessi and Reigner, 2007). Burnside et al. (1986) applied herbicide to a corn field for five years and reduced 95% of the weed seedbank, then tried the sixth year herbicide free and ended up with 90% of the original weed seedbank, suggesting that the frequent use of herbicides is necessary to keep weed populations at an acceptable level against economic and technical constraints. A point worth noting is that use of herbicides could affect the relative abundance of a particular species, but cannot eliminate it (Cousens and Mortimer, 1995). Weed eradication is likely impractical for technical, financial and environmental reasons because weeds evolve resistance (Buhler et al., 1997). Relying solely on mechanical control can create another direction for weeds to evolve resistance. Repetition of mechanical weed control resulted in avoidance strategies like crop mimicry at important life stages to escape eradication (Barrett, 1983). For example, where hand weeding is more popular than chemical herbicide, noxious weeds like junglerice (*Echinochloa colona* L. Link) have evolved biophysiological traits similar to cultivated rice and cause significant crop yield loss (Fischer et al., 1997). Therefore, it would be more realistic to aim for maintaining weeds at low population densities that do not damage yield rather than for a weed-free condition. This is consistent with an agroecological perspective, as our research and previous projects have illustrated. Maintaining weeds at low densities would require a change in mindset that recognizes various benefits of weeds by seeing their potentials and seeks to manage them instead of seeing them as obstacles only (Hyvonen and Huusela-Veistola, 2008; Jordan and Vatovec, 2004; Legere, 2009).

Hareau et al. (2006) used an economic model to assess development in Uruguay and found that strategic partnerships for a larger regional market (rather than only local markets) were required to encourage multinational seed companies to adapt existing herbicide resistant crops to local conditions. This result is highly relevant to the socio-economic setting of Vietnam's Mekong Delta region, because Uruguay and Vietnam are both low middle income countries, and thus should be referenced in making a macro-economic national plan for weed control in rice with herbicide resistant varieties. Decentralization of governance authorities in which concerned citizens have their voice heard should provide an enabling environment for citizens and groups from various backgrounds to actively participate in decision-making processes (Work, 2002). Community-driven development programs that strengthen the linkage between smallholders and high-value markets with special conditions to avoid elite capture of resources could ensure equal benefits for underrepresented groups (Food and Agriculture Organization, 2009).

Altieri (2004) recognized the importance of cooperation between agroecologists and traditional farmers in order to employ more traditional knowledge for improving existing agricultural systems. Effective agroecological models that are highly productive and facing less complicated pest problems have been practiced by subsistence farmers around the world and do not require high levels of technological inputs. Taking these findings together, herbicide resistant crops and herbicide packages are not the “silver bullet” in weed management and poorer accessibility to modern technology such as transgenic crop and pesticide packages or precision agriculture in developing countries should not be regarded as the barrier to having more sustainable agricultural systems.

Developing countries in general and Vietnam’s Mekong Delta region in particular do not have to go through all the steps that have happened in the U.S. and other developed regions in customizing an effective weed management program. The duck-rice and fish-rice systems mentioned in Section 2.2.1 are agroecologically-based and have proven profitability in local contexts. Embracing agroecology right at the beginning of agricultural industrialization would help the Mekong Delta region avoid inviting environmentally destructive effects similar to the ones that have occurred in the U.S. Midwest. Non-selective application of agricultural industrialization in developing countries could enlarge the economic development gap between developed and developing countries, especially under the forecasted circumstances of climate change (Fischer et al., 2005). An extreme case of economic failure due to inadequate research and development can be seen in Argentina, where 16 million hectares of arable land were planted to glyphosate resistant soybean cultivars and a “transgenic treadmill” was created, in which glyphosate resistant johnsongrass (*Sorghum halepense* L.) covered approximately 10,000 hectares of crop land (Binimelis et al., 2009). This failure indicates that mono-crop agriculture and overreliance on one associated means of weed control are not practical and reliable.

Relating to rice as one of the top export commodities and main staple grain in Vietnam, the “rice first” policy would likely to be problematic over the long term if packages of transgenic herbicide resistant rice cultivars and herbicides proliferate there. As discussed by Hanson et al. (2014), herbicide resistant gene transfer from a crop to its wild relatives in a crop’s non-native habitat is much lower than that in its native habitat. Conversely, the risk of having herbicide resistance genes transferred from herbicide resistant rice to rice’s wild relatives are higher in Asia than in other regions where rice is not native. Gene flow from transgenic, glufosinate resistant rice to red rice (*Oryza sativa L.*) was observed in field conditions (Busconi et al., 2014; Gressel and Valverde, 2009). Therefore, rigorous research and development is required before incorporating transgenic herbicide resistant rice into current rice production systems (Gressel and Valverde, 2009; Olofsdotter et al., 2000).

Overall, expansion of a reduced external input philosophy through practical examples would help farmers become less dependent on agricultural input manufacturers, regardless of the level of technology to which they have access.

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