

The Pennsylvania State University

The Graduate School

College of Agricultural Sciences

**THE EFFECT OF CROPPING ROTATION AND MANAGEMENT ON ARBUSCULAR  
MYCORRHIZAL FUNGI IN A SUSTAINABLE DAIRY CROPPING SYSTEM**

A Thesis in

Ecology

by

Kristin R. Haider

© 2014 Kristin R. Haider

Submitted in Partial Fulfillment  
of the Requirements  
for the Degree of

Master of Science

August 2014

The thesis of Kristin R. Haider was reviewed and approved\* by the following:

Roger T. Koide  
Professor of Horticultural Ecology  
Thesis Advisor

Douglas B. Beegle  
Distinguished Professor of Agronomy

David Mortensen  
Professor of Weed and Applied Plant Ecology

David Eissenstat  
Professor of Woody Plant Physiology  
Chair, Intercollege Graduate Degree Program in Ecology

\*Signatures are on file in the Graduate School.

## Abstract

As concerns about biodiversity loss, soil loss, nutrient imbalance, energy use, and climate change grow, there has been an increasing effort to develop cropping systems that minimize these environmental impacts while remaining economically viable. Indicators such as crop yield and quality, weed and insect populations, nutrient conservation, greenhouse gas emissions, energy use and production, and overall farm profitability are commonly measured to assess the performance of these systems, but it is also important to consider the impact cropping systems may have on non-target organisms, especially those that play an important role in agricultural systems. One such group of non-target organisms is the arbuscular mycorrhizal fungi (AMF). We conducted two studies to examine the impact of cropping rotation and management on AMF within one such cropping systems trial that was developed to identify sustainable dairy cropping practices in the northeast United States.

In the first study, oats (*Avena sativa* L.) were planted as a mycorrhizal companion crop for fall planted canola (*Brassica napus* L.), a non-mycorrhizal crop, to determine if intercropping oats with canola was an effective method of maintaining AMF populations. The colonization of corn (*Zea mays* L.) bioassay plants was assessed in plots of canola with and without oats as a companion crop after the canola was harvested. The colonization of the two crops following canola in the crop rotation, rye (*Secale cereal* L.) and soybeans (*Glycine max* (L.) Merr.) respectively was also assessed. There was no significant difference among

treatments in the colonization of any crops assessed. The lack of the effect of oats as a companion crop may have been due to the oats being winter-killed prior to establishing significant biomass, or the low planting density (22.4 kg ha<sup>-1</sup>) of the oats. Alternatively, compounds produced by the canola plants, called isothiocyanates, may have suppressed the colonization of the oats by AMF. Therefore, intercropping canola with a low density of winter-killed oats does not appear to be sufficient method of increasing mycorrhizal colonization in crops following winter canola.

Additionally, in the first study, the oat companion crop treatment was nested within an herbicide treatment that compared reduced and standard herbicide practices. The reduced herbicide treatment was tilled with a moldboard plow before canola was planted while the standard herbicide treatment was not tilled. Colonization of the corn bioassay plants, planted after canola was harvested, was significantly reduced in the reduced herbicide (tilled) treatment. There was no difference in the colonization of the subsequent two crops, rye and soybeans. There was also no interaction between the herbicide management treatment and the oat companion crop treatment. The rapid disappearance of the impact of tillage on AMF colonization in this cropping system suggests that sporadic tillage may have little consequence for mycorrhiza-dependent crops if they do not directly follow tillage in the rotation.

The second study focused on the overall effect of crop rotation and management in the dairy cropping system on AMF colonization of corn grown within three rotations, a six-year grain crop rotation with an herbicide management

treatment, a six-year forage crop rotation with a manure management treatment, and a two-year corn-soy rotation with a manure management treatment. The colonization of corn seedlings was assessed for all corn plots in the system when the seedlings reached the third leaf stage.

Within the three rotations, I also made comparisons between management treatments including a manure management treatment (broadcast vs. injected manure), an herbicide treatment (reduced vs. standard herbicide), and a cover crop treatment (red clover (*Trifolium pretense* L.) vs. hairy vetch (*Vicia villosa* Roth) and oats). Overall there was no difference in the colonization of corn between manure management treatments, herbicide treatments, or cover crop treatments.

Three different varieties of corn were used in the three rotations: one conventional variety, one variety with a single transgenic trait for herbicide resistance, and another with three transgenic traits including one for expressing the insecticide protein *Bacillus thuringiensis* (Bt). To determine if corn variety impacted AMF colonization, the three corn varieties were grown in a greenhouse in sterile soil inoculated AMF spores. There were no significant differences in the amount of mycorrhizal colonization of the three varieties.

Although there was no significant impact of corn variety or treatments within the rotations on AMF colonization, corn seedlings in the corn-soy and grain rotations were colonized significantly less than those in the forage rotation. The observed differences in the colonization of corn among the rotations may have been driven by the presence of a cover crop preceding corn in the rotation. Because AMF are dependent on their host plants for energy, long periods during which host plants

are absent or only poor hosts are present are detrimental to their populations. In the corn-soy rotation there was a fallow during the winter preceding corn, in the grain rotation there was a rye (a potentially weak AMF host) cover crop, and in the forage rotation there were alfalfa (*Medicago sativa* L.) and red clover/hairy vetch cover crops. Based on these results, it appears that the prior crop has the largest impact on colonization of corn by AMF in this system, and that cover crops that form strong AMF associations should be used instead of fallows and plants that form weak AMF associations to promote the colonization of subsequent plants.

## TABLE OF CONTENTS

List of Figures.....	viii
List of Tables.....	ix
Acknowledgements.....	x
Chapter 1. SUSTAINABLE DAIRY CROPPING SYSTEM SUMMARY.....	1
Introduction.....	1
Strategies for Enhancing Soil Quality.....	2
Strategies for Closing Nutrient Cycles .....	4
Strategies for Reducing Chemical Inputs.....	6
Strategies for Reducing Agricultural Impacts on Climate Change.....	9
Sustainable Dairy Cropping System Design.....	11
Effect of Management and Crop Rotation Strategies on Arbuscular Mycorrhizal Fungi.....	13
Chapter 2. THE IMPACT OF INTERCROPPING CANOLA ( <i>Brassica napus</i> L.) WITH OATS ( <i>Avena sativa</i> L.) ON ARBUSCULAR MYCORRHIZAL FUNGI IN A DAIRY CROPPING SYSTEM.....	15
Introduction.....	15
Materials and Methods.....	20
Results.....	28
Discussion.....	32
Conclusion.....	35
Chapter 3. THE IMPACT OF CROP ROTATION AND MANAGEMENT ON ARBUSCULAR MYCORRHIZAL FUNGI IN A DAIRY CROPPING SYSTEM.....	37
Introduction.....	37
Materials and Methods.....	40
Results.....	52
Discussion.....	59
Conclusion.....	63
Appendix: Sustainable Dairy Cropping System Description.....	64
Bibliography.....	70

## LIST OF FIGURES

2.1 Soil map units for the cropping systems trial.....	21
2.2 Lay out of one of four replicate split-split plots.....	22
2.3 Six-year crop rotation within a sustainable dairy cropping systems trial.....	23
2.4 3.1 Soil map units for the cropping systems trial.....	21
A.1 Lay out of one of four replicate split-split plots.....	58
A.2 Six-year grain rotation.....	59
A.3 Six-year forage rotation.....	60
A.4 Two-year conventional corn and soybean rotation.....	61



## LIST OF TABLES

2.1 Mean (standard error) colonization of corn in canola vs. canola-oats and standard vs. reduced herbicide split plots.....	28
2.2 Mean (standard error) colonization of rye planted subsequent to canola and canola-oats in the standard and reduced herbicide split-split plots.....	29
2.3 Mean (standard error) colonization of soy planted in the growing season following canola and canola-oats in the standard and reduced herbicide split-split plots. n = 4.....	30
3.1 Mean (standard error) colonization of corn varieties grown in greenhouse pot cultures inoculated with arbuscular mycorrhizal fungi.....	49
3.2 Mean (standard error) colonization of corn planted in standard and reduced herbicide split plots within the grain rotation.....	50
3.3 Mean (standard error) colonization of corn planted in broadcast and injected manure split plots within the corn-soy rotation.....	51
3.4 Mean (standard error) colonization of corn planted in broadcast and injected manure split plots within the forage rotation at the first entry point.....	52
3.5 Mean (standard error) colonization of corn manure management and cover crop treatment split-split plots within the forage rotation at the second entry point.....	53
3.6 Mean (standard error) colonization of corn grown in the grain, corn-soy and forage rotations.....	54

## ACKNOWLEDGEMENTS

First, I would like to thank graduate advisor, Dr. Roger Koide. Your guidance, patience, humor, and support through this process has been tremendous. I have gained a lot and grown a lot from working with you.

I would also like to thank the other members of the Koide lab, Christopher Fernandez, Krittika Petprakob, and Matthew Peoples, and my committee members, Dr. David Mortensen and Dr. Douglas Beegle, for your advice and assistance. It was a pleasure learning from and working with all of you.

Finally, I would like to thank my friends and family for your love and support. I know you will be as happy with the culmination of this work as I am.

## **Chapter 1**

### Sustainable dairy cropping systems summary

#### **Introduction**

Pennsylvania is one of the largest dairy producing states in the U.S. with approximately 536 thousand cows that produce 10.5 billion pounds of milk annually. In 2012, there were only four other states in the nation that ranked higher in milk production (USDA NASS 2013). Thus, dairy is an important industry for Pennsylvania's economy, generating about \$2.3 billion in cash receipts annually (USDA ERS 2012). However, despite its role in the economy, there are a number of external environmental costs associated with the industry, and as concerns about biodiversity loss, soil loss, nutrient imbalance, energy use, and climate change grow, there is an increasing interest in minimizing its environmental costs while maintaining its economic viability (Millennium Ecosystem Assessment 2005, Tilman et al. 2001). The Sustainable Dairy Cropping Systems Project (Northeast Sustainable Agriculture Research and Education Grant #: NLNE90-291, Kobell 2010) at the Pennsylvania State University is aimed at identifying sustainable food and bioenergy crop production strategies for dairy farms in the Northeast United States.

The overarching goal of the Sustainable Dairy Cropping Systems Project is to assess cropping systems capable of producing the feed, forage, and fuel needed on a typical Pennsylvania dairy farm while minimizing environmental impacts, reducing

off farm inputs, and remaining productive and profitable. To accomplish this, a sustainable dairy cropping system trial was developed based on ecological principles and processes with the intent of having a profitable dairy farm while:

1. enhancing soil quality,
2. tightening nutrient cycles,
3. reducing agrichemical inputs,
4. and reducing agricultural impacts on climate change.

### **Strategies for Enhancing Soil Quality**

Reduced soil quality, resulting from conventional agricultural practices, is a major threat to the sustainable intensification of global food systems (Millennium Ecosystem Assessment 2005). Soil organic matter (SOM) loss and soil erosion are major contributors to soil degradation in agricultural systems. This can have negative consequences for agricultural production as SOM is important for many soil processes and properties including cation exchange capacity (Schnitzer 1965), nutrient retention and release (Tiessen et al. 1994, Tiessen et al. 1992), soil structure and bulk density (Shepherd et al. 2002), water-holding capacity (Olness and Archer 2005), and microbial activity (Schnürer et al. 1985). Moreover, SOM loss results in the loss of carbon from the soil, which can result in increased atmospheric CO<sub>2</sub> concentrations (Sauerbeck 2001, Halvorson et al. 2002).

The conversion of land for agricultural production results in increased rates of soil erosion. The rate of soil loss in conventionally plowed agricultural fields is approximately 1-2 orders of magnitude greater than that in natural systems and significantly outpaces soil production (Montgomery 2007). Soil loss degrades agricultural lands, sometimes to the point where the land is no longer productive due to reductions in soil fertility, water availability, soil depth, and soil biota. Soil loss can also result in soil salinization and acidification (Lal et al. 2007, Pimentel and Kounang 1998, Quinton et al. 2010). Soil erosion causes numerous off-farm environmental problems such as sedimentation and siltation of lakes and rivers and pollution of waterways by fertilizers and pesticides carried in the soil (Pimentel and Kounang 1998, Uri 1999). All told, the financial cost of soil erosion is significant, an estimated \$44 billion annually in the United State alone (Pimentel et al. 1995).

To enhance soil quality in the trial and to reduce the negative off-farm impacts of soil erosion, the Sustainable Dairy Cropping Systems Project looks at reduced tillage, the incorporation of cover crops into rotations, and the use of a roller crimper to help manage crop residues. Because tillage loosens the soil and exposes it to rain and wind, reducing the amount of tillage in agricultural systems can help prevent soil erosion, which also helps maintain SOM and soil nutrient pools (Holland 2004; Lal 2006). Reducing tillage also helps maintain crop residues on the soil surface, reducing erosion and helping to build SOM (Locke and Bryson 1997). Incorporating cover crops into an agricultural system can provide numerous ecosystem benefits including improved soil quality (Schipanski et al. 2013). Cover crops provide a vegetative cover for the soil when economic crops are not being

grown. This reduces the erosive effects of rain on the soil surface, which can dislodge soil particles, and increases water infiltration, which reduces runoff (Hartwig and Ammon 2002). Cover crops and their residues also help increase soil organic matter and soil nutrient pools (Dabney et al. 2001, Kuo et al. 1997, Scholberg et al. 2010). The Sustainable Dairy Cropping Systems Project manages some of the cover crop residue using a roller crimper. The roller crimper lays down, then crimps the stems of the cover crop (Mirsky et al. 2009). The cover crop forms a protective layer on the soil surface and the rolling and crimping process leaves the crop relatively intact, which increases the amount of time the crop residue remains on the soil surface (Mirsky et al. 2011).

### **Strategies for Tightening Nutrient Cycles**

Today, most conventional agricultural systems rely on synthetic fertilizers to replace nutrients that are removed from the system when crops are harvested or when soil is eroded, rather than relying on organic amendments, crop rotation, cover crops, and biologic activity to maintain soil nutrients (Drinkwater and Snapp 2007). The shift to synthetic fertilizers typically reduces the amount of time living plants are present in a system, resulting in reductions in soil organic matter and the nutrient holding capacity of the system (Matson et al. 1997). This may increase the reliance on external sources of phosphorus and nitrogen fertilizers to maintain crop

yields and the subsequent higher propensity for loss of nutrients to the environment by leaching and denitrification (Drinkwater and Snapp 2007).

The increased specialization of many farms, including the separation of crop and livestock production, has led to the large-scale disruption of nutrient cycles. The decoupling of crop and animal agriculture occurred in the United States over the last century due to changes in agricultural policies, advances in farming technology, and the availability of cheap fossil fuels and fertilizers (Hilimire 2011). In many cases, this has resulted in an excess of nutrients near livestock production facilities, which causes an increase in air and water pollution in those areas.

Excess nitrogen lost to the environment, from the over application of fertilizers or the buildup of nutrients near livestock production, can enhance denitrification resulting in increased production of nitrous oxide, a greenhouse gas (Vitousek et al. 1997). Excess nitrogen can also increase the productivity of nitrogen-limited systems where it is deposited, which generally results in decreased biodiversity (Tilman 1987). Finally, both phosphorus and nitrogen pollution from agricultural systems can damage both marine and freshwater ecosystems by causing eutrophication (Carpenter et al. 1998, Tilman et al. 2001).

To tighten nutrient cycles and reduce the associated environmental damage, the Sustainable Dairy Cropping Systems Project integrates the crop and livestock aspects of the project and attempts to minimize off farm feed and nutrient inputs. Furthermore, the Sustainable Dairy Cropping Systems Project uses management practices intended to maximize nutrient use efficiency and minimize nutrient loss in the system. To avoid the over application nutrients, the Sustainable Dairy Cropping

Systems Project uses soil nutrient testing to help determine the correct amount of manure and fertilizer that is needed for a given crop. The Sustainable Dairy Cropping Systems Project is also evaluating the effectiveness of subsurface manure application as a method for improving nutrient use efficiency in the system. Subsurface manure application reduces nitrogen loss by significantly reducing ammonia volatilization compared broadcast manure application (Dell et al. 2011). Moreover, this low disturbance manure application method helps retain the benefits of no-till crops.

### **Strategies for Reducing Chemical Inputs**

During the green revolution the variety and amount of synthetic fertilizers and pesticides used in agricultural systems increased rapidly (Stanley and Bunyan 1979, Horrigan et al. 2002). Synthetic fertilizers are used to maintain soil fertility in agricultural systems (as previously discussed) and pesticides, including insecticides, herbicide, and fungicides, are used to control insects, weeds, and pathogens that are harmful to crops. Unfortunately, while these materials can increase agricultural yields, at least in the short term, they may also have negative impacts on both environmental and human health.

The use of synthetic pesticides in agricultural systems has produced a number of environmental problems. Pesticides make their way into groundwater, fresh water, and soils where they can harm or kill non-target organisms including



non-target plants (Mortensen et al. 2012) fish, birds, microorganisms, livestock, and beneficial insect populations (Pimentel et al. 1992). When pesticides harm non-target organisms it can have wider impacts on ecosystems functioning (Pimentel et al. 1982). For example, if insecticides destroy beneficial insects and other natural enemies of agricultural pests, their use may lead to secondary pest problems because the natural controls on pest populations are removed. Frequently this may require the application of additional pesticides to control the new pest outbreaks (Harper 1991, Pimentel et al. 1992).

Pesticide exposure is a human health threat. The acute toxicity of most pesticides has been well studied and in the United States there are a number of occupational and other safety standards for the use of pesticides to help prevent pesticide poisoning and illness. Despite preventative regulations, in United States there are an average of 7385 emergency department visits, 1419 hospitalizations, and 23 deaths annually as a result of pesticide acute exposure (Langley and Amiss Mort 2012). There is a growing body of evidence correlating chronic long-term pesticide exposure with an increased risk of a number of cancers and neurologic diseases (Alavanja et al. 2004, Mostafalou & Abdollahi 2013, Pimentel et al. 1992). In total, the external cost of health problems and environmental damage caused by pesticide use in the United States is approximately \$12 billion (Pimentel and Paoletti 2009).

The Sustainable Dairy Cropping Systems Project employs a number of strategies to reduce the amount of synthetic fertilizers and pesticides used in the system. First, the project relies heavily on dairy manure and green manure crops

for managing soil fertility. Then the Sustainable Dairy Cropping Systems Project also uses soil testing to determine the appropriate fertilizer recommendations and attempting to synchronize nutrient availability and crop demand to increase nutrient use efficiency.

To reduce the use of synthetic pesticides, the Sustainable Dairy Cropping Systems Project uses integrated pest management (IPM) to manage agricultural pests. IPM is built on preventative measures in an attempt to keep pest populations below and economic threshold. Such measures include diversifying crop rotations to increase natural enemies (Horrigan et al. 2002) and staggering temporal periods of crop growth and disturbance to reduce weed populations. If preventative measures alone are not sufficient, then pest populations are, and control tactics, are deployed based on predetermined thresholds.

The Sustainable Dairy Cropping Systems Project is working to promote natural enemies of crop pests in order to prevent crop damage. Winter cover crops, which are used extensively in the system, provide resources for natural enemies earlier and later in the growing season than most cash crops and provide habitat where natural enemies may overwinter. Perennial crops, which are also included in the system, have reduced levels of disturbance compared to annual crops and may also provide important habitat for natural enemies. Furthermore, the systems is almost entirely no-till which is less disruptive to natural enemy populations (Landis et al. 2011).

Promoting natural enemies may also help reduce weed pressure and herbicide use as some natural enemies are weed seed predators. Cover crops can

also help lessen weed pressure by reducing the amount of light and space available for undesired plants or by producing allelopathic chemicals (Bàrberi and Mazzoncini 2001). Furthermore, the use of the roller crimper to terminate cover crops in the system may also help suppress weeds by creating high residue mulch, limiting the available light and creating a physical barrier for germinating seeds. The use of the roller crimper to terminate crops instead of relying on chemical termination will reduce herbicide use in the system. Other strategies to reduce herbicide use in the system include planting triticale (*x Triticosecale rimpaui* Wittm.) and peas (*Pisum sativum* L.) with alfalfa (*Medicago sativa* L.) and orchard grass (*Dactylis glomerata* L.) as nursery crops to suppress weeds while the stand of alfalfa and orchard grass is becoming established and using tillage instead of herbicide to terminate alfalfa, a perennial crop, in the rotation.

### **Strategies for Reducing Agricultural Impacts on Climate Change**

During the past century, average global temperatures have risen from some of the coldest to some of the warmest of the Holocene (the past 11,500 years) (Marcott et al 2013). This warming trend is largely driven by anthropogenic increases in atmospheric concentrations of four greenhouse gases including carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), and halocarbons (IPCC 2007). Agriculture accounts for 13.5 % of global anthropogenic greenhouse gas emissions and is the fourth largest source of emissions following energy supply (25.9 %),

industry (19.4 %), and forestry (17.4 %) (IPCC 2007). It is likely that the recent increase in global N<sub>2</sub>O concentrations, compared to their natural ranges for the last 650,000 years, is driven primarily by agriculture, and increases in global CH<sub>4</sub> concentrations is driven by both agriculture and fossil fuel use (IPCC 2007).

In agricultural systems, N<sub>2</sub>O emissions represent the greatest percentage of total greenhouse warming potential, followed by CH<sub>4</sub> emissions and then CO<sub>2</sub> emissions (Snyder et al. 2009). Microbial processes naturally result in soil N<sub>2</sub>O emissions, but numerous agricultural practices including tillage, the production of nitrogen fixing crops, and the application of nitrogen fertilizers and manure can increase emissions (Aneja et al. 2009). CH<sub>4</sub> emissions in agriculture are largely the result of natural processes of ruminants during digestion and from the anaerobic decomposition of organic materials, including manure (Aneja et al. 2009). Emission of CO<sub>2</sub> due to agriculture is largely the result of fossil fuel use in farm operations and the production of nitrogen fertilizer. Even at maximum efficiency, nitrogen fixation via the Haber-Bosch process produces 1.38 kg of CO<sub>2</sub> for every gram of nitrogen produced (Snyder et al. 2009).

The project is attempting to build SOM through the reduction of soil erosion in the system, inclusion of cover crops in the rotations, careful management of cover crop residue, and reduction of tillage are expected to reduce the contribution to climate change (Lal 2011). Reducing tillage in the system will also have the added benefit of reducing the fuel demands in the system. Additionally, to reduce CO<sub>2</sub> emissions from on-farm fuel use, the Sustainable Dairy Cropping Systems Project is including canola (*Brassica napus* L.), an oil seed crop, in the rotations to produce

biofuel. The seeds of the canola will be harvested, pressed and utilized directly in a vegetable oil-powered tractor for farm operations. The goal of the Sustainable Dairy Cropping Systems Project is to produce enough vegetable oil to meet the fuel demands for all farm operations.

Finally, the Sustainable Dairy Cropping Systems Project's goal to increase nutrient use efficiency in the system should reduce the systems greenhouse gas emissions. Increasing nutrient use efficiency will reduce the need for nutrient inputs and consequently the CO<sub>2</sub> emissions associated with nitrogen production. Unfortunately, subsurface manure application, one of the strategies used in the system to increase nutrient use efficiency, has the potential to increase N<sub>2</sub>O emissions. Within the bands of injected, readily-metabolized manure, there are high rates of concentrated microbial activity which can lead to the anaerobic conditions needed for the denitrification of soil and manure nitrates (Dell et al 2011). The Sustainable Dairy Cropping Systems Project is monitoring N<sub>2</sub>O emissions from plots with subsurface manure injection and plots with broadcast manure application to determine if N<sub>2</sub>O emissions become a significant source of greenhouse gas pollution in the system.

### **Sustainable Dairy Cropping System Design**

The strategies discussed above for enhancing soil quality, tightening nutrient cycles, reducing synthetic chemical inputs, and reducing agricultural impacts on

climate change were implemented in by the Sustainable Dairy Cropping Systems Project in the spring of 2010 in a long-term sustainable dairy cropping system trial (Appendix 1). The sustainable dairy cropping system trial consists of a diverse six-year grain rotation, a diverse six-year forage rotation, and a conventional two-year corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr) rotation. A comparison between two herbicide management strategies (conventional vs. reduced) was made in the six-year grain rotation and a comparison between two nutrient management strategies (injected vs. broadcast manure) were made in the six-year forage rotation. The two-year corn-soybean rotation was included to examine the effect of the diverse forage and grain rotations on insect pests and beneficial insect populations.

Each entry point of all three rotations is represented during each growing season and, within each rotation, comparisons are made between split plot and split-split plot treatments to assess the effectiveness of the different cropping and management strategies discussed above. To assess the performance of the cropping system strategies, all of the plots are monitored for soil fertility, weed populations, crop yield, and crop feed/forage quality. To assess herbicide and nutrient management strategies in the system, weed biomass is assessed in all of the plots in the grain rotation and N<sub>2</sub>O emissions are measured in the forage rotation. Additionally, as part of the integrated pest management strategy used in the system, scouting is done for a number of pests including slugs, potato leafhoppers, soybean aphids, wireworms, and European corn borer and beneficial invertebrates were monitored in select plots. Finally, farm energetics and greenhouse gas emissions

are analyzed using a farm energy analysis tool and system economics to determine the profitability of the system.

While the monitoring and analysis discussed above will help the Sustainable Dairy Cropping Systems Project determine if the goals of minimizing environmental impacts, reducing off farm inputs, and remaining productive and profitable are being met, another goal of the project is to understand how the cropping rotation and management choices impact other non-targeted aspects of the system. This includes the impacts on arbuscular mycorrhizal fungi. The remainder of this thesis will focus on the impact of select management and crop rotation strategies in the system on arbuscular mycorrhizal fungi, during the first two years of the trial.

### **Effect of Management and Crop Rotation Strategies on**

#### **Arbuscular Mycorrhizal Fungi**

Arbuscular mycorrhizal fungi (AMF) are biotrophic organisms that form a symbiotic relationship with most plants. They are abundant in both natural and agricultural ecosystems (Gianinazzi et al. 2010). The fungi occupy primarily the cortical region of the root while growing into the soil. The symbiosis is characterized by the movement of nutrients, mainly phosphorus, from the fungi to the plant and the movement of carbohydrate from the plant to the fungus. While plants vary in their response to mycorrhizal colonization (Bryla and Koide 1990, Koide 1991), AMF often play an important role in plant nutrient uptake, particularly

in the acquisition of phosphorus, and have been shown to improve plant water relations and pathogen resistance in some instances (Smith and Read 2008). The fungi, on the other hand, receive up to 20 percent of the host plant's photosynthate (Smith and Read 2008). Additionally, AMF can benefit plant growth by improving soil structure via the physical entanglement of soil particles by hyphae (Miller and Jastrow 1990) and the production of glomalin, a glycoprotein, which improves soil aggregate stability (Wright and Upadhyaya, 1998).

Although AMF associate with the majority of plants and are abundant in most soils, several agricultural practices such as fertilization, tillage, fallowing, and the inclusion of non-mycorrhizal crops in rotations can reduce the vigor of the fungi and their ability to form associations with host crops. Other practices, such as the incorporation of cover crops into a cropping rotation and help promote AMF and their associated ecosystem services within agricultural systems (Schipanski et al. 2013) To better understand how AMF respond to sustainable cropping practices, we are comparing the effects of different cropping and management strategies on AMF inoculum potential in the system.



## Chapter 2

The impact of intercropping canola (*Brassica napus* L.) with oats (*Avena sativa* L.) and the impact of tillage on arbuscular mycorrhizal fungi in a dairy cropping rotation

### Introduction

Currently, canola (*Brassica napus* L.) is the leading oilseed crop in Europe and soybean (*Glycine max* (L.) Merr.) is the leading oilseed crop in the United States (Haas 2005). Canola seeds contain approximately 40% oil (Raymer 2002). Soybean seeds, on the other hand, only contain 20-30% oil, theoretically resulting in a lower oil yield per acre than canola (Smith et al. 2007). Unfortunately, significant seed losses can occur prior to or during canola harvest, due to the tendency of the siliques to shatter when ripe and the stand to ripen unevenly. Winter canola yield losses have been reported to range from 10 to 25 percent (Price et al. 1996). Despite this problem, canola production is gaining momentum in the United States. Over the past 20 years, the number of acres in canola production in the United States has increased tenfold reaching just over 1.6 million acres in 2011 (NASS 2007). This trend is likely to continue considering the increasing interest in biofuels, the potentially high oil yield of canola, and continued efforts to improve canola cultivars to reduce shattering (Hossain et al 2012).

Canola oil can be used to produce biodiesel or it can be used as straight vegetable oil fuel in some applications. The integration of canola into cropping rotations on small dairy farms, therefore, has the potential to increase the environmental and economic sustainability of these farms by reducing their reliance on fossil fuels. Biodiesel production is an energetically efficient process with significantly more energy produced than is required for production (Hill et al. 2006). Moreover, replacing petroleum-derived diesel with biodiesel can reduce greenhouse gas emissions by up to 41% (Hill et al. 2006). In addition to these environmental benefits, on-farm fuel production can help farmers reduce external inputs and become economically more self-sufficient. This will become increasingly important as the price of petroleum-derived fuels continues to rise. Adding to the value of growing canola on dairy farms are the associated by-products of canola oil production, seed meal and straw (Smith et al. 2007), potentially used in dairy rations or as bedding, respectively.

One barrier to the adoption of canola into cropping rotations is that some crops grow poorly following canola (Koide and Peoples 2012; Gavito and Miller 1998a; Krupinsky et al. 2006). For example, Gavito and Miller (1998a) found that corn (*Zea mays* L.) planted after canola had reduced shoot phosphorus content and reduced shoot weight in the early stages of development compared to corn in plots previously planted in corn. This resulted in a reduced harvest index of the corn that followed canola. This is not surprising as phosphorus nutrition of corn seedlings has been shown to be an important factor in corn yields (Barry and Miller 1989, Kabir and Koide 2002).

One potential explanation for the negative impact of canola on subsequent crops is an allelopathic interaction (Koide and Peoples 2012). Canola, as do many members of the mustard family, produces glucosinolates (mustard oils) that may have allelopathic effects on other plants as they degrade into isothiocyanates (Bialy et al. 1990, Moyer and Huang 1997). While canola has been bred for reduced glucosinolate content, there is evidence that canola residue can still impede the growth of other plants (Moyer and Huang 1997, Yasumoto et al. 2010)

Another possible explanation for the negative impact that canola has on subsequent crops is that canola reduces the ability of arbuscular mycorrhizal fungi (AMF) to form associations with the following crops (Gavito and Miller 1998b). Arbuscular mycorrhizal fungi are biotrophic organisms that form symbiotic relationships with the majority of crop species (Gianinazzi et al. 2010). Canola, however, is a member of the mustard family (Brassicaceae) and, as with most mustards, it is incapable of forming associations with AMF (Schreiner and Koide 1993). Because AMF are dependent on their host plants for energy, long periods during which host plants are absent are detrimental to their populations (Douds et al. 2011). While plants vary in their response to mycorrhizal colonization (Bryla and Koide 1990), AMF often play an important role in plant nutrient uptake, particularly in the acquisition of phosphorus (Smith and Read, 2008). Therefore, reductions in AMF populations could have negative impacts on mycorrhizal crops planted after canola. Additionally, AMF may actually reduce the allelopathic effects of mustards on plants that form mycorrhizal associations making it even more important to maintain AMF populations (Barto et al. 2010).

In order for canola to be a viable option for dairy farmers, it is important to determine if there are ways to incorporate canola into dairy rotations without negatively impacting the yield of subsequently planted crops. If the negative impact of canola on subsequent crops is largely the result of reductions in AMF populations, one potential way of mitigating the negative effects would be to use a mycorrhizal companion crop to maintain AMF populations while canola is on the field. The primary purpose of this study, therefore, was to examine the impact of a potential mycorrhizal companion crop, oats (*Avena sativa* L.), in order to determine whether their presence in a fall planted canola crop helps maintain AMF populations in the soil.

This study was conducted as part of a larger sustainable dairy cropping systems trial, which included two weed management treatments, a standard herbicide treatment and a reduced herbicide treatment. The standard herbicide treatment plots were managed without tillage and the reduced herbicide treatment plots were tilled once during the six-year rotation with a moldboard plow for weed management. It has been well documented that tillage negatively impacts AMF colonization in agricultural systems (Castillo et al. 2006, Kabir et al. 1997, McGonigle and Miller 1996). However, there is little information on how long the impact of tillage on AMF colonization persists. Therefore, the secondary purpose of this study was to examine the effect of a single tillage event on AMF colonization in this system and to determine how long tillage effects persist.

## **Materials and Methods**

### **Experimental conditions**

I utilized treatments that were already established for the grain rotation of a large, sustainable dairy cropping systems trial at the Russell Larsen Research and Education Center of the Pennsylvania State University, located in Rock Springs, PA, USA (40°43'17.40"N, 77°55'12.19"W )(Appendix). The soil map units at the site include Buchanan channery loam, 3 to 8 percent slope, Hagerstown silty clay loam, 3 to 8 percent slope, Murril channery silt loam, 0 to 3 percent slope, and Murril channery silt loam, 3 to 8 percent slope (Figure 2.1)(USDA NRCS 2013). In May 2011, average soil phosphorus in the experimental blocks, determined by a Mehlich 3 soil test (Sims et al. 2002), was 76 parts per million (ppm) and average soil potassium was 121.5 ppm. In Pennsylvania, the optimum soil phosphorus level is 30-50 ppm for all agronomic crops and the optimum soil potassium level is 100-150 ppm for grain crops and 100-200 ppm for forage crops (Kirsten 2013).

The experiment had a split-split plot design. The main split plot treatment was a weed management treatment, which compared standard and reduced herbicide regimes. The split-split plot treatment was a mycorrhiza management treatment that compared canola to canola and oats (Figure 2.1). The treatments were replicated in four blocks.

The mycorrhiza management treatment was the main focus of this study but, because of the design of the sustainable dairy cropping systems trial, the impact of the weed management treatment on arbuscular mycorrhizal fungal inoculum

potential was also examined during this study. The treatments were assessed immediately after canola was harvested, and during the two crops that followed canola in the rotation, rye and soybeans (Figure 2.2).

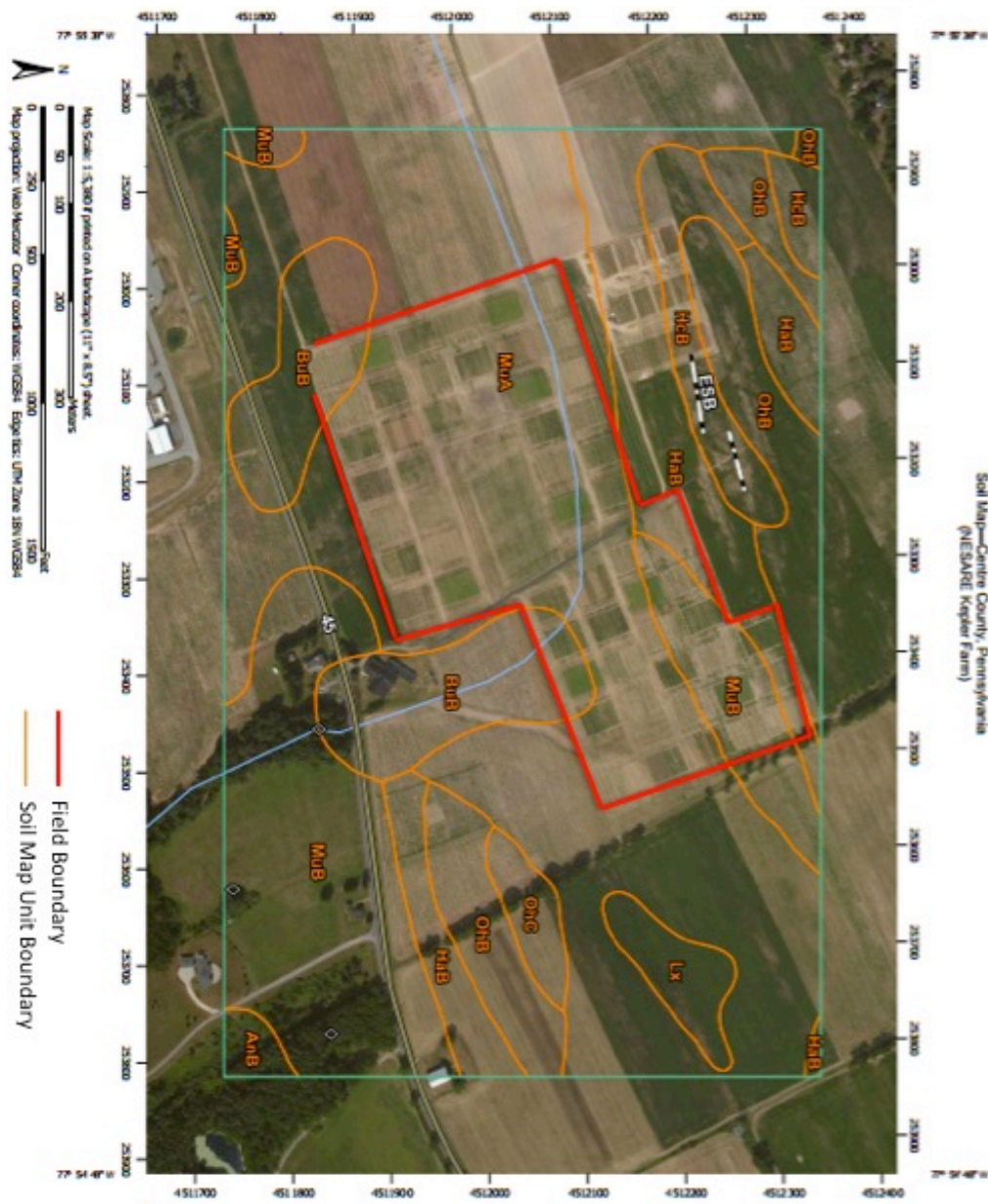


Figure 2.1 Natural Resource Conservation Service soil map units for the cropping systems trial at the Russell Larsen Research and Education Center of the Pennsylvania State University, Rock Springs, PA, USA (40°43'17.40"N, 77°55'12.19"W). Soil map units within the trial boundary include Buchanan channery loam, 3 to 8 percent slope (BuB), Hagerstown silty clay loam, 3 to 8 percent slope (HaB), Murril channery silt loam, 0 to 3 percent slope (MuA), and Murril channery silt loam, 3 to 8 percent slope (MuB).

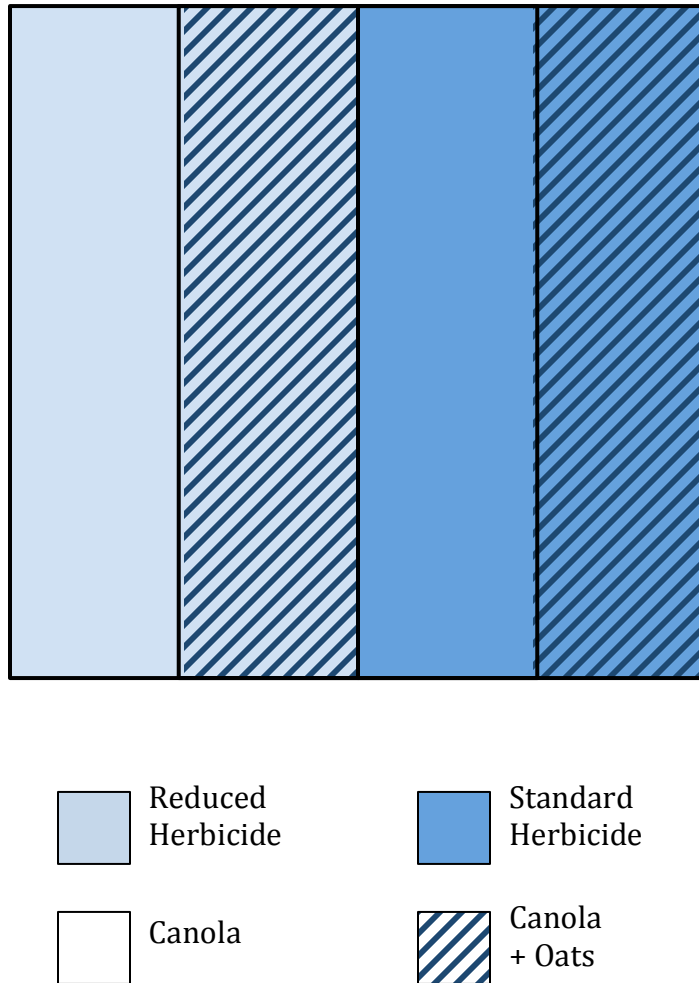


Figure 2.2 Lay out of one of four replicate split-split plots. The crop treatment (canola or canola + oats) split-split plots are nested within herbicide treatment (standard or reduced) split plots.



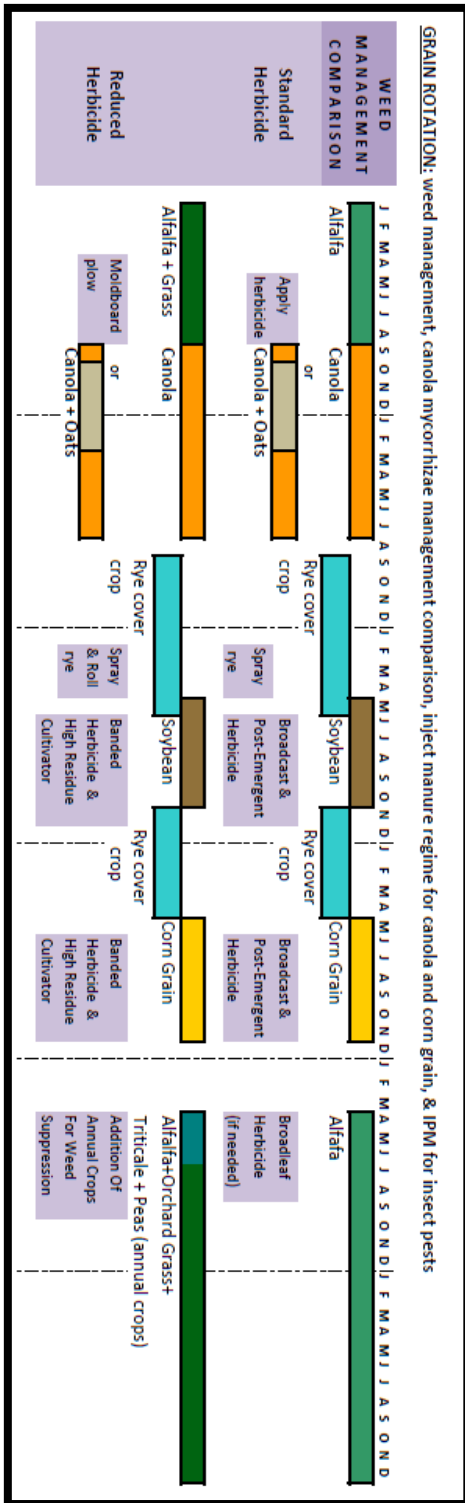


Figure 2.3 Six-year rotation with in a sustainable dairy cropping systems trail.

### **Canola vs. canola-oats**

Alfalfa (*Medicago sativa* L.) was grown in the plots prior to canola. To prepare the field to plant canola, the alfalfa plants were killed on 17 Aug 2010 using 0.6 liters ha<sup>-1</sup> 2,4-D herbicide (2,4-dichlorophenoxyacetic acid), 1.1 liters ha<sup>-1</sup> glyphosate herbicide (N-(phosphonomethyl) glycine) and 2.2 kg ha<sup>-1</sup> ammonium sulfate. The reduced herbicide split plots were then broadcast with approximately 40 tonnes ha<sup>-1</sup> of manure on 30 Aug 2010. The reduced herbicide split plots were tilled on 31 Aug 2010 with a moldboard plow, disked and tined on 1 Sep 2010, and culti-mulched on 11 Sep 2010. The standard herbicide split plots were injected with approximately 40 tonnes ha<sup>-1</sup> of manure using a shallow disk injector on 30 Aug 2010. These plots were not tilled.

'Wichita' canola was planted in all split-split plots on 11 Sep 2010 at a rate of 7.3 kg ha<sup>-1</sup>. The seeds were sown 1.9 cm deep in 19.3 cm rows. 'Blaze' oats were planted in the canola – oat split-split plots on 11 Sep 2010 at a rate of 22.4 kg ha<sup>-1</sup>. The oats were planted 1.9 cm deep in 19.3 cm rows that were centered between the rows of canola. The oats were killed by frost in Jan 2011 and the canola was harvested on 5 Jul 2011.

To assess AMF inoculum potential, 'Pioneer 35F48AM' corn seed was planted in all treatment split-split plots on 17 July 2011. This corn variety possessed three recombinant traits including Herculex I, LibertyLink, and Roundup Ready. Herculex I is a trait that produces the Bt delta-endotoxin Cry1F for lepidopteran control (Glaser and Matten 2003), the LibertyLink trait provides resistance to glufosinate herbicides, and the Roundup Ready trait provides resistance to glyphosate

herbicides. The seeds were planted approximately 5 cm deep in eight randomly placed locations in each split-split plot. When planting the corn seeds, a 1.5 meter buffer around the edge of the plot was avoided as well as a 4.6 meter yield strip at the center of the plot that was needed for yield measurements of the sustainable dairy cropping systems trial.

Average rainfall for the region for the month of July is 8.9 cm but in 2011 the area only received 2.4 cm of rain. To make up for the droughty conditions, each bioassay plant seed was watered by hand using a watering can four times after planting approximately with 3 liters of water each time. The corn bioassay plants emerged on 21 Jul 2011 and the seedlings were carefully harvested eight days after emergence with an effort to harvest as much of the root system as possible.

Once the corn seedlings were harvested, the roots and shoots were rinsed in distilled water and separated. Five seedlings were selected randomly from each split-split plot and pooled for analysis. The roots were preserved in 50% ethanol until they could be processed. The roots were cleared with 10% KOH and stained with Trypan Blue in order to assess root colonization by arbuscular mycorrhizal fungi using the line intersect method (Koide and Mooney 1987). Early colonization of the roots was used as a proxy for AMF inoculum potential (Boswell et al. 1998).

### **Subsequent rye winter cover crop**

In order to prepare for planting rye (*Secale cereal* L.), all of the split-split plots were rotary harrowed on 30 July 2011 and sprayed on 27 Aug 2011 with 0.6 liters ha<sup>-1</sup> 2,4-D, 1.1 liters ha<sup>-1</sup> glyphosate herbicide, and 2.3 kg ha<sup>-1</sup> ammonium

sulfate. 'Aroostock' rye was planted on 2 Sep 2011 at a rate of 134.5 kg ha<sup>-1</sup>. The seeds were planted 2.5 to 3.2 cm deep in 19.1 cm rows. Six seedlings were harvested from each split-split plot on 3 Nov 2011. The seedlings were handled exactly as for corn, above, and the same methods were used for assessing percent colonization.

### **Soy following rye**

The rye in the standard herbicide split plots was killed with 0.8 liters ha<sup>-1</sup> glyphosate herbicide and 0.6 liters ha<sup>-1</sup> 2,4-D herbicide on 21 Apr 2012. The rye in the reduced herbicide split plots was sprayed with the same herbicide mix on 12 May 2012 after the rye reached the heading out stage. The rye in the reduced herbicide split plots was then turned into dead mulch on the top of the field using a roller crimper on 17 May 2012. The roller crimper rolled down the rye and crimped the stem, killing the plants (Mirsky et al. 2009). To help control weeds, 0.02 liter ha<sup>-1</sup> flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione), 0.01 liter ha<sup>-1</sup> chlorimuron ethyl (ethyl 2-[(4-chloro-6-methoxypyrimidin-2-yl)carbamoylsulfamoyl]benzoate), and 1.6 liter ha<sup>-1</sup> S-metolachlor (2-Chloro-N-(2-ethyl-6-methylphenyl)-N-[(2*S*)-1-methoxy-2-propanyl]acetamide) herbicide were applied in 25 cm bands on 76 cm rows in the reduced herbicide split plots on 31 May 2012. The standard herbicide plots also received an additional herbicide treatment of 0.06 liter ha<sup>-1</sup> flumioxazin and 0.02 liter ha<sup>-1</sup> chlorimuron ethyl on 31 May 2012.

'Growmark HS28A12' soybeans were planted in all of the split-split plots on 31 May 2012 at a rate of 81,000 seeds ha<sup>-1</sup>. The seeds in the reduced herbicide plots were drilled 2.5 to 3.8 cm deep and the seeds in the standard herbicide plots were planted 2.5 to 3.8 cm deep using a corn planter.

Ten days after the soybean seeds germinated, six seedlings were harvested from each split-split plot. An effort was made to harvest as much of the root system as possible. After being harvested, the roots and shoots of the soybean seedlings were washed and separated. The six seedlings from each split-split plot were pooled for analysis. The roots were handled exactly as for corn, above, and the same methods were used for assessing percent colonization. All plots were harvested on 25 Oct 2012 and plot yield was measured from a 1.5 meter yield strip.

### **Statistical analysis**

Statistical analyses were performed using the R Statistical Program (R Development Core Team, 2012). The treatments were analyzed using a split plot analysis of variance using each block as a replicate. Bartlett's test for homogeneity was used to verify the assumption of equal variance across samples and the data were transformed if needed.

## **Results**

### **Canola vs. canola-oats**

Intercropping oats with canola had no significant ( $p=.07$ ) impact on the colonization of corn bioassay seedlings by arbuscular mycorrhizal fungi. However, colonization was lower in the reduced herbicide plots, which had been tilled with a moldboard plow, than in the standard herbicide plots, which had not been tilled. Additionally, there was no interaction between the crop treatment and the herbicide treatment (Table 2.1).

### **Subsequent rye winter cover crop**

Intercropping oats with canola had no significant impact on the colonization by arbuscular mycorrhizal fungi of subsequently planted rye. Additionally, herbicide treatment, standard versus reduced herbicide, had no significant impact on the colonization of subsequently planted rye by arbuscular mycorrhizal fungi and there was no interaction between the crop and herbicide treatments (Table 2.2).

### **Soybeans following rye**

Intercropping oats with canola had no impact on mycorrhizal colonization of soybeans planted the following growing season. Similarly, herbicide treatment, standard versus reduced herbicide, had no significant impact on the colonization of soybeans by arbuscular mycorrhizal fungi and there was no interaction between the crop and herbicide treatments (Table 2.3).

Table 2.1 Mean (standard error) colonization of corn bioassay plants harvested 28 Jul 2011 in canola vs. canola-oats and standard vs. reduced herbicide split plots.

n = 4

<b>Herbicide Treatment</b>	<b>Crop</b>	<b>Colonization (% of root length)</b>
Standard Herbicide	Canola	39.0 (0.04)
Standard Herbicide	Canola + Oats	27.6 (0.03)
Reduced Herbicide	Canola	20.1 (0.02)
Reduced Herbicide	Canola + Oats	17.1 (0.03)
<i>Analysis of variance</i>		
Herbicide Treatment		p = <b>0.0409</b>
Crop		p = 0.0658
Interaction		p = 0.2384

Table 2.2. Mean (standard error) colonization of rye planted subsequent to canola and canola-oats on 2 Sep 2011 in the standard and reduced herbicide split-split plots. Plants used to assess colonization were harvested on 3 Nov 2011. n = 4

<b>Herbicide Treatment</b>	<b>Crop</b>	<b>Colonization (% of root length)</b>
Standard Herbicide	Canola	5.7 (0.7)
Standard Herbicide	Canola + Oats	7.7 (1.6)
Reduced Herbicide	Canola	5.2 (0.8)
Reduced Herbicide	Canola + Oats	5.3 (1.1)
<i>Analysis of variance</i>		
Herbicide Treatment		p = 0.324
Crop		p = 0.395
Interaction		p = 0.433



Table 2.3 Mean (standard error) colonization of soy planted on 31 May 2012 following rye in the canola and canola-oats in the standard and reduced herbicide split-split plots. Plants were harvested 10 days after emergence. n = 4

<b>Herbicide Treatment</b>	<b>Crop</b>	<b>Colonization (% of root length)</b>
Standard Herbicide	Canola	50.0 (0.8)
Standard Herbicide	Canola + Oats	52.4 (3.7)
Reduced Herbicide	Canola	47.8 (2.4)
Reduced Herbicide	Canola + Oats	46.7 (3.7)
<i>Analysis of variance</i>		
Herbicide Treatment		p = 0.229
Crop		p = 0.810
Interaction		p = 0.509

## **Discussion**

### *Intercropping Canola with Oats*

Contrary to expectations, there was no significant difference in the mycorrhizal colonization of crops grown after canola intercropped with oats compared to those grown after canola monocultures. The average of colonization of the corn bioassay plants in all treatments of this study were similar to or less than the colonization of corn plants grown following canola in a study Koide and Peoples (2012) conducted at a nearby field site. In the same study Koide and Peoples observed approximately 20 percent greater colonization of corn plants grown subsequent to soy as compared to canola. This suggests that the colonization of the corn bioassay plants in all treatments potentially were negatively impacted by canola and that the oats were not effective in maintaining more robust AMF populations in this study. This is contrary to research by Kabir and Koide (2002), on the effectiveness of different winter cover crops at improving AMF colonization of subsequent crops compared with a fallow that showed that planting oats as a cover crop resulted in the increased colonization of sweet corn. While planting oats as a cover crop may be effective in increasing the colonization of subsequent crops in some instances, there are several possible explanations for the lack of treatment effects observed in this study.

First, while oats grow vigorously after they are planted in the fall, they have a low cold tolerance. In this experiment, the oats were killed by frost in January 2011 and the corn bioassay plants were not sown until July 17, 2011. As a result, while

intercropping oats with canola did extend the time a host crop was present on the field compared to plots planted only with canola there was still approximately six months where host crops were absent from the field. In the 2002 study by Kabir and Koide, the oat cover crop was killed by frost in January and corn was planted on May 26 of the same year leaving approximately four months where host crops were absent from the field. Because AMF are dependent on their host plants for energy, the longer period of time host plants were absent from the field may help account for the lack of treatment differences. Douds et al. (2011) found that there was no difference in the colonization of leek (*Allium porrum* L.) plants grown in soil from plots that had been covered and kept free of plants for 21 months and those grown in soil from adjacent, plots growing mycorrhizal crops. The lack of difference in colonization observed in this study may be a result of the timing. Douds et al. (2011) did not assess mycorrhizal colonization of the leeks until nine weeks after planting and, as a result, they may have missed differences in the earliest phase of colonization. They did find that AMF propagule density was significantly reduced in the soil from the covered plots.

The planting density of oats may also have been too low to have a significant effect on the vigor of the mycorrhizal fungi. In the study by Kabir and Koide (2002) oats were seeded at a rate of 100 kg ha<sup>-1</sup>. This was over four times the rate that oats were seeded in this study (22.4 kg ha<sup>-1</sup>). Moreover, in the current study, the growth of the oats may have been reduced due to competition with the canola. The greater density of host plants in the Kabir and Koide (2002) study, may have resulted in a

more robust AMF population which was able to more rapidly colonize the subsequently planted sweet corn.

Additionally, the lack of treatment effect observed in this study may be the result of allelochemicals produced by canola. Allelochemicals produced by canola may have negative effects on subsequent crops (Koide and Peoples 2012, Moyer and Huang 1997, Yasumoto et al. 2010) and on mycorrhizal fungi themselves (Schreiner and Koide, 1993). Thus, the production of these allelochemicals by canola may have negated any ameliorative effect of the oats on the mycorrhizal fungi.

Finally, the initial high levels of soil phosphorus may have contributed to the low levels of AMF colonization and the lack of a treatment effect. In some instances phosphorus fertilization has been shown to reduce AMF colonization in corn (Sheng et al. 2012). However, high levels of colonization have been observed even when available phosphorus was at levels well above what was required for maximum yield (Miller et al. 1995). Without a low phosphorus treatment, it is difficult to determine what, if any, impact the high background soil phosphorus levels had on this study.

### *Tillage*

Tillage can be an important tool for reducing weed pressure and herbicide use in agricultural systems (Teasdale 2007). Unfortunately, tillage generally has a negative effect on AMF populations and mycorrhizal colonization of the crop (Kabir et al. 1997, Galvez et al. 2001). In the current study, the reduced herbicide plots

were tilled using a moldboard plow, disked, tined, and culti-mulched before canola was planted. Consistent with previous studies, the corn bioassay plants from the reduced herbicide plots had significantly reduced colonization compared to those in the standard herbicide plots that were not tilled. The corn bioassay plants were planted in July, eleven months after that tillage event.

Somewhat surprisingly, there was no difference in the colonization of the subsequently planted rye cover crop in the standard (untilled) and reduced herbicide (tilled) plots. The rye was planted in September but colonization was not assessed until November. This delay in the assessment in the colonization of the rye plants may have made it impossible to detect differences in earliest phases of colonization. Additionally, the colonization of the rye was very low overall and it is possible that there would have been a significant treatment effect in a more highly colonized crop. However, when soybeans were planted, approximately 20 months after the tillage event there was no difference in the colonization of plants in standard and reduced herbicide plots.

### **Conclusion**

Intercropping canola with a low density of winter-killed oats may not be sufficient to promote mycorrhizal colonization in crops following winter canola. Crops that are highly dependent on mycorrhizal fungi for healthy growth, therefore, should probably not follow a winter canola crop if AMF inoculum potential is low.

Additionally, the rapid disappearance of the impact of tillage on AMF colonization in this cropping system suggests that sporadic tillage may have little consequence for mycorrhiza-dependent crops if they do not directly follow tillage in the rotation.

## **Chapter 3**

The impact of crop rotation and management on  
arbuscular mycorrhizal fungi in a dairy cropping system

### **Introduction**

Pennsylvania is one of the largest dairy producing states in the U.S. with approximately 536 thousand cows that produce 10.5 billion pounds of milk annually. In 2012, there were only four other states in the nation that ranked higher in milk production (USDA NASS 2013). Thus, dairy is an important industry for Pennsylvania's economy, generating about \$2.3 billion in cash receipts annually (USDA ERS 2012). However, despite its role in the economy, there are a number of external environmental costs associated with the industry, and as concerns about biodiversity loss, soil loss, nutrient imbalance, energy use, and climate change grow, there is an increasing interest in minimizing its environmental costs while maintaining its economic viability (Millennium Ecosystem Assessment 2005, Tilman et al. 2001). In response, the Sustainable Dairy Cropping Systems Project at the Pennsylvania State University designed a dairy cropping systems trial to examine sustainable food and bioenergy crop production strategies for dairy farms in the Northeast United States.

The overarching goal of the trial is to produce all of the feed, forage, and fuel needed for a typical Pennsylvania dairy farm while minimizing environmental

impacts, reducing off farm inputs, and remaining productive and profitable. To accomplish this goal, the Researched Group developed a sustainable dairy cropping system trial based on ecological principles and processes with the intent of having a profitable dairy farm while enhancing soil quality, tightening nutrient cycles, reducing chemical inputs, and reducing agricultural impacts on climate change.

The sustainable dairy cropping system trial consists of two diverse, six-year rotations and a corn-soy grain conventionally managed rotation (Appendix 1). The first six-year rotation is a grain rotation that incorporates weed management strategies designed to reduce herbicide use including occasional tillage, the use of a high residue cultivator, cover crops, the use of a roller crimper to turn cover crops into a dead mulch (Mirsky et al. 2009), and companion crops. The second six-year rotation is a forage rotation that incorporates a number of nutrient management strategies including legumes, cover crops, perennials, no-till, and manure injection with a shallow disk injector (Dell et al. 2011).

Indicators including crop yield and quality, weed populations, nutrient conservation, greenhouse gas emissions, energy use and production, and overall farm profitability are measured to assess the performance of the strategies used in the trial. While such indicators are typically the main foci of agricultural research, it can also be important to consider the impact cropping systems may have on non-target organism, especially those that play an important role in agricultural systems such as arbuscular mycorrhizal fungi (AMF).

AMF are abundant in both natural and agricultural systems, where they form symbiotic associations with the roots of most plant species (Gianinazzi et al. 2010).



While plants vary in their response to mycorrhizal colonization (Bryla and Koide 1990, Koide 1991), AMF often play an important role in plant nutrient uptake, particularly in the acquisition of phosphorus, and have been shown to improve plant water relations and pathogen resistance in some instances (Smith and Read 2008). The fungi receive up to 20 percent of the host plant's photosynthate (Smith and Read 2008). AMF can also benefit plant growth by improving soil structure via the physical entanglement of soil particles by hyphae (Miller and Jastrow 1990) and the secretion of glomalin, a glycoprotein, which improves soil aggregate stability (Wright and Upadhyaya 1998).

Despite their abundance in most soils, AMF are sensitive to several agricultural practices including phosphorus fertilizer applications (Lu et al. 1994), intensive tillage (Kabir et al. 1997, Kabir 2005), fallow periods (Harinikumar and Bagyaraj 1988, Kabir and Koide 2002) and the cultivation of non-mycorrhizal crops (Gavito and Miller 1998). To better understand how AMF respond to the cropping practices used in the sustainable dairy cropping system trial as a whole, this study concerned the effects of the three rotations on the colonization of corn (*Zea mays* L.).

## **Materials and Methods**

### **Overall experimental design**

I utilized treatments that were established for a large, sustainable dairy cropping systems trial at the Russell Larsen Research and Education Center of the Pennsylvania State University, located in Rock Springs, PA, USA (40°43'17.40"N, 77°55'12.19"W). The soil map units at the site include Buchanan channery loam, 3 to 8 percent slope, Hagerstown silty clay loam, 3 to 8 percent slope, Murril channery silt loam, 0 to 3 percent slope, and Murril channery silt loam, 3 to 8 percent slope (Figure 2.1)(USDA NRCS 2013). In May 2011, average soil phosphorus in the experimental blocks used for this study, determined by a Mehlich 3 soil test (Sims et al. 2002), was 72 parts per million (ppm) and average soil potassium was 87 ppm. In Pennsylvania, the optimum soil phosphorus level is 30-50 ppm for all agronomic crops and the optimum soil potassium level is 100-150 ppm for grain crops and 100-200 ppm for forage crops (Kirsten 2013).

The systems trial was initiated in the spring of 2010 (Appendix) and consisted of three rotations including a six-year grain rotation, a two-year corn-soy rotation, and a six-year forage rotation. Every entry point of each rotation was represented during each growing season and each was replicated in four blocks.

Contrasting management treatments were established within each of the three rotations using a split-plot experimental design. Within the six-year grain rotation, a contrast was made between a standard herbicide treatment and a reduced herbicide treatment. Within the two-year corn-soy rotation, a contrast was made

between two manure management strategies, broadcast manure and injected manure. Within the six-year forage rotation, the same manure management strategy contrast was made. Additionally, in one entry point in the six-year forage rotation, a split-split plot treatment was nested within the manure management treatment. The split-split plot treatment compared two winter cover crop treatments: red clover vs. a mixture of hairy vetch and oats.

The purpose of this study was to examine the impact of distinct crop rotations on mycorrhizal colonization of corn (*Zea mays* L.), a crop common to all three rotations. Corn occurs once in both the six-year grain rotation and the two-year corn-soy rotation, and twice during the six-year forage rotation. The rotations were established in 2010 and sampling for this study took place in the spring of 2011 so the results presented herein represent only those following a single year of each rotation. In order to minimize the effects of prior cropping history on mycorrhizal colonization, in the fall of 2009, prior to the rotations being established, all plots of all rotations were chisel plowed and planted to 'Aroostock' rye (*Secale cereale* L.).

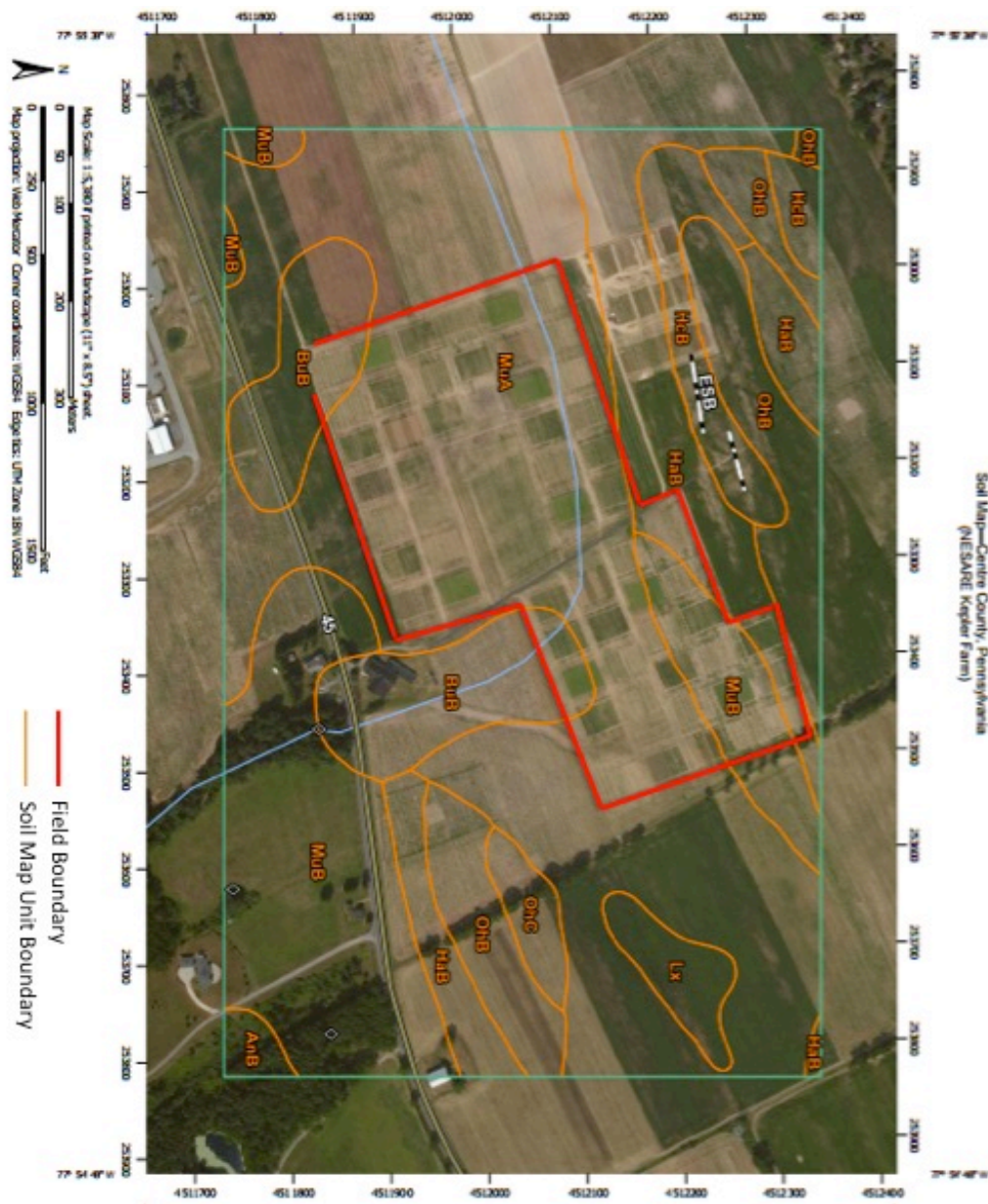


Figure 3.1 Natural Resource Conservation Service soil map units for the cropping systems trial at the Russell Larsen Research and Education Center of the Pennsylvania State University, Rock Springs, PA, USA (40°43'17.40"N, 77°55'12.19"W). Soil map units within the trial boundary include Buchanan channery loam, 3 to 8 percent slope (BuB), Hagerstown silty clay loam, 3 to 8 percent slope (HaB), Murril channery silt loam, 0 to 3 percent slope (MuA), and Murril channery silt loam, 3 to 8 percent slope (MuB).

### Six-year grain rotation

To kill the rye that was planted in 2009 the plots in the grain rotation were sprayed with 1.6 liters ha<sup>-1</sup> glyphosate herbicide (N-(phosphonomethyl) glycine) on 19 May 2010. Then, on 21 May 2010, the rye rolled down to form dead mulch using a roller crimper (Mirsky et al. 2009).

The reduced herbicide split plots were planted with soybeans on 25 May 2010. On the day of planting, the plots were treated with 0.02 liter ha<sup>-1</sup> flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione), 0.01 liter ha<sup>-1</sup> chlorimuron ethyl (ethyl 2-[(4-chloro-6-methoxypyrimidin-2-yl)carbamoylsulfamoyl]benzoate), and 0.5 liter ha<sup>-1</sup> S-metolachlor (2-Chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxy-2-propanyl]acetamide) applied in 30.5 cm bands for weed control. Then 'Growmark HiSoy 2766' soybeans were planted 2.5 to 3.8 cm deep in 75 cm rows at a rate of 81,000 seeds per hectare. 'Growmark HiSoy 2766' soybeans possess a Roundup Ready recombinant trait.

The standard herbicide split plots were planted with soybeans (*Glycine max* (L.) Merr.) on 27 May 2010. On the day of planting, 0.07 liters ha<sup>-1</sup> flumioxazin and 0.03 liters ha<sup>-1</sup> chlofimuron ethyl herbicides of broadcast in the standard herbicide split plots. Then, 'Growmark HiSoy 2766' soybeans were planted 2.5 to 3.8 cm deep in 19 cm rows at a rate of 81,000 seeds per hectare.

To control weeds during the growing season, a high residue cultivator was used in the reduced herbicide split plots on 25 Jun 2010 and 2 Jul 2010. Glyphosate herbicide was sprayed on the standard herbicide split plots at a rate of 0.8 liters ha<sup>-1</sup>

on 29 June 2010. Both the standard and reduced herbicide treatment split plots were harvested on 22 October 2010.

After the soybeans were harvested 'Aroostock' rye was planted in both the standard and reduced herbicide treatment split plots on 24 October 2010. The rye was planted 2.5 to 3.2 cm deep in 19 cm rows at a rate of 134.5 kg ha<sup>-1</sup>. The rye was killed on 6 May 2011 with 0.8 liters ha<sup>-1</sup> glyphosate herbicide and 0.6 liter ha<sup>-1</sup> 2,4-D herbicide (2,4-dichlorophenoxyacetic acid).

On 13 May 2011, approximately 40 tonnes ha<sup>-1</sup> of manure were injected in both of the standard and reduced herbicide treatment split plots using a shallow disk injector (Dell et al. 2011). On 26 May 2011, 'Pioneer 35F38' corn was planted in all split plots at a rate of 13,000 seeds ha<sup>-1</sup> in 76 cm rows, 3.8 cm deep. The seeds had been treated with the fungicides fudioxonil, mefanoxam, and azoxystrobin and the insecticide thiamethoxam. When tested under field conditions, fudioxonil and mefanoxam fungicide seed treatments did not impact AMF colonization (Murillo-Williams and Pedersen 2008). At planting, a fertilizer containing 19 kg ha<sup>-1</sup> nitrogen, 16 kg ha<sup>-1</sup> phosphorus, 9 kg ha<sup>-1</sup> potassium, and 11 kg ha<sup>-1</sup> sulfur was applied to all treatment split plots. An additional 33.6 kg ha<sup>-1</sup> nitrogen was applied to all split plots in the form of a urea ammonium nitrate solution. At the time of planting, the plots were also treated with herbicides to help control weed populations. pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine), S-metolachlor, and mesotrione (2-(4-mesy-2-nitrobenzoyl)cyclohexane-1,3-dione) herbicides were banded in the reduced herbicide split plots at the rate of 0.45 liters ha<sup>-1</sup>, 0.55 liters ha<sup>-1</sup>, and 0.03 liters ha<sup>-1</sup>

respectively. The standard herbicide split plots were sprayed with 1.4 liters ha<sup>-1</sup> pendimethalin herbicide and 1.7 liters ha<sup>-1</sup> S-metolachlor herbicide.

When the corn seedlings reached the 3 leaf stage, on 10 Jun 2011, ten seedlings were randomly sampled from each herbicide treatment (split plots). An effort was made to harvest as much of the root system as possible. The ten samples from each split plot were pooled, and the roots and shoots were rinsed in distilled water and separated. The roots were preserved in 50% ethanol until they could be processed.

### **Two-year corn-soy rotation**

To kill the rye that was planted in 2009 the plots in the grain rotation were sprayed with glyphosate herbicide at a rate of 0.8 liters ha<sup>-1</sup> on 19 May 2010. On 27 May 2010, both the injected and broadcast manure split plots were planted with 'Growmark HS32A90' soybeans. The soybeans were planted 2.5 to 3.8 cm deep in 19 cm rows at a rate of 81,000 seeds per hectare. 'Growmark HS32A90' soybeans possess a Roundup Ready recombinant trait. To control weeds during the growing season, all of the split plots were sprayed with 1.6 liters ha<sup>-1</sup> glyphosate herbicide on June 2010. They soybeans were harvested on 2 Nov 2010, and the split plots were left fallow until the spring of 2011.

On 13 May 2011, approximately 45 tonnes ha<sup>-1</sup> manure was injected in the injected manure split plots using a shallow disk injector (Dell et al. 2011) and approximately 45 tonnes ha<sup>-1</sup> manure was broadcast in the broadcast manure split plots. On 1 Jun 2011, 'Pioneer 35F48AM' corn was planted in all split plots at a rate

of 13,000 seeds ha<sup>-1</sup> in 76 cm rows, 3.8 cm deep. This corn variety possessed three recombinant traits including Herculex I, LibertyLink, and Roundup Ready. Herculex I is a trait that produces the Bt delta-endotoxin Cry1F for lepidopteran control (Glaser and Matten 2003), the LibertyLink trait provides resistance to glufosinate herbicides, and the Roundup Ready trait provides resistance to glyphosate herbicides. Additionally, the seeds were treated with the fungicides fudioxonil, mefenoxam, azoxystrobin, and tebuconazole and insecticide the insecticide thiamethoxam. At planting a fertilizer containing 19 kg ha<sup>-1</sup> nitrogen, 16 kg ha<sup>-1</sup> phosphorus, 9 kg ha<sup>-1</sup> potassium, and 11 kg ha<sup>-1</sup> sulfur was applied to all treatment split plots. An additional 33.6 kg ha<sup>-1</sup> nitrogen was applied to all split plots in the form of a urea and ammonium nitrate solution.

When the corn seedlings reached the 3 leaf stage, on 20 June 2011, ten seedlings were randomly sampled from each split plot. The harvested corn seedlings were handled as above.

### **Six-year forage rotation – first corn entry point**

On 14 April 2010, the plots in both the broadcast and injected manure treatments were sprayed with glyphosate herbicide, at a rate of 0.8 liters ha<sup>-1</sup>, to kill the rye that was planted in 2009. On April 15, a mixture of 'Genoa' alfalfa (*Medicago sativa* L.) and 'Extend' orchardgrass (*Dactylis glomerata* L.) were planted in all split plots at a rate of 10.1 kg ha<sup>-1</sup> and 3.9 kg ha<sup>-1</sup> respectively. The seed mix was planted 0.6 to 1.3 cm deep in 19 cm rows. The alfalfa seeds were treated with the fungicide Mefenoxam.



During the first week of June 2011, 4-(2,4 Dichlorophenoxy)butyric acid), an herbicide, was applied to all split plots at a rate of 1.0 liters ha<sup>-1</sup>. On 29 Jun 2011, both reduced herbicide and standard herbicide split plots were treated with 0.29 liters ha<sup>-1</sup> of Warrior, an insecticide, to control leafhopper (*Empoasca* sp.) pests. Additionally, both treatment split plots were fertilized twice during the summer: 10 kg ha<sup>-1</sup> nitrogen, 20 kg ha<sup>-1</sup> phosphorus, and 28 kg ha<sup>-1</sup> potassium was applied on 8 July 2010 and 196 kg ha<sup>-1</sup> potassium was applied on 24 Sept 2010. The alfalfa and orchardgrass were cut three times during the summer, 29 Jun 2010, 3 Aug 2010, and 14 Sep 2010 and were killed the following spring on 6 May 2011 with 1.1 liters ha<sup>-1</sup> glyphosate herbicide, 0.6 liters ha<sup>-1</sup> 2,4-D herbicide, 0.3 liters ha<sup>-1</sup> Dimethylamine salt of dicamba (3,6-dichloro-o-anisic acid) Herbicide, and 2.2 kg ha<sup>-1</sup> ammonium sulfate.

On 10 May 2011, approximately 45 tonnes ha<sup>-1</sup> manure was injected in the injected manure split plots using a shallow disk injector and approximately 45 tonnes ha<sup>-1</sup> manure was broadcast in the broadcast manure split plots. Then on 25 May 2011, 'TA290-08' corn was planted in all split plots. This corn variety possessed a recombinant LibertyLink trait that provides resistance to glufosinate herbicides. Additionally, the seeds were treated with the fungicides Captan, Allegiance, and Trilex, and the insecticide Clothianidin. At planting a fertilizer containing 19 kg ha<sup>-1</sup> nitrogen, 16 kg ha<sup>-1</sup> phosphorus, 9 kg ha<sup>-1</sup> potassium, and 11 kg ha<sup>-1</sup> sulfur was applied to all treatment split plots. An additional 33.6 kg ha<sup>-1</sup> nitrogen was applied to all split plots in the form of a urea and ammonium nitrate

solution. The corn seeds were planted at a rate of 13,000 seeds ha<sup>-1</sup> in 76 cm rows, 3.8 cm deep.

When the corn seedlings reached the 3 leaf stage, on 9 June 2011, ten seedlings were randomly sampled from each split plot. The harvested corn seedlings were handled as above.

### **Six-year forage rotation – second corn entry point**

There was a split-split plot contrast between red clover and the mixture of hairy vetch and oats cover crops prior to the second corn entry point. On 15 Apr 2010, 'Freedom' red clover (*Trifolium pretense* L.) was drill seeded into the rye that was planted the previous fall in half of the split-split plots at a depth of 0.95 cm in 19.1 cm rows at a rate of 13.5 kg ha<sup>-1</sup>. The rye in the remaining split-split plots was sprayed with glyphosate herbicide at a rate of 0.8 liters ha<sup>-1</sup>. On 1 Sept 2010, a mixture of 22.4 kg ha<sup>-1</sup> 'Auburn' hairy vetch (*Vicia villosa* Roth) and 22.4 kg ha<sup>-1</sup> 'Blaze' oats (*Avena sativa* L.) was planted in these split-split plots in 19 cm rows approximately 2.5 cm deep.

The oats in the hairy vetch – oat split-split plots died during the winter. On 12 May 2011, all of the split-split plots were sprayed with 0.8 liters ha<sup>-1</sup> glyphosate herbicide, 0.6 liters ha<sup>-1</sup> 2,4-D herbicide, 0.6 liters ha<sup>-1</sup> Dimethylamine salt of dicamba herbicide, and 2.2 kg ha<sup>-1</sup> ammonium sulfate to kill the red clover and hairy vetch that were planted in 2011. Then on 1 June 2011, approximately 50 tonnes ha<sup>-1</sup> manure was injected in the injected manure split plots using a shallow

disk injector and approximately 50 tonnes ha<sup>-1</sup> was broadcast in the broadcast manure split plots.

On 3 June 2011 'TA290-08' Corn (*Zea mays* L.) was planted in all split-split plots at a rate of 13,000 seeds ha<sup>-1</sup> in 76 cm rows, 3.8 cm deep. At planting, fertilizer containing 19 kg ha<sup>-1</sup> nitrogen, 16 kg ha<sup>-1</sup> phosphorus, 9 kg ha<sup>-1</sup> potassium, and 11 kg ha<sup>-1</sup> sulfur was applied to all treatment split plots. An additional 33.6 kg ha<sup>-1</sup> nitrogen was applied to all split-split plots in the form of a urea ammonium nitrate solution.

When the corn seedlings reached the 3 leaf stage, on 20 June 2011, ten seedlings were randomly sampled from each split plot. The harvested corn seedlings were handled as above.

### **Postharvest analyses**

The roots were cleared with 10% KOH and stained with Trypan Blue in order to assess root colonization by arbuscular mycorrhizal fungi using the line intersect method (Koide and Mooney 1987). Early colonization of the roots was used as a proxy for AMF inoculum potential (Boswell et al. 1998).

### **Greenhouse corn variety comparison**

Different corn varieties were used in each of the three rotations. Because the goal was to use the corn as a bioassay for mycorrhizal inoculum potential to compare the three rotations, it was necessary to determine whether the corn varieties differed in their ability to become colonized by mycorrhizal fungi. Soil for

this experiment was collected from alfalfa plots in the grain rotation using 1.9 cm soil cores to a depth of 10 cm in Sep 2011. The field-collected soil was then used to initiate pot cultures of arbuscular mycorrhizal fungi in Sep 2011. The field-collected soil was mixed 1:1 with autoclaved sand and potted in 12.7 cm diameter round pots. The pots were planted with several seeds of sorghum (*Sorghum bicolor* (L.) Moensch). The pot cultures were grown for 4 months in the greenhouse. During this time they were watered with a drip irrigation system. At the end of four months, water was withheld, the sorghum was allowed to die and the pots dried down naturally in the greenhouse.

The shoots of the plants were removed and the soil and the sorghum root system was collected. Spores were collected from the pot cultures soil and were used to set up 18 pots (3 corn varieties x 6 blocks). For each block the spores were extracted from 250 ml of soil, 50 ml at a time using a wet sieving and decanting technique (INVAM 2013). The top sieve used for collecting the decanted material was 500  $\mu\text{m}$  and the bottom sieve was 45  $\mu\text{m}$ . The material collected in the top sieve was moved to a large petri dish where it was viewed under a dissecting microscope. All arbuscular mycorrhizal spores present in the sample were collected and the remaining material was discarded.

The material collected in the bottom sieve was collected in a beaker and transferred to 50 ml Falcon Centrifuge Tubes containing a 20/60% sucrose gradient. The tubes were centrifuged at 960 x g for two minutes. The supernatant, containing spores and other organic material, was transferred back into the 45  $\mu\text{m}$  sieve, rinsed with distilled water, and transferred to a petri dish for viewing under a

dissecting microscope (INVAM). As many spores as possible were collected from the sample.

When spores were collected from 250 ml of pot culture soil, the spores were added to 1.5 liters of autoclaved field soil. The soil and spores were thoroughly mixed and added to 1.5 liters of autoclaved sand. The soil and sand mixture was divided evenly among three 12.7 cm round pots. Each of the three pots was planted with five seeds of one of the following corn varieties (one variety per pot): 'TA290-08' corn, Pioneer 35F48AM' corn, and 'Pioneer 35F38' corn. This process was repeated until there were six replicate blocks.

The pots were arranged by blocks in the greenhouse on 20 Jul 2012 and were watered using a drip irrigation system. One plant was harvested from each pot on 1 Aug 2012, 8 Aug 2012, and on 17 Aug 2012 to track mycorrhizal colonization through time. On 21 Aug 2012, the remaining two plants in each pot were harvested and pooled. The roots were cleared with 10% KOH and stained with Trypan Blue in order to assess root colonization by arbuscular mycorrhizal fungi using the line intersect method (Koide and Mooney 1987).

## **Statistics**

Statistical analysis was performed using the R Statistical Program (R Development Core Team, 2012). The treatments were analyzed using a split plot analysis of variance using each block as a replicate. Bartlett's test for homogeneity was used to verify the assumption of equal variance across samples and the data was transformed if needed.

## **Results**

In the greenhouse experiment, there was no significant effect of corn variety on mycorrhizal colonization (Table 3.1). Additionally, there were no significant effects of any of the split plot or split-split plot treatments within the rotations in the field on mycorrhizal colonization (Tables 3.2, 3.3, 3.4, 3.5).

Mycorrhizal colonization of the corn bioassay plants in both corn entry points in the six-year forage rotation was significantly greater than in the six-year grain rotation or the two-year corn soy rotation (Table 3.6). There was no significant difference in mycorrhizal colonization between the two corn entry points in the six-year forage rotation, and there was no significant difference between the corn-soy rotation and the grain rotation.

Table 3.1 Mean (standard error) colonization of corn varieties grown in greenhouse pot cultures inoculated with arbuscular mycorrhizal fungi. n = 6

<b>Corn Variety</b>	<b>Colonization (% of root length)</b>	<b>Shoot weight (g)</b>
Pioneer 35F48AM	28.1 (2.6)	0.9 (0.06)
Pioneer 35F38	24.2 (3.6)	0.8 (0.05)
TA290-80	28.0 (2.8)	1.0 (0.2)
<i>Analysis of variance</i>		
Rotation	p =0.623	p =0.173

Table 3.2 Mean (standard error) colonization of corn planted in standard and reduced herbicide split-split plots within the grain rotation on 26 May 2011. The plants assessed for colonization plants were harvested at the third leaf stage, on 10 June 2011. n = 4

<b>Herbicide Treatment</b>	<b>Colonization (% of root length)</b>
Reduced Herbicide	43.1 (3.0)
Standard Herbicide	47.6 (5.3)

*Analysis of variance*

Rotation	p = 0.48
----------	----------



Table 3.3 Mean (standard error) colonization of corn planted in broadcast and injected manure split plots within the corn-soy rotation. On 1 Jun 2011. The plants assessed for colonization plants were harvested at the third leaf stage, on 20 Jun 2011. n = 4

<b>Manure Management Treatment</b>	<b>Colonization (% of root length)</b>
Broadcast Manure	53.1 (5.5)
Injected Manure	46.6 (0.5)

*Analysis of variance*

Rotation	p = 0.18
----------	----------

Table 3.4 Mean (standard error) colonization of corn planted in broadcast and injected manure split plots within the forage rotation at the first entry point on 25 May 2011. The plants assessed for colonization plants were harvested at the third leaf stage, on 9 Jun 2011. n=4

<b>Manure Management Treatment</b>	<b>Colonization (% of root length)</b>
Broadcast Manure	66.2 (5.1)
Injected Manure	64.1 (0.1)

*Analysis of variance*

Rotation	p = 0.28
----------	----------

Table 3.5 Mean (standard error) colonization of corn manure management and cover crop treatment split-split plots within the forage rotation at the second entry point on 3 Jun 2011. Manure management treatments were broadcast or injected manure and cover crop treatments were hairy vetch and oats or red clover. The plants assessed for colonization plants were harvested at the third leaf stage, on 20 Jun 2011. n=4n=4

<b>Manure Management Treatment</b>	<b>Cover Crop Treatment</b>	<b>Colonization (% of root length)</b>
Broadcast Manure	Hairy Vetch + Oats	61.6 (3.7)
Broadcast Manure	Red Clover	65.0 (4.1)
Injected Manure	Hairy Vetch + Oats	68.4 (5.4)
Injected Manure	Red Clover	68.2 (5.7)

*Analysis of variance*

Manure Management Treatment	p = 0.15
Cover Crop Treatment	p = 0.56
Interaction	p = 0.52

Table 3.6 Mean (standard error) colonization of corn grown in the grain, corn-soy and forage rotations. Significantly different means are denoted with different letters ( $P < 0.05$ ), taken from a Fisher's Least Significant Difference post hoc test. The bioassay plants were harvested once they reached the 3 leaf stage on June 10, June 20, June 9, and June 20 respectively.  $n=4$

<b>Rotation</b>	<b>Colonization (% of root length)</b>
Grain	45.6 (3.3) a
Corn-Soy	49.8 (2.8) a
Forage 1	65.1 (2.6) b
Forage 2	65.8 (4.2) b

*Analysis of variance*

Rotation	$p < 0.001$
----------	-------------

## **Discussion**

Before comparing AMF inoculum potential between the three rotations in the cropping system, we first examined the within rotation split plot and split-split plot treatments to determine if these impacted AMF inoculum potential. These included a manure management treatment in the corn-soy and forage rotations, a cover crop treatment in forage rotation, and a herbicide treatment in the grain rotation.

There was no significant difference between the manure management treatments, broadcast application and shallow disk injection, in the corn-soy and forage rotation. Shallow disk injection is a low disturbance, subsurface manure application method that is thought to reduce ammonia (NH<sub>3</sub>) volatilization and nutrient runoff compared to broadcast application (Maguire et al. 2011). It is not surprising that the manure injection treatment did not significantly impact AMF colonization compared to the broadcast for two main reasons. First, shallow disk manure injection causes minimal soil disturbance (Dell et al. 2012) making the application unlikely to have any significant impact on AMF populations. Next, there does not seem to be strong evidence that organic nutrient rich zones impact plant colonization. For example, Hodge et al. (2000) found that adding a nutrient rich organic patch to the rooting zone of narrowleaf plantain (*Plantago lanceolata* L.) generally had no significant impact on colonization in a microcosm.

The cover crop treatment, a comparison between a red clover cover crop and a hairy vetch and oat cover crop mixture, in the forage rotation, also had no significant impact on the colonization of corn and there was no interaction between

the cover crop treatment and the manure management treatment. Because there appears to be specificity between some AMF species and their host plants (Eom et al. 2000, Burrows and Pflieger 2002), growing different species had the potential to impact the overall fungal community and could result in differences in inoculum potential depending on AMF community composition (Hart and Reader 2002). However, it seems unlikely that one season of a cover crop would cause a significant shift in the AMF community in this system due to its agricultural legacy, which likely spans numerous decades (Oehl et al. 2003). Furthermore, the cover crop treatment lasted less than one year and we do not know how long AMF propagules remain viable at this site.

Finally, within the grain rotation there was an herbicide treatment where split plots were either managed with standard or reduced herbicide regimes. While some herbicides including glyphosate and alachlor, have been demonstrated to negatively impact arbuscular mycorrhizal fungi (Pasaribu et al. 2013, Druille et al. 2013a, Druille et al. 2013b) there was no significant difference between the two herbicide treatments in this experiment. While herbicide use was typically less in the reduced herbicide, twenty days before corn was planted in the split plots both treatments were sprayed with glyphosate herbicide at the same rate for the purpose of killing a rye cover crop. Since we lacked a control plot where herbicide was not applied we were not able to look at the overall impact of any use herbicide on colonization. Finally, it is important to note that the this study took place in the second year of a long-term trial and it would be interesting to determine if long

term-term differences in herbicide use impact AMF colonization once the trial is well established.

Since there were no significant differences in the split plot and split-split plot treatments within the rotation we were able to next able to compare the colonization of corn by AMF between the rotations. At the three leaf stage, colonization of corn in the corn-soy and grain rotations was significantly less than corn in the two entry points in the forage rotations. There were no significant differences between the corn-soy and the grain rotation and no significant difference between the two entry points in the forage rotation.

While different varieties of corn, including genetically modified varieties, were used in each rotation, corn variety does not appear to be the main driver of the differences in corn colonization we observed between the rotations. The variety of corn planted in the corn-soy rotation had three recombinant traits including Herculex I, which includes a gene for expressing the insecticide protein *Bacillus thuringiensis* (Bt). There is some evidence that AMF colonization is reduced in plants expressing Bt genes (Cheeke et al. 2012), however the majority of research has found that there is no impact of Bt gene expression on AMF colonization (De Vaufleury et al. 2007, Knox et al. 2008, Tan et al. 2011). The results of the greenhouse trial, comparing the three corn varieties used in this study, is consistent with the latter studies. As such, we do not believe corn variety accounts for the differences in colonization we observed between the rotations.

Instead, it appears the differences in the colonization of corn between the rotations may have been driven by the cover crop or lack of cover crop preceding

corn in the rotation. Because AMF are dependent on their host plants for energy, long periods during which host plants are absent or only poor hosts are present are detrimental to their populations (Douds et al. 2011). This may have been what occurred in the corn-soy and grain rotations. In the corn-soy rotation there was a fallow period preceding corn, and in the grain rotation a rye cover crop preceded corn. We did not directly examine the colonization of rye by AMF in this study so it is difficult to determine if it was a poor host. Rye colonization has been reported to be as low as 3.6 percent of total root length when measured after 14 weeks (Hetrick et al. 1992). However, some plant varieties can vary greatly in their colonization by AMF (Hetrick et al. 1992), and a wide range of colonization levels have been reported for rye (Gollner et al. 2011, Hetrick et al. 1992).

In this study we were not able to compare the yield of corn grown in the different rotations because the corn in the grain and the corn-soy rotations was harvested for grain and the corn in the forage rotation was harvested for silage. However, AMF colonization appears to be important for corn phosphorus nutrition and reduced colonization in the seedling stage has been correlated with reduced corn yield (Kabir and Koide 2002, Karasawa et al. 2001, Miller 2000). Therefore, having a fallow period or planting a cover crop that is a poor AMF host prior to growing corn may have implications for yield and should be of concern to growers.



## **Conclusion**

Within this system, winter cover crop selection seems to be the strongest management practice impacting the colonization of corn by AMF. Corn variety was not significant with respect to colonization, as were the manure and herbicide management treatments used in this study. While corn variety, manure management, and herbicide management may have important implications for other aspects of the cropping system, well planned crop rotations that include cover crops that form strong AMF associations may be the best way to promote strong AMF inoculum potential.

## Appendix

### Sustainable Dairy Cropping System Description

The sustainable dairy cropping systems trial was established at the Russell E. Larson Agricultural Research Farm at Rock Springs, PA (40°43'17.40"N, 77°55'12.19"W ) in the spring of 2010. The trial consisted of three rotations, with each entry point of each rotation represented during each growing season. Within each rotation, comparisons were made between split plot and split-split plot treatments. Each plot (36.6 meters by 27.4 meters) was divided into four 9.1 meters by 27.4 meters split-split plots (Figure A.1). The split plot treatments were randomly assigned within each plot for each entry point of the three rotations and the split-split plot treatments were randomly assigned within the split plots. The entire experiment was replicated in four blocks with each entry point of each rotation randomly assigned within each block.

The first rotation was a six-year grain rotation (Figure A.2). The split plot contrast in this rotation was between two weed management strategies. One strategy employed a standard herbicide treatment and the other, a reduced herbicide treatment, made use of cultural practices such as tillage to reduce weed pressure (standard vs. reduced herbicide treatment). One cultural practice used to try to reduce weed pressure in the reduced herbicide treatment split-plots was increasing crop richness in these split-plots in the alfalfa entry points. Therefore, the herbicide treatment split-plot comparison is synonymous with the crop richness

treatment comparison, where the standard herbicide treatment split-plots are the same at the low crop richness treatment split-plots and the reduced herbicide treatment split-plots are the same as the high crop richness treatment split-plots. A split-split plot contrast was added during the first fall of the rotation, consisting of a mycorrhizal management treatment in order to test the impact of intercropping oats (*Avena sativa* L.), a mycorrhizal crop, with canola (*Brassica napus* L.), a non-mycorrhizal crop, on arbuscular mycorrhizal fungal populations. Thus, within each weed management treatment, the split-split plots were planted with either canola or canola and oats.

The second rotation was a six-year forage rotation (Figure A.3). The split plot contrast in this rotation was between two manure management strategies (broadcast manure vs. manure injected with a shallow disk injector (Dell et al. 2011)). The split-split plot contrast was added during the second year of the rotation and consisted of a comparison between two legume cover crops. Thus, within each manure management treatment, split-split plots were planted with either red clover (*Trifolium pretense* L.) or hairy vetch (*Vicia villosa* Roth) and oats.

The third rotation in the trial was a two-year conventional corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) rotation (Figure A.4). This rotation was included to examine the effect of the diverse forage and grain rotations on insect pests and beneficial insect populations. The split plot contrast in this rotation was a contrast between the two manure management strategies (see above). There was no split-split plot treatment in this rotation.

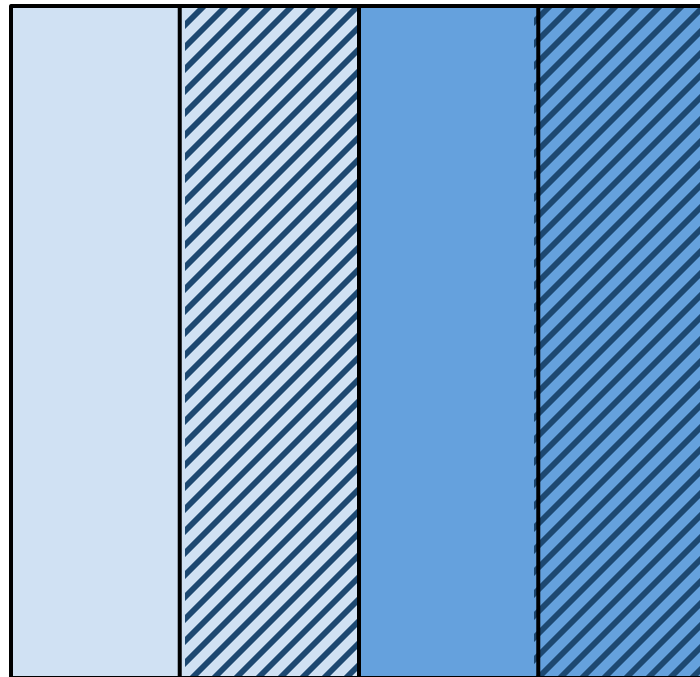


Figure A.1 Lay out of one of four replicate split-split plots.

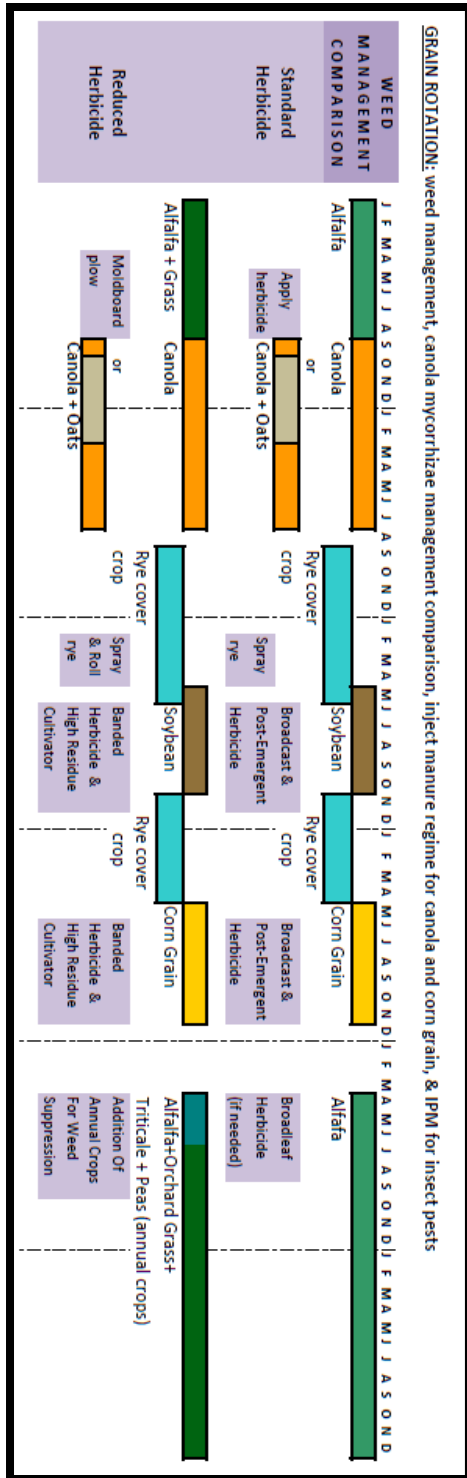


Figure A.2 Six-year grain rotation.

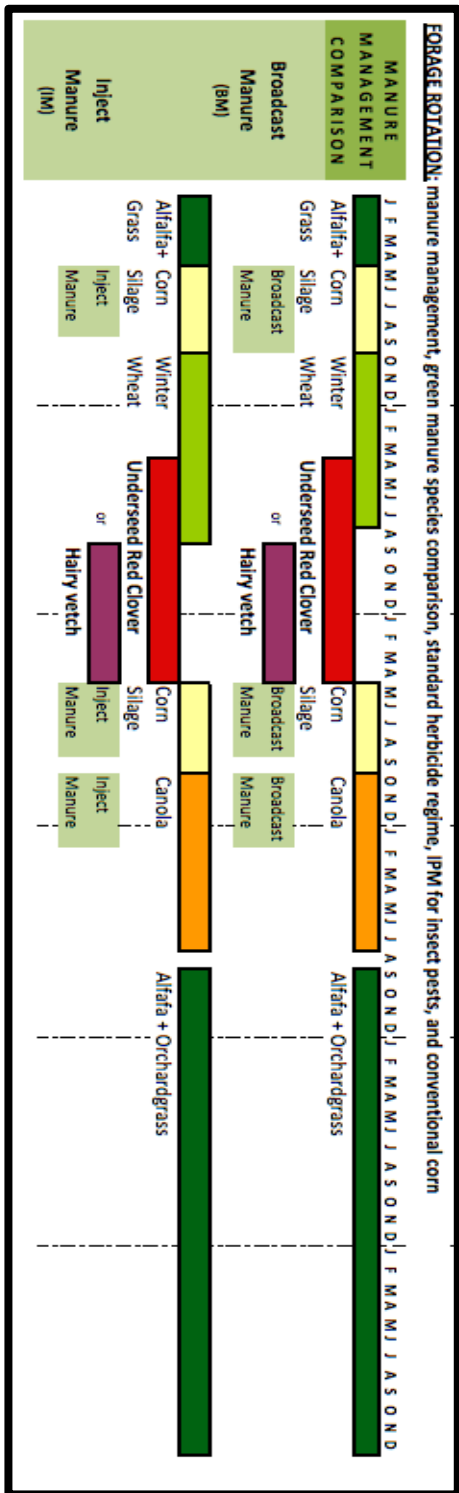


Figure A.3 Six-year forage rotation.

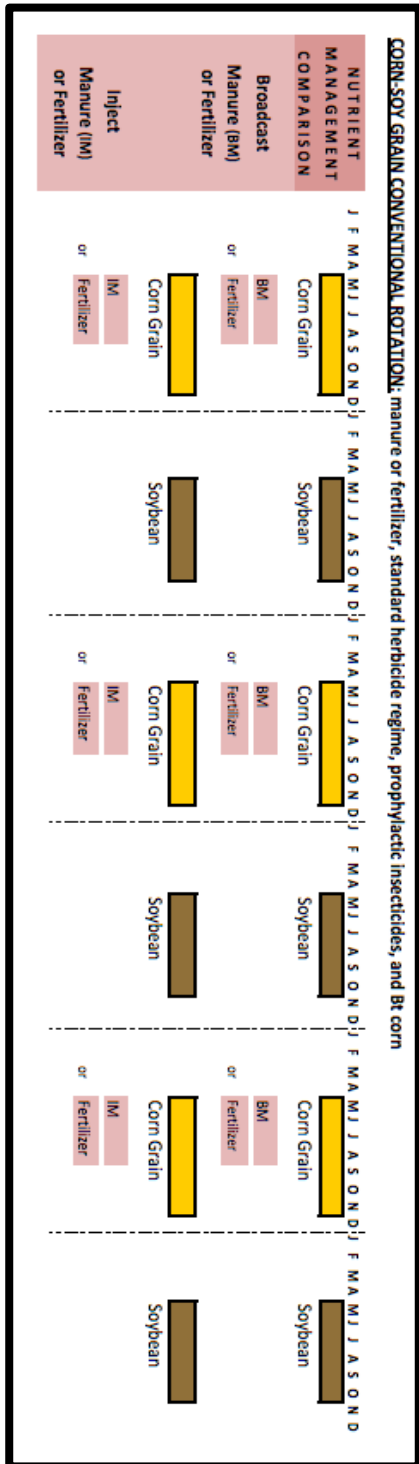


Figure A.4 Two-year conventional corn and soybean rotation.

## Bibliography

- Alavanja, M.C.R, Hoppin, J.A., & Kamel, F. (2004) Health effects of chronic pesticide exposure: cancer and neurotoxicity. *Annual Review of Public Health*, 25: 155-197.
- Aneja, V.P, Schlesinger, W.H., & Erisman, J.W. (2009). Effects of agriculture upon the air quality and climate: research, policy, and regulations. *Environmental Science and Technology*, 43: 4234-4240.
- Bialy, Z., Oleszek, W., Lewis, J., & Fenwick, G.R. (1990). Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. *Plant and Soil*, 192(2): 277-281.
- Barak, P., Jobe, B.O., Krueger, A., Peterson, L.A., and Laird, D.A. (1998). Effects of long-term soil acidification due to agricultural inputs in Wisconsin. *Plant and Soil*, 197: 61-69.
- Bàrberi, P. & Mazzoncini, M. (2001). Changes in weed community composition as influenced by cover crop and management system in continuous corn. *Weed Science*, 49: 491-499.
- Barry, D.A.J, & Miller, M.H. (1989). Phosphorus Nutritional Requirement of Maize Seedlings for Maximum Yield. *Agronomy Journal*, 81: 95-99
- Barto, K., Friese, C., & Cipollini, D. (2010). Arbuscular mycorrhizal fungi protect a native plant from allelopathic effects of an invader. *Journal of Chemical Ecology*, 36: 351-360.
- Boswell, E.P., Koide, R.T., Shumway, D.L., & Addy, H.D. (1998) Winter wheat cover cropping, VA mycorrhizal fungi and maize growth and yield. *Agriculture, Ecosystems, and Environment*, 67: 55-65.
- Bryla, D.R. & Koide, R.T. (1990). Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. *Oecologia*, 84: 82-92.
- Burrows, R.L. & Pflieger, F.L. (2002). Host responses to AMF from plots differing in plant diversity. *Plant and Soil*, 240: 169-179.
- Castillo, C.G., Rubio, R., Rouanet, J.L., & Borie, F. (2006). Early effects of tillage and crop rotation on arbuscular mycorrhizal fungal propagules in an Ultisol. *Biology and fertility of soils*, 43(1): 83-92.



- Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., & Smith, V.H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8(3): 559-568.
- Cheeke, T.E., Rosenstiel, T.N., & Cruzan, M.B. (2012). Evidence of reduced arbuscular mycorrhizal fungal colonization in multiple lines of Bt maize. *American Journal of Botany*, 99(4): 700-707.
- Dabney, S.M., Delgado, J.A., & Reeves, D.W. (2001). Using winter cover crops to improve soil and water quality. *Communications in Soil Science and Plant Analysis*, 32(7-8):1221-1250.
- De Vaufleury, A., Kramarz, P. E., Binet, P., Cortet, J., Caul, S., Andersen, M. N., ... & Krogh, P. H. (2007). Exposure and effects assessments of Bt maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in microcosms. *Pedobiologia*, 51(3): 185-194.
- Dell, C. J., Kleinman, P. J., Schmidt, J. P., & Beegle, D. B. (2012). Low-disturbance manure incorporation effects on ammonia and nitrate loss. *Journal of environmental quality*, 41(3): 928-937.
- Dell, C.J., Meisinger, J.J., & Beegle, D.B. (2011) Subsurface application of manure slurries for conservation tillage and pasture soils and their impact on the nitrogen balance. *Journal of Environmental Quality*, 40: 352-361
- Douds, D.D., Nagahashi, G., Wilson, D.O., & Moyer, J. (2011). Monitoring the decline in AM fungus populations and efficacy during a long term bare fallow. *Plant and Soil*, 342: 319-326.
- Drinkwater, L.E. and Snapp, S.S. (2007). Nutrients in agroecosystems: rethinking the management paradigm. In Sparks (ed.) *Advances in Agronomy* (pp. 163-186). San Diego, CA: Elsevier Academic Press Inc.
- Druille, M., Cabello, M. N., Omacini, M., & Golluscio, R. A. (2013a). Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi. *Applied Soil Ecology*, 64: 99-103.
- Druille, M., Omacini, M., Golluscio, R. A., & Cabello, M. N. (2013b). Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application. *Applied Soil Ecology*: 72, 143-149.
- Eom, A.H., Hartnett, D.C., & Wilson, G.W.T. (2000). Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia*, 122: 435-444.

- Galvez, L., Douds, D.D., Drinkwater, L.E., & Wagoner, P. (2001). Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil*, 228: 299-308.
- Gavito, M.E. & Miller, M.H. (1998a). Early phosphorus nutrition, mycorrhizae development, dry matter partitioning and yield of maize. *Plant and Soil*, 199: 177-186.
- Gavito, M.E. & Miller, M.H. (1998b). Changes in mycorrhiza development in maize induced by crop management practices. *Plant and Soil*, 198: 85-192.
- Gianinazzi, S., Gollotte, A., Binet, M.N., van Tuinen, D., Redecker, D., & Wipf, D. (2010). Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza*, 20: 519-530.
- Glaser, J.A. & Matten, S.R. (2003). Sustainability of insect resistance management strategies for transgenic Bt corn. *Biotechnology Advances*, 22: 45-69.
- Gollner, M.J., Wagentristsl, H., Liebhard, P., & Friedel, J.K. (2011). Yield and arbuscular mycorrhiza of winter rye in a 40-year fertilisation trial. *Agronomy for Sustainable Development*, 31: 373-378.
- Haas, M.J. (2005). Improving the economics of biodiesel production through the use of low value lipids as feedstocks: vegetable oil soapstock. *Biodiesel Processing and Production*, 86(10): 1087-1096.
- Halvorson, A.D., Wienhold, B.J., & Black, A.L. (2002). Tillage, nitrogen, and cropping system effects on soil carbon sequestration. *Soil Science Society of America Journal*, 66:906-912.
- Harinikumar, K.M. & Bagyaraj, D.J. (1988). Effect of crop rotation on native vesicular arbuscular mycorrhizal propagules in soil. *Plant and Soil*, 110: 77-80.
- Harper, C.R. (1991). Predator-prey systems in pest management. *Northeastern Journal of Agricultural and Resource Economics*, 20(1): 15-23.
- Hart, M. M., & Reader, R. J. (2002). Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist*, 153(2): 335-344.
- Hartwig, N.L. and Ammon, H.U. (2002). Cover crops and living mulches. *Weed Science*, 50(6): 688-699.
- Hetrick, B. A. D., Wilson, G. W. T., & Cox, T. S. (1992). Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. *Canadian Journal of Botany*, 70(10): 2032-2040.

- Hilimire, K. (2011). Integrated crop/livestock agriculture in the United States: a review. *Journal of Sustainable Agriculture*, 35(4): 376-393.
- Hill, J., Nelson, E., Tilman, D., Polasky, S., & Tiffany, D. (2006). Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proceedings of the National Academy of Sciences*, 103: 11206-11210.
- Hodge, A., Robinson, D., & Fitter, A.H. (2000). An arbuscular mycorrhizal inoculum enhances root proliferation in, but not nitrogen capture from, nutrient-rich patches in soil. *New Phytologist*, 145: 575-584.
- Holland, J.M. (2004). The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. *Agriculture, Ecosystems and Environment*, 103: 1-25
- Horrigan, L., Lawrence, R.S., & Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives*, 110(5): 145-156.
- Hossain, S., Kadkol, G.P., Raman, R., Salisbury, P.A., and Raman, H. (2012). Breeding *Brassica napus* for Shatter Resistance. In Abdurakhmonov (Ed.), *Plant Breeding* (pp. 313-332). InTech.
- IPCC. (2007). *Climate change 2007: synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change*. Geneva, Switzerland: Pachauri, R.K. & Reisinger, A. (eds.).
- International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM). (2013). Extraction of spores. Retrieved from <http://invam.wvu.edu/methods/spores/spore-extraction>.
- Isse, A.A., MacKenzie, A.F., Stewart, K., Cloutier, D.C., & Smith, D.L. (1999). Cover crops and nutrient retention for subsequent sweet corn production. *Agronomy Journal*, 91(6): 934-939.
- Kabir, Z. (2005). Tillage or no-tillage: impact on mycorrhizae. *Canadian Journal of Plant Science*, 85: 23-29.
- Kabir, Z., O'Halloran, I.P., Fyles, J.W., & Hamel, C. (1997). Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization. *Plant and Soil*, 192: 285-293.
- Kabir, Z., & Koide, R.T. (2002). Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of maize in Pennsylvania, USA. *Plant and Soil*, 238:205-215.

- Karasawa, T., Kasahara, Y., & Takebe, M. (2001). Variable response of growth and arbuscular mycorrhizal colonization of maize plants to preceding crops in various types of soils. *Biology and Fertility of Soils*, 33(4): 286-293.
- Kirsten, A. (Eds.)(2013). *The Penn State Agronomy Guide, 2013-2014*.
- Knox, O. G. G., Nehl, D. B., Mor, T., Roberts, G. N., & Gupta, V. V. S. R. (2008). Genetically modified cotton has no effect on arbuscular mycorrhizal colonisation of roots. *Field Crops Research*, 109(1): 57-60.
- Kobell, R. (2010, September 1). Penn State project's goal is for pollution-free dairy farms to crop up in landscape. *Bay Journal*.
- Koide, R.T. (1991). Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist*, 117:365-386.
- Koide, R.T. & Mooney, H.A. (1987). Spatial variation in inoculum potential of vesicular-arbuscular mycorrhizal fungi caused by formation of gopher mounds. *New Phytologist*, 107: 173-182.
- Koide, R.T. & Peoples, M.S. (2012). On the nature of temporary yield losses in maize following canola. *Plant and Soil*, 360(1-2): 259-269.
- Krupinsky, J.M., Tanaka, D.L., Merrill, S.D., Liebig, M.A., & Hanson, J.D. (2006). Crop sequence effects of 10 crops in the northern Great Plains. *Agricultural Systems*, 88: 227-254.
- Kuo, S., Sainju, U.M., & Jellum, E.J. (1997). Winter cover crops effects on soil organic carbon carbon and carbohydrate in soil. *Soil Science Society of America Journal*, 61: 145-152.
- Lal, R. (2006). Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. *Land Degradation and Development*, 17: 197-2009
- Lal, R. (2011). Sequestering carbon in soils of agro-ecosystems. *Food Policy*, 36: 533-539.
- Lal, R., Reicosky, D.C., & Hanson, J.D. (2007). Evolution of the plow over 10,000 years and the rationale for no-till farming. *Soil & Tillage Research*, 93: 1-12.
- Landis, D.A., Wratten, S.D., & Gurr, G.M. (2000). Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*, 45: 175-201.

- Langley, R.L., & Amiss Mort, S. (2012). Human exposure to pesticides in the United States. *Journal of Agromedicine*, 17: 300-315.
- Locke, M.A. & Bryson, C.T. (1997). Herbicide-soil interactions in reduced tillage and plant residue management systems. *Weed Science*, 45(2): 307-320.
- Lu, S., Braunberger, P.G., & Miller, M.H. (1994). Response of vesicular arbuscular mycorrhizas of maize to various rates of P addition to different rooting zones. *Plant and Soil*, 158:119-128.
- Maguire, R.O., Kleinman, P.J., Dell, C. J., Beegle, D.B., Brandt, R.C., McGrath, J.M., & Ketterings, Q.M. (2011). Manure application technology in reduced tillage and forage systems: A review. *Journal of Environmental Quality*, 40(2): 292-301.
- Marcott, S.A., Shakun, J.D., Clark, P.U., & Mix, A.C. (2013). A reconstruction of regional and global temperatures for the past 11,300 years. *Science*, 339: 1198-1201.
- Matson, P.A., Parton, W.J., Power, A.G., & Swift, M.J. (1997). Agricultural intensification and ecosystem properties. *Science*, 227(5325): 504-509
- McGonigle, T. P. & Miller, M. H. (1996). Mycorrhizae, phosphorus absorption, and yield of maize in response to tillage. *Soil Science Society of America Journal*, 60(6): 1856-1861.
- Millennium Ecosystem Assessment (2005). *Ecosystems and Human Well-being: Policy Responses*. World Resources Institute, Washington, DC.
- Miller, M.H. (2000). Arbuscular mycorrhizae and the phosphorus nutrition of maize: a review of Guelph studies. *Canadian Journal of Plant Science*, 80(1): 47-52.
- Miller, M.H., McGonigle, T.P., & Addy, H.D. (1995). Functional ecology of vesicular-arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem. *Critical Reviews in Biotechnology*, 15(3-4):241-255.
- Miller, R.M. & Jastrow, J.D. (1990). Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biology and Biochemistry*, 22(5):579-584.
- Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R., & Shumway, D.L. (2009). Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agronomy Journal*, 101: 1589-1596.
- Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R., & Shumway, D.L. (2011). Timing of cover-crop management effects on weed suppression in no-till planted soybean using a roller-crimper. *Weed Science*, 59(3): 380-389.

- Montgomery, D.R. (2007). Soil erosion and agricultural sustainability. *Proceedings of the National Academy of Sciences*, 104(33): 13268-13272.
- Mortensen, D.A., Egan, J.F., Maxwell, B.D., Ryan, M.R., & Smith, R.G. (2012). Navigating a critical juncture for sustainable weed management. *BioScience*, 62(1): 75-84.
- Mostafalou, S. & Abdollahi, M. (2013). Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. *Toxicology and Applied Pharmacology*, 268(2): 157-177.
- Moyer, J.R. & Huang, H.C. (1997) Effect of aqueous extracts of crop residues on germination and seedling growth of ten weed species. *Botanical Bulletin-Academia Sinica*, 38: 131-139.
- Murillo-Williams, A. & Pedersen, P. (2008). Arbuscular mycorrhizal colonization response to three seed-applied fungicides. *Agronomy Journal*, 100(3): 795-800.
- National Agricultural Statistics Service (NASS) (2007). *Canola Quick Stats*. U.S. Department of Agriculture, Washington, D.C.
- Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., & Wiemken, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology*, 69(5): 2816-2824.
- Olness, A. & Archer, D. (2005). Effect of organic carbon on available water in soil. *Soil Science*, 170(2): 90-101.
- Pasaribu, A., Mohamad, R. B., Hashim, A., Rahman, Z. A., Omar, D., & Morshed, M. M. (2013). Effect of herbicide on sporulation and infectivity of vesicular arbuscular mycorrhizal (*Glomus mosseae*) symbiosis with peanut plant. *Journal of Animal and Plant Sciences*, 23(6): 1671-1678.
- Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A., & D'Amore, M. (1992). Environmental and economic costs of pesticide use. *Bioscience*, 42(10): 750-760.
- Pimentel, D., Harvey, C., Resosudarmo, P., Sinclair, K., Kurz, D., McNair, M., Crist, S., Shpritz, L., Fitton, L., Saffouri, R., & Blair, R. (1995). Environmental and economic costs of soil erosion and conservation benefits. *Science*, 267(5201): 1117-1123.
- Pimentel, D. and Kounang, N. (1998). Ecology of soil erosion in ecosystems. *Ecosystems*, 1(5): 416-426.

- Pimentel, D. and Paoletti, M.G. (2009). Developing a 21<sup>st</sup> century view of agriculture and the environment. In Ferry, N. and Gatehouse, A.M.R. (eds.) *Environmental Impact of Genetically Modified Crops*. (pp. 42-58) CABI.
- Price, J.S., Hobson, R.N., Neale, M.A. & Bruce, D.M. (1996). Seed losses in commercial harvesting of oilseed rape. *Journal of Agricultural Engineering Research*, 65: 183-191.
- Quinton, J.N., Govers, G., Van Oost, K., & Bardgett, R.D. (2010). The impact of agricultural soil erosion on biogeochemical cycling. *Nature Geoscience*, 3: 311-314.
- R Development Core Team (2012). R: A Language and Environment for Statistical Computing. <http://www.R-project.org/>
- Raymer, P.L. (2002). Canola: an emerging oilseed crop. In Janick and Whipkey (eds.) *Trends in New Crops and New Uses* (pp. 122-126). Alexandria, VA: ASHS Press.
- Sauerbeck, D.R. (2001). CO<sub>2</sub> emissions and C sequestration by agriculture – perspectives and limitations. *Nutrient Cycling in Agroecosystems*, 60(1-3):253-266.
- Schnitzer, M. (1965). Contribution of organic matter to the cation exchange capacity of soils. *Nature*, 207: 667-668
- Scholberg, Dogliotti, Leoni, Cherr, Zotarelli, & Rossing (2010). Cover crops for sustainable agrosystems in the Americas. In Lichtfouse (ed.) *Genetic Engineering, Biofertilisation, Soil Quality, and Organic Farming* (pp. 23-58). Sustainable Agricultural Reviews 4.
- Schipanski, M.E., Barbercheck, M., Douglas, M.R., Finney, D.M., Haider, K., Kaye, J.P., Kemanian, A.R., Mortensen, D.A., Ryan, M.R., Tooker, J. & White, C. (2013). A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, 125:12-22.
- Schreiner, R.P., & Koide, R.T. (1993). Mustards, mustard oils and mycorrhizas. *New Phytologist*, 123: 107-113.
- Sheng, M., Lalande, R., Hamel, C., Ziadi, N., & Shi, Y. (2012) Growth of corn roots and associated arbuscular mycorrhizae are affected by long term tillage and phosphorus fertilization. *Agronomy Journal*, 104(6): 1672-1678.
- Shepherd, M.A., Harrison, R., & Webb, J. (2002). Managing soil organic matter – implications for soil structure on organic farms. *Soil Use and Management*, 18: 284-292.

- Smith, E.G., Janzen, H.H., & Newlands, N.K. (2007). Energy balances of biodiesel production from soybean and canola in Canada. *Canadian Journal of Plant Science*, 87: 793-801.
- Smith, S.E., & Read, D.J. (2008). *Mycorrhizal symbiosis*, 3rd edition. Academic, Amsterdam
- Snapp, S.S., Swinton, S.M., Labarta, R., Mutch, D., Black, J.R., Leep, R., Nyiraneza, J., & O'Neil, K. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy Journal*, 97(1): 322-332.
- Snyder, C.S., Bruulsema, T.W., Jensen, T.L., & Fixen, P.E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture, Ecosystems, & Environment*, 133(3-4): 247-266.
- Stanley, P.I. & Bunyan, P.J. (1979) *Agricultural chemicals and the environment – review of the impact of agricultural chemical usage on flora and fauna.* *Agricultural Development and Advisory Service Quarterly Review*, 33:115-128.
- Schnürer, J., Clarholm, M., & Rosswall, T. (1985). Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biology and Biochemistry*, 17(5): 611-618.
- Sims, J.T., Maguire, R.O., Leytem, A.B., Gartley, K.L., & Pautler, M.C. (2002). Evaluation of Mehlich 3 as an agri-environmental soil phosphorus test for the Mid-Atlantic United States of America. *Soil Science*, 66(6): 2016-2032.
- Teasdale, J.R. (2007). Strategies for soil conservation in no-tillage and organic farming systems. *Journal of Soil and Water Conservation*, 62(6): 144A-147A.
- Tiessen, H., Cuevas, E., & Chacon, P. (1994). The role of soil organic matter in sustaining soil fertility. *Nature*, 371: 783-785
- Tiessen, H., Salcedo, I.H., & Sampaio E.V.S.B. (1992). Nutrient and soil organic matter dynamics under shifting cultivation in semi-arid northeastern Brazil. *Agriculture, Ecosystems and Environment*, 38(3): 139-151
- Tilman, D. (1987). Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecological Monographs*, 57(3): 189-214
- Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., & Swackhamer, D. (2001) Forecasting agriculturally driven global environmental change. *Science*, 292: 281-284.



- United States Department of Agriculture Economic Research Service (USDA ERS) (2012). *State fact sheets: Pennsylvania*. Retrieved from [http://www.ers.usda.gov/dataproducts/state-fact-sheets/state-data.aspx?StateFIPS=42&StateName=Pennsylvania#Pcfbb8d864dd24d0e9b8cf6563675c42b\\_2\\_4`99iT18C0x0](http://www.ers.usda.gov/dataproducts/state-fact-sheets/state-data.aspx?StateFIPS=42&StateName=Pennsylvania#Pcfbb8d864dd24d0e9b8cf6563675c42b_2_4`99iT18C0x0)
- United States Department of Agriculture National Agricultural Statistics Service (USDA NASS) (2013). *Milk Production*. Retrieved from <http://usda01.library.cornell.edu/usda/current/MilkProd/MilkProd-02-20-2013.pdf>
- United States Department of Agriculture, Natural Resources Conservation Service (2013) Official Soil Series Descriptions (OSD) with series extent mapping capabilities. <http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>. Accessed 11 July 2014
- Uri, N.D. (2001). The environmental implications of soil erosion in the United States. *Environmental Monitoring and Assessment*, 66: 293-312.
- Vitousek, P.M., Mooney, H.A., Lubchenco, J., & Melillo, J.M. (1997). Human Domination of Earth's Ecosystems. *Science*, 277(5325): 494-499.
- Wright, S.F. & Upadhyaya, A. (1998). A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, 198: 97-107.
- Yasumoto, S., Matsuzaki, M., Hirokane, H., & Okada, K. (2010). Glucosinolate content in rapeseed in relation to suppression of subsequent crop. *Plant Production Science*, 13(2): 150-155.