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EFFECTS OF DIFFERENT AGRICULTURAL MANAGEMENT SYSTEMS ON
ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY, COMMUNITY
STRUCTURE, AND ECOSYSTEM SERVICES

By

Charles Bradford Gottshall

B.A. University of Louisville, 2009

A Dissertation

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April 30, 2015

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ABSTRACT

EFFECTS OF DIFFERENT AGRICULTURAL MANAGEMENT SYSTEMS ON
ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY, COMMUNITY
STRUCTURE, AND ECOSYSTEM SERVICES

Charles B. Gottshall

April 30, 2015

Disturbances associated with row-crop agricultural management systems include mechanical (tillage and cultivation) and chemical (fertilizer, pesticides, herbicides, fungicides) inputs and are often co-occurring. Many soil microbes are sensitive to these disturbances, including arbuscular mycorrhizal fungi (AMF), important plant mutualists in agricultural systems. AMF associate with many crop plants and provide direct benefits through root pathogen protection, drought resistance, nutrient acquisition and uptake, as well as contribute to ecosystem services by improving overall soil fertility. Examining how different row-crop management system disturbances affect the AMF community is important for understanding and enhancing benefits provided by these important mutualists, and key to developing sustainable agro-ecosystems.

For this work I surveyed AMF community composition, structure, and AMF related functions in no-till, biologically-based/organic, early succession, and conventional management plots at the Kellogg Biological Station-Long Term Ecological Study Main Cropping System Experiment. I examined the effects of tillage and chemical inputs on AMF through an intensive sampling from 2010 to 2012. I also examined the historical

effects of these different row crop agricultural management systems on AMF community and function by surveying archived soil samples taken annually following establishment of this site in 1989. Finally, I examined effects of the different management systems on the functioning of whole soil microbial communities in a controlled greenhouse experiment.

Overall, I found that AMF communities respond differently to tillage and chemical input disturbances associated with management. Although long term trends indicate a reduction in both AMF richness and diversity for all row crop management systems, short term richness and diversity were higher in conventional, organic, and reduced input plots, as compared to the no-till and early succession systems. I found AMF community structure to be differently affected by tillage and chemical inputs. For example, AMF community composition and structure was most similar between the conventional and no-till row crop systems, and the reduced input and organic systems, when controlling for year/crop effects, indicating an effect of chemical input on the AMF community. I found measures of AMF function, specifically plant root colonization, to be robust to management system inputs. Under row-crop management, ecosystem services linked to soil carbon sequestration and water-stable aggregate formation and provided by AMF derived soil glomalin, were lower in conventional compared to the organic systems. All active agricultural systems had lower levels of soil glomalin as compared to old fields (agricultural abandonment). My results suggest AMF community composition, structure, and function are altered by these different row crop agricultural management systems, and ecosystem services currently provided by AMF are limited by both historical (+100 years) and continued management input disturbances. Following total abandonment of

agricultural management, there is some restoration of AMF community structure and function and increased AMF contribution to ecosystem services, but these improvements in function are likely not similar to the functioning of the original soil microbial community.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGMENTS_____	iii
ABSTRACT_____	iv
LIST OF TABLES_____	viii
LIST OF FIGURES_____	ix
CHAPTER I: INTRODUCTION_____	1
ORGANIZATION OF DISSERTATION_____	3
SITE DESCRIPTION_____	4
CHAPTER II: LONG-TERM RESPONSES OF ARBUSCULAR MYCORRHIZAL COMMUNITIES FOLLOWING CONVERSION TO MORE SUSTAINABLE ROW CROP AGRICULTURAL MANAGEMENT SYSTEMS_____	6
SUMMARY_____	6
INTRODUCTION_____	7
METHODS_____	10
RESULTS_____	13
DISCUSSION_____	14
CHAPTER III: RESPONSES OF ARBUSCULAR MYCORRHIZAL FUNGI TO DIFFERENT AGRICULTURAL MANAGEMENT SYSTEMS INCLUDE ALTERED COMMUNITY COMPOSITION AND ECOSYSTEM SERVICES _____	22
SUMMARY_____	22
INTRODUCTION_____	23
METHODS_____	26
RESULTS_____	30
DISCUSSION_____	32

CHAPTER IV: SUSTAINABLE AGRICULTURAL MANAGEMENT ALTERS SOIL MICROBIAL EFFECTS ON CROP GROWTH	43
SUMMARY	43
INTRODUCTION	44
METHOD	46
RESULTS	49
DISCUSSION	50
CHAPTER V: SUMMARY AND FUTURE DIRECTIONS	57
SUMMARY	57
FUTURE DIRECTIONS	58
REFERENCES	59
CURRICULUM VITAE	71

LIST OF TABLES

TABLE	PAGE
1. Morpho-Group List and Blocked ISA Table: Results from Blocked Indicator Species Analysis_____	18
2. ANOVA Table: Results from two factor ANOVA for all response variables_____	38
3. Morpho-Group List and Blocked ISA Table: Morpho-group list of characteristics and results from Blocked Indicator Species Analysis_____	39
4. ANOVA Table: Results from a two factor ANOVA separated by crop_____	53

LIST OF FIGURES

FIGURE	PAGE
1. Trends in: a) AMF diversity (H'); b) Total soil glomalin (BRSP); and c) AMF spore abundance (spores 50 g soil), for the time series 1989 to 2008_____	19
2. CAP analysis visualization of AMF community structure associated with each agronomic treatment, controlling for effect of year from 1989-2008_____	20
3. Average between-year heterogeneity of AMF communities in the different agronomic treatments for the 1989-2008 time series_____	21
4. Results for a) AMF species richness; b) AMF diversity (H'); and c) AMF spore abundance (spores 50 g soil), for 2010-2012_____	40
5. AMF activity measured as a-b) percent root colonized by year and agricultural management, c) ERH, and d) total soil glomalin_____	41
6. CAP analysis visualization of AMF community structure associated with each agronomic treatment, controlling for differences between sampling years_____	42
7. Corn growth responses to soil inoculum source and type as determined from ANOVAs_____	55
8. Wheat growth responses to soil inoculum source and type as determined from ANOVAs_____	56

CHAPTER I

INTRODUCTION

Land use change and agricultural management of both arable and marginal lands has intensified during the past 100 years, leading to reduced biodiversity at local, regional and global scales and increased negative environmental consequences associated with conventional agricultural production systems (Robertson and Vitousek, 2009; Levine et al., 2011). Ecosystem services provided by row-crop agricultural systems, such as food and fiber, are important to human societies, but environmental trade-offs can be high. Some negative effects to ecosystem services caused by agricultural management are due to initial land use change (DuPont et al., 2010; Paula et al., 2014), but continued intensive land management furthers the loss of biodiversity, often reducing soil fertility to a point where applications of inorganic fertilizers are necessary to maintain productivity (Drinkwater and Snapp, 2007a; Power, 2010). This type of intensive agricultural management has been shown to alter soil microbial community structure and ecosystem functioning across a wide variety of systems, affecting land restoration attempts (Holtkamp et al., 2008; Kardol et al., 2008), and potentially affecting overall sustainability and productivity of the system (Culman et al., 2010; Kardol et al., 2011).

Some soil microbes are beneficial mutualists, providing plants access to nutrient pools or protection from water stress, such as arbuscular mycorrhizal fungi (AMF). AMF are a group of beneficial soil microbes present in both natural and agricultural ecosystems

and associate with many important row crops (Douds and Millner, 1999; Smith and Read, 2008). As obligate mutualist fungi, AMF associate with plant roots and form a network of extra-radicle hyphae (ERH). In this way, AMF-ERH act as an extension of the host plant root system and increases plant nutrient acquisition capabilities beyond the rhizosphere (Johnson and Gehring, 2007; Camenzind and Rillig, 2013). AMF also produce glomalin, a recalcitrant C-N rich glycoprotein. Glomalin has been shown to make up 3-8% of total organic carbon in some ecosystems, including agriculturally managed land (Treseder and Turner, 2007; Wilson et al., 2009). Together, AMF-ERH and glomalin contribute to water stable aggregate formation, and increase overall soil fertility (Rillig, 2004b; Wu et al., 2014).

AMF should be considered important for row-crop agriculture. The benefits of AMF include increased nutrient use and uptake, root pathogen protection, and stress tolerance (Clark and Zeto, 2000; Jansa et al., 2003; Lewandowski et al., 2013), but these contributions are often ignored. However, recently the potential for AMF contributions to increase ecosystem services provided by row crop agriculture, specifically C sequestration, has received increased attention. Additionally, the role of beneficial soil microbes like AMF may become even more important for row-crop agriculture in the future, as effects of climate change create insecurity for many areas used for crop production (Funk et al., 2008; Chen et al., 2011). Understanding how conventional and alternative row crop management systems affect AMF community structure and function is important considering the multiple contributions AMF make to ecosystem services, as well as their overall importance to soil fertility and system sustainability.

ORGANIZATION OF DISSERTATION

This dissertation is focused on how the AMF community responds to four different row crop agricultural management systems, as well as AMF community response to agricultural abandonment. I examine the effects of mechanical (tillage) and chemical (fertilizer, pesticide, herbicide) input disturbances associated with these different row crop systems on AMF community composition, structure, and function, and also look at ecosystem services provided by AMF.

Chapter two of this dissertation examines how conversion from conventional agricultural management to more sustainable systems affects AMF diversity, function, and ecosystem services, using archive soil samples representing 20 years of management. I evaluate long-term trends in AMF diversity and abundance, community structure, and glomalin production. I also compare between-year AMF community stability (heterogeneity) for the time series 1989 to 2008. My results indicate AMF have an overall negative response to tillage disturbance, leading to less diverse and less stable AMF communities in these types of row crop systems.

Chapter three focuses on the effects of a three year crop rotation cycle through an intensive survey of the AMF community. I examine how the different row crop management systems affect overall AMF community composition and structure, and also effects on AMF function and contribution to soil C. My results indicate AMF respond to both mechanical and chemical disturbances, but not in predictable ways, and ecosystems services provided by AMF are limited under all row crop agricultural management systems.

In the fourth chapter I examine the effects of row crop agricultural management on the functioning of whole soil microbial communities. In a complimentary greenhouse experiment, I look at how three different row crop management systems alter the functioning of soil microbes. I show how whole soil sourced from the different systems differentially affects plant growth and C partitioning, and how removal of AMF and other soil biota to create a “microbial wash” negatively affects plant growth. Overall, our results indicate how these row crop systems can shift the functioning of the soil microbial community, but organic row crop management may work to reduce negative effects of conventionally managed tilled and no-till systems.

Chapter five is a general summary and discussion of the findings of my dissertation research. Current and future research interests are presented.

SITE DESCRIPTION

The W.K. Kellogg Biological Station Long Term Ecological Research (KBS-LTER) site located in Michigan, USA (42°24'N, 85°24'W) was established in 1989 to evaluate the ecology of row-crop agricultural management typical of the north central USA grain producing region. The Main Cropping Systems Experiment (MCSE) consists of eight agronomic management systems including: conventional (tillage and chemical inputs at recommended rates; CONV), reduced input (tillage/cultivation, but 50% reduction of all chemical inputs; LOW), no-till (conventional chemical inputs, but no tillage; NOTILL or NT), biologically-based/organic (tillage/cultivation, but no organic or inorganic inputs; ORG), and fire maintained early succession (i.e. abandoned agricultural field; ES). All treatments are replicated five times as 1 ha fields. The full site description

and experimental design is detailed in Robertson (1991). Site history prior to 1989 is well documented and consisted of mixed agricultural and horticultural cropping for 100+ years, with the most recent years dominated by conventional corn and soybean production.

CHAPTER II
LONG-TERM RESPONSES OF ARBUSCULAR MYCORRHIZAL COMMUNITIES
FOLLOWING CONVERSION TO MORE SUSTAINABLE ROW CROP
AGRICULTURAL MANAGEMENT SYSTEMS
SUMMARY

Conventional agricultural management has been shown to alter soil microbial community structure and ecosystem functioning. Many microbes are sensitive to chemical and mechanical disturbances associated with agriculture, including important plant mutualists like arbuscular mycorrhizal fungi (AMF). AMF provide benefits to agricultural production through multiple mechanisms including pathogen resistance, nutrient access and acquisition, and also act to increase total soil organic carbon through production of glomalin. Alternative agronomic management systems such as biologically-based/organic agriculture and no-till may reduce the negative effects of conventional management to AMF. To address the question of how conversion to more sustainable management systems affects AMF diversity and function, we surveyed AMF spore diversity, community structure and stability, and glomalin production over 20 years in no-till, biologically-based/organic, early succession, and conventional management plots at the Kellogg Biological Station-Long Term Ecological Study Main Cropping System Experiment. Our results indicate that conversion from intensive conventional agricultural management to a no-till system creates a more biodiverse and stable

agricultural system, but only total abandonment of agricultural management results in increased AMF abundance and soil glomalin concentration.

INTRODUCTION

Decades of ecological research looking at conventional agricultural management systems have highlighted important negative environmental consequences associated with intensive agricultural production (McLaughlin and Mineau, 1995; Robertson and Vitousek, 2009). These negative consequences include direct effects on regional biodiversity and ecosystem services due to land use change, but also indirect effects which have reduced soil fertility across large areas of arable land (Grandy et al., 2006; Power, 2010). Intensive agricultural management has been shown to alter soil microbial community structure and ecosystem functioning across a wide variety of systems. For example, biofuel crop production in a tropical system reduced arbuscular mycorrhizal fungi (AMF) diversity and soil aggregate stability (Alguacil et al., 2012). Another study found conventional agricultural management reduced bacterial functional gene diversity (Xue et al., 2013). Loss of soil biodiversity has been shown to increase both greenhouse gas production and nutrient losses from leaching (de Vries et al., 2011). These types of changes may have current and future consequences for crop production, as effects of climate change create uncertainty for agricultural productivity (Funk et al., 2008; Culman et al., 2010).

Many soil microbes are sensitive to chemical and mechanical disturbances associated with agriculture, including important plant mutualists like AMF (Brito et al., 2012). AMF are present in nearly all terrestrial ecosystems and are known to associate

with many agricultural crops (Douds and Millner, 1999). AMF can provide benefits to crops through multiple mechanisms including pathogen resistance and nutrient acquisition (Wehner et al., 2010, Veresoglou and Rillig 2012). AMF create an extra-radicle hyphal (ERH) nutrient transfer network in the soil which contains a C-N rich glycoprotein known as glomalin (Wright and Upadhyaya, 1996). Glomalin is a recalcitrant soil protein produced by AMF-ERH and may represent 4-8% of soil organic carbon in natural ecosystems (Treseder and Allen, 2000) and 2-4% soil organic carbon in agricultural systems (Borie et al., 2006). Recent research in agricultural systems has shown positive correlations between glomalin and AMF diversity and abundance (Rillig, 2004; Veresoglou et al., 2012). Soil fertility and crop performance benefit from the formation of water stable soil aggregates (Piotrowski et al., 2004), which are associated with soil glomalin concentration (Rillig et al., 2010). Unfortunately, these potential benefits are often ignored as farmers rely on chemical and fertilizer inputs to maintain productivity (Drinkwater and Snapp, 2007).

Alternative agricultural management systems such as biologically-based/organic and no-till may reduce the negative effects of conventional management to AMF and other soil microbes. One study examining management effects on AMF communities associated with grain producing farmlands in England found AMF diversity was much reduced under conventional management as compared to organic management, which harbored communities more similar to natural grasslands (van der Gast et al., 2011). However, alternative agricultural systems may still affect soil microbial communities through different combinations of chemical and mechanical (tillage) disturbance. AMF community diversity and abundance have been shown to vary under organic management

employing different tillage regimes (Säle et al., 2015), and in one case, Hijri and colleagues (2006) found low AMF diversity in an organically managed system; similar to what they observed in an intensive conventional system. Management systems which increase overall soil microbial diversity and abundance may also work to increase ecosystem stability and productivity (Caruso and Rillig, 2011; de Vries and Shade, 2013), but little is understood about how the AMF community responds to implementation of these different management systems for previously cultivated land.

In this study we looked at how the AMF community has responded following conversion from long term intensive conventional agricultural management to more sustainable no-till and biologically-based/organic systems. We focused on two main questions. 1) How have AMF community diversity and function been altered by conversion from conventional to no-till and biologically-based/organic management over time, and 2) do more sustainable alternative agricultural systems increase AMF community stability over time? For our first question, we hypothesized that conversion from long-term conventional management to no-till and biologically-based/organic management would a) increase AMF diversity due to stopping tillage (no-till) and the reduction of chemical inputs (biologically-based/organic), and b) increase soil glomalin concentration as AMF diversity and abundance increase. For the second question, we hypothesized the AMF community would become more stable if tillage was stopped (no-till), if chemical inputs were stopped (biologically-based/organic), or if both were stopped (early succession). Agricultural system sustainability may be positively affected by increasing the overall stability of AMF community structure (Wu and Xia, 2006; Li et al., 2013). Results from our study provide more understanding of how conversion to

these more sustainable agricultural systems may change AMF communities and related functioning, and whether adoption of these alternative agricultural systems may work to reverse negative effects of conventional management.

METHODS

Site Description:

The W.K. Kellogg Biological Station Long Term Ecological Research (KBS-LTER) site located in Michigan, USA (42°24'N, 85°24'W) was established in 1989 to evaluate the ecology of row-crop agricultural management typical of the north central USA grain producing region. The Main Cropping Systems Experiment (MCSE) consists of eight fully replicated agronomic management systems including: conventional (CONV), no-till (NT), biologically-based/organic (ORG), and fire maintained early succession (i.e. abandoned agricultural field; ES). The full site description and experimental design is detailed in Robertson (1991). The site history prior to 1989 is well documented and consisted of mixed agricultural and horticultural cropping for 100+ years, with the most recent years dominated by conventional corn and soybean production.

Soil Samples:

In 2012, we took 50 g subsamples of eight archived soil samples from each agronomic treatment described above spanning 20 years (1989, 1990, 1991, 1992, 1994, 1998, 2003, and 2008), following field conversion and experiment initiation. The original soil sampling dates were March or April, with the exception of 1989 which was taken in November. Full details of the LTER soil sampling protocol are available at:

ter.kbs.msu.edu/protocols/112. Briefly, five samples for each treatment were taken using a standard soil probe (2 cm x 30 cm) from five distinct sampling sites within each of five replicated hectare fields. The samples were pooled at the replicate field level, sieved to 4 mm, and air-dried. Archived soil samples were dried at 60°C for 48 hours and stored in glass jars at room temperature.

Glomalin Extraction:

We extracted total soil glomalin from one gram of soil using the 0.8mM sodium citrate buffer and autoclaving method described in Janos et al., (2008), and then quantified the Bradford reactive fraction (Bio Rad, Hercules, CA, USA) using bovine serum as a standard (Koide and Peoples, 2013). Total soil glomalin has several extractible fractions (Cornejo et al., 2008), and Bradford reactive soil protein (BRSP) has been shown to consistently represent the largest fraction (approximately 95%) of total extracted soil protein. Therefore we chose to use BRSP to operationally define glomalin for this work.

AMF Spores:

Each remaining 49 g sample was wet-sieved through stacked 500 µm and 38 µm sieves, and AMF spores were extracted using the sucrose gradient-centrifugation method described in Gerdemann and Nicholson (1963). Spores were visually quantified and placed into one of nine species groups (Table 1) based on AMF morphotypes described by Schussler and Walker (2010) and others (Walker et al., 2007; Morton and Msiska, 2010b, a; Redecker et al., 2013). The morphospecies groups we used for this work are based on AMF functional differences associated with AMF at the family organizational level as described by van der Heijden and Scheublin (2007).

Analyses:

To examine differences in AMF diversity and function across 20 years of agricultural management, spore diversity (Shannon's H'), total soil glomalin (BRSP mg g^{-1}), and AMF abundance (spores g^{-1} soil) data were analyzed using general linear models with agronomic treatment and year as factors. To look at differences in AMF community structure between the different agricultural treatments over time, we used a blocked MRPP with pairwise comparisons (McCune and Grace, 2002). A blocked indicator species analysis (ISA) was performed to see if individual AMF morphospecies were associated with a particular agricultural treatment (McCune and Grace, 2002). To visualize differences in AMF community structure due to agricultural management, we performed constrained ordination analysis (CAP). This procedure allowed us to look at effects of agricultural management on AMF community structure while constraining the effects of a blocking factor (in this case, year/time) (Anderson and Willis, 2003). To test if conversion from conventional agricultural management to no-till, biologically-based/organic, or early succession alters AMF community stability, we calculated mean Euclidean distances between years for each treatment (Collins and Smith, 2006). We then used a one-way ANOVA to test for differences between the agronomic treatment groups. All GLM and ANOVA analyses were performed using Systat12 (SYSTAT v. 12 2008, SYSTAT Software Inc., Chicago, IL.). CAP analysis was performed using Primer v. 6 (Anderson et al., 2008), and the blocked MRPP and ISA were done using PC-ORD v.6.08 (McCune and Mefford, 1999).

RESULTS

AMF Diversity and Function:

There was a strong interaction between agronomic treatment and time for AMF diversity (H') (Year $F_{1,24} = 4.709$, $p = 0.0401$; MCSE management $F_{3,24} = 1.729$, $p = 0.1877$; Year x MCSE management $F_{3,24} = 3.426$, $p = 0.0332$). AMF diversity increased over time in the no-till treatment, but diversity decreased in all other treatments (Figure 1a). BRSP concentration increased in the early successional treatment (by 78%) compared to the other three treatments over time (Year $F_{1,24} = 5.123$, $p = 0.0329$; MCSE management $F_{3,24} = 1.729$, $p = 0.0925$; Year x MCSE management $F_{3,24} = 4.601$, $p = 0.0111$; Figure 1b). Similarly, AMF abundance increased in the early succession treatment compared to the other three treatments over time (Year $F_{1,24} = 9.205$, $p = 0.0057$; MCSE management $F_{3,24} = 2.519$, $p = 0.0820$; Year x MCSE management $F_{3,24} = 2.847$, $p = 0.0587$; Fig 1c).

AMF community composition:

CAP analysis indicated tillage disturbance rather than chemical inputs is driving differences in AMF community structure, with clear separation between tillage management systems: conventional and biologically-based organic clustering together, and no-till and early succession clustering together (Figure 2). This is further demonstrated in the blocked MRPP which showed significant AMF community differences between conventional and both no-till ($A = 0.2233$, $p = 0.0156$) and early succession systems ($A = 0.2407$, $p = 0.0144$), but no difference between AMF community structure under no-till and early succession ($A = 0.0376$, $p = 1.0$). There is some indication that the conventional and biologically-based/organic management plots

are separated along CAP axis 2, although the blocked MRPP indicated no significant difference in AMF community structure between these treatments ($A = 0.0925$, $p = 0.1172$).

Overlay of AMF morphospecies in the CAP analysis indicated there are different AMF associated with the different agricultural management systems, and this finding was supported by the blocked indicator species analysis. Results for the blocked ISA indicated that the *Diversispora* species group was significantly associated with the biologically-based/organic system, the *Acaulospora* species group was significantly associated with conventional management, no-till was significantly associated with the *Gigaspora* species group as compared to the other systems, and both *Rhizophagus* and *Claroideoglossum* species were indicators for early succession treatment (Table 1).

AMF community stability:

For the time series of our experiment, 1989 – 2008 ($N = 7$ for each sample), AMF communities were most stable under no-till and early succession agricultural systems and least stable under the biologically-based/organic system ($F_{3,23} = 4.559$, $p < 0.05$).; conventional management seemed to have an intermediate, stabilizing effect on the AMF community (Figure 3).

DISCUSSION

For this work we surveyed AMF community structure and function under 20 years of different agricultural management systems following conversion from conventional management previously in a soybean-corn rotation. The post-conversion management systems of no-till, biologically-based/organic, and early succession

represented in this study are alternatives currently available to farmers in mid-west and north-central grain producing regions of the USA. Overall, our results show conversion from conventional agricultural management to these alternative systems alters AMF community diversity, structure, and ecological functioning, but not in consistent ways. Research by others has shown that conventional horticultural and agricultural cropping systems generally reduce AMF diversity (Alguacil et al., 2012), but this is not always the case (e.g. Hijri et al., 2006).

For the 20 year time series of our study, AMF diversity has increased following conversion to no-till management, but has been reduced by all other agricultural management systems we examined. Our results provide strong evidence of tillage disturbance effects on the overall AMF community composition as seen in other agricultural research comparing conventional or organic to no-till (Oehl et al., 2004). No-till management is expected to facilitate the growth and function of more disturbance sensitive AMF. In our study, *Gigaspora* species, an AMF known for colonizing mainly from spores and producing large amounts of soil hyphae (Powell et al., 2009), was associated with no-till treatment. Stopping tillage also increased stability of the AMF community under no-till and early successional management; likely a result of removing the mechanical disturbance to hyphal networks (de la Providencia et al., 2007). Such stability is not always a positive thing since overall losses in AMF diversity and the selection for more resilient, less mutualistic species could also stabilize communities (Reynolds et al., 2006).

Our results also indicate that conversion from conventional to organic management may not improve AMF community structure and function, although similar

research by Verbruggen and others (2010), showed conversion to organic management significantly improved AMF richness and diversity. We found no effects of conversion to organic management on AMF diversity (H'), abundance, or total soil glomalin, and AMF communities were not different from those associated with conventional systems, even after 20 years. AMF community stability from year to year was also low, again probably due to agricultural disturbances associated with prior land use at this site causing a “bottle-neck effect” similar to that described by Verbruggen and colleagues (2012).

Stopping both tillage and chemical inputs in the early successional system resulted in increased AMF abundance and soil glomalin, shifts in AMF community composition, and a more stable AMF community from year to year. Contrary to our initial predictions, AMF diversity (H') did not increase in the early succession system. Changes in host plant presence in the early succession plots may be affecting AMF community assembly and diversity (Engelmoer and Kiers, 2015). Also, there are non-mycorrhizal plant species present in the early succession system, i.e. *Cyperus esculentus* L. and *Amaranthus retroflexus* L., but their specific influence on AMF diversity is unknown. Differences between AMF diversity in the no-till and early succession systems could also be due to selection pressure associated with chemical inputs in the no-till system as described by Vandenkoornhuyse and others (2003).

It is worth noting that differences in AMF communities between the management systems in our study may in part be due to seasonal and temporal variability in crops and climate for the sample year (Panwar et al., 2011; Jie et al., 2013; Lee et al., 2013). While we could not separate effects due to factors such as crop identity, phenology, or climate for this study, it is important to consider the role that these factors may play in AMF

community dynamics (Lutgen et al., 2003; Bohrer et al., 2004; Santos-Gonzalez et al., 2007).

In conclusion, we have found that conversion from long-term conventional agricultural management to the no-till or biologically-based/organic systems used in our study did not uniformly improve AMF diversity, community structure, or function. Conversion from conventional management does change AMF community properties and alter some related functioning, but adoption of these alternative agricultural systems does not work to reverse all the negative effects of prior conventional management. Our findings indicate that conversion to no-till management was best for improving AMF diversity and community stability, but only complete abandonment of agricultural management in the early succession system resulted in general improvements in AMF abundance, community structure, and function. Overall, our results provide evidence of negative long term effects of prior conventional agricultural management on the AMF community and demonstrate that conversion to more sustainable agronomic systems may not quickly restore important AMF functions.

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Table 1.

AMF families	Morpho-group	Morphological Characteristics	ISA Associated Management
Glomeraceae	Glomus sp.	Spores ranging from light to med-dark yellow, matte or hyaline, 100-150µm	N.S.
	Rhizophagus sp.		Early Succession
Claroideoglomeraceae	Claroideoglomus sp.	Pale yellow w/ segregated interior, 100-150µm	Early Succession
Gigasporaceae	Gigaspora sp.	Large spores > 150µm, "bulbous" hyphal attachment	No-Till
Acaulosporaceae	Acaulospora sp.	Dark red-brown, hyaline sp. ≤ 150µm	Conventional
Diversisporaceae	Diversispora sp.	Yellow-orange spores, 80-120µm	Organic
Paraglomeraceae	Paraglomus sp.	White, "dirty" w/hyphae 100-120µm	N.S.
Archaeosporaceae	Archaeospora sp.	White (clear) hyaline spores 60-100µm	N.S.

Morpho-group classifications, descriptions, and results from blocked indicator species analysis (ISA). Significant indicator morpho-group and associated agricultural management system listed in bold. (N.S. = Not a significant indicator)

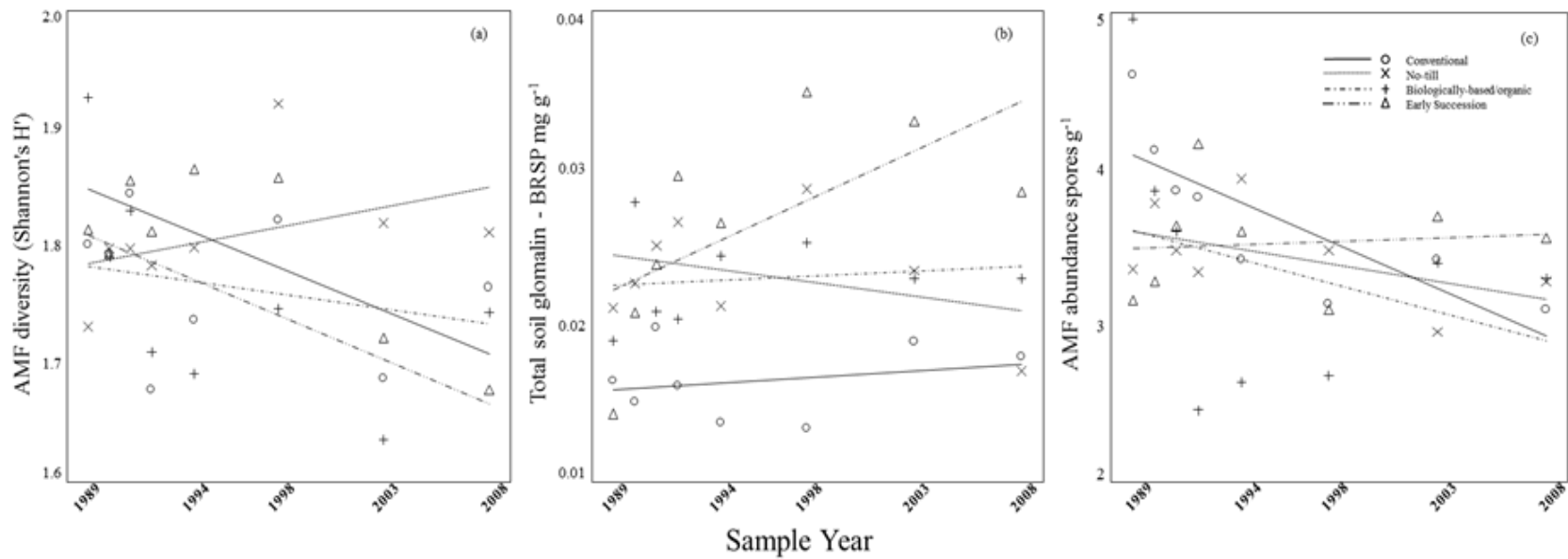


Figure 1. Trends in **a)** diversity (H') of the AMF community; **b)** total soil glomalin – BRSP; and **c)** AMF abundance, across 20 years associated with different agricultural management strategies. Different MCSE management represented by different symbols and line styles. Lines of best fit are provided to show trends, even if linear regressions were not significant.

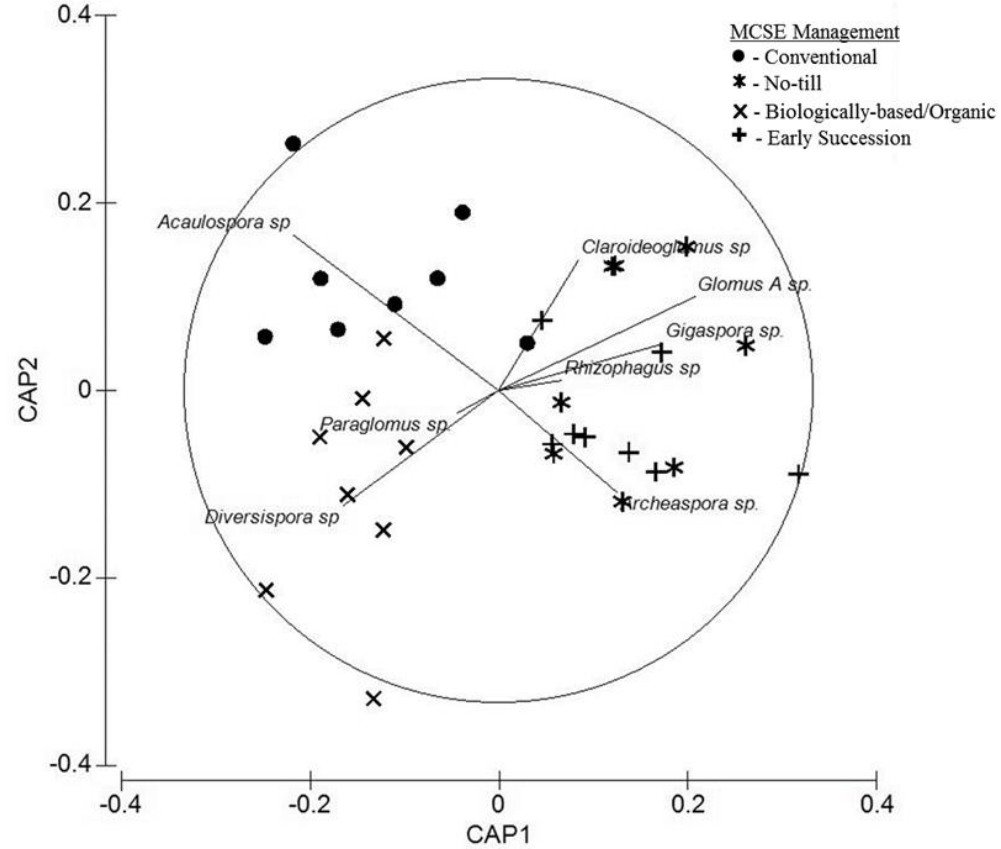


Figure 2. CAP analysis visualization of AMF community structure associated with each agricultural management system, controlling for differences between sampling years. Bi-plot lines represent significant correlations between morphogroups and ordination axes. CAP results based on $m = 7$, and explained 68.75% of the variation in the data with a misclassification of 31.25%, and with a trace statistic $p = 0.001$ for 999 permutations.

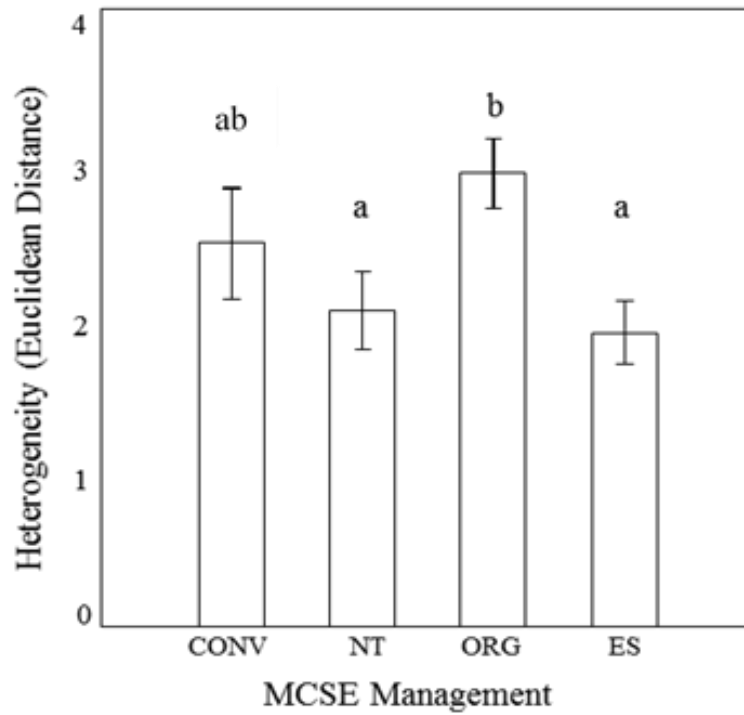


Figure 3. Average heterogeneity of AMF communities in the MCSE agricultural systems during the time series 1989 – 2008. Different letters indicate significant differences AMF community structure as determined by pairwise Fisher's LSD. Error bars are \pm S.E. (CONV = conventional; NT = no-till; ORG = organic; ES = early succession).

CHAPTER III

RESPONSES OF ARBUSCULAR MYCORRHIZAL FUNGI TO DIFFERENT AGRICULTURAL MANAGEMENT SYSTEMS INCLUDE ALTERED COMMUNITY COMPOSITION AND ECOSYSTEM SERVICES

SUMMARY

Agricultural management has been shown to alter soil microbial community structure and ecosystem services. Tillage and chemical inputs negatively affect soil carbon and nitrogen cycling by uncoupling soil microbial communities from their associated ecological functioning. Important plant mutualists like arbuscular mycorrhizal fungi (AMF) associate with many agricultural crops and provide benefits to agricultural production through multiple mechanisms including pathogen protection, nutrient access and acquisition, and also act to increase total soil organic carbon through production of glomalin. To address the question of how different agricultural management systems affect AMF community structure, composition, and function, we surveyed no-till, reduced input, biologically-based organic, early succession, and conventional management plots at the Kellogg Biological Station-Long Term Ecological Study Main Cropping System Experiment. Our results indicate that AMF respond to both tillage and chemical disturbance associated with different management systems. However, the often reported negative effects of conventional agricultural management on AMF diversity were not apparent during our three year study. Also, organic management did not

improve AMF diversity, richness, or overall community function. Moreover, ecosystem services provided by AMF are limited under all agricultural management systems and even total abandonment of agricultural management does not act to quickly restore the AMF community.

INTRODUCTION

Agricultural management of arable and marginal lands has steadily increased during the past decades leading to increased negative environmental consequences associated with conventional agricultural production (Robertson and Vitousek, 2009; Levine et al., 2011). Some negative effects of agricultural management are due to initial land use change (DuPont et al., 2010; Paula et al., 2014), but continued intensive land management further reduces soil fertility to a point where applications of inorganic fertilizers are necessary to maintain productivity (Drinkwater and Snapp, 2007; Power, 2010). This type of intensive agricultural management has been shown to alter soil microbial community structure and ecosystem functioning across a wide variety of systems. For instance, arid and semi-arid lands used for crop production in both China (Danfeng et al., 2006) and Italy (Salvati et al., 2015), were shown to be at greater risk for desertification, likely due to loss of important soil microbes. Another study in a tropical system showed biofuel crop production reduced arbuscular mycorrhizal fungi (AMF) diversity and associated soil aggregate stability (Alguacil et al., 2012). Loss of soil microbial biodiversity and ecosystem functioning due to intensive agricultural management may be reversible, therefore growers may want to consider alternative management strategies to restore benefits of soil microbes. For example, altering

management to enhance beneficial soil microbes such as N-fixing rhizobial bacteria and AMF restored soil stability and ecosystem function in a semi-arid system (Requena et al., 2001). The roles of soil microbes in agricultural systems may become even more important in the future, as effects of climate change create insecurity in many areas used for crop production (Funk et al., 2008; Culman et al., 2010; Chen et al., 2011).

AMF are one group of beneficial soil microbes present in both natural and agricultural ecosystems and associate with many agricultural crops (Douds and Millner, 1999; Smith and Read, 2008). AMF are important plant mutualists shown to provide benefit through multiple mechanisms, including root pathogen resistance and drought tolerance (Asrar and Elhindi, 2011; Veresoglou and Rillig, 2012). However as obligate mutualists, AMF must colonize their host plant root and create a nutrient transfer network of extra-radicle soil hyphae (ERH) to exchange plant derived carbon for soil nutrients foraged by the AMF-ERH, typically organic N and P (Cavagnaro et al., 2005). In this way, AMF-ERH act as an extension of the host plant root system and increases plant nutrient acquisition capabilities beyond the rhizosphere (Johnson and Gehring, 2007; Camenzind and Rillig, 2013). Additionally, AMF-ERH cell walls contain a recalcitrant C-N rich glycoprotein known as glomalin, shown to be tightly correlated with water stable aggregate formation and may account for 3-8% of soil organic carbon in natural and agricultural systems (Wright et al., 1996; Rillig et al., 2002). Through this pathway, glomalin produced by AMF-ERH contributes to soil organic carbon and nitrogen pools and improved soil fertility, potentially providing an important ecosystem service for agricultural systems (Nichols and Wright, 2006; D'Hose et al., 2014).

Through disturbances associated with tillage and chemical inputs, agricultural management has been shown to negatively affect soil carbon and nitrogen cycling by uncoupling soil microbial communities from their associated ecological functioning (McLauchlan, 2006; Liiri et al., 2012). Alternatives including no-till, reduced input, and biologically-based organic systems are considered more sustainable, however these management systems may still affect soil microbial communities through different combinations of chemical and mechanical (tillage) disturbance. For instance, under reduced tillage organic management S ale and colleagues (2015) saw increased AMF diversity and abundance, but in another case, Hijri and colleagues (2006) found AMF diversity in an organically managed system similar to an intensive conventional system, most likely due to the overriding effects of mechanical tillage. Understanding how different agricultural management systems affect the AMF community is important for being able to properly manage these important mutualists to maintain or improve soil fertility, agro-ecosystem stability, and productivity (Caruso and Rillig, 2011; Alguacil et al., 2014).

In this study we looked at how different row-crop agricultural management systems have influenced AMF community diversity and function during a three year cropping rotation, and compared these effects to an early succession system abandoned from agriculture for >20 years. Our focus was on three main questions: 1) Do different row crop agricultural management systems affect AMF community structure and abundance? 2) do more sustainable agricultural systems enhance AMF ecosystem services? and 3) does cessation of agricultural management further restore AMF ecosystem services? For our first question, we hypothesized that no-till and biologically-

based organic management would increase AMF abundance and diversity due to stopping mechanical disturbance (in no-till) or chemical inputs (in biologically-based organic). For the second question, we hypothesized that no-till management would enhance ecosystem services provided by AMF through increased ERH and associated glomalin (both correlated with water stable aggregates/soil organic carbon/soil fertility), and the systems that included tillage would continue to have much reduced AMF-ERH and glomalin-associated ecosystem services. For the third question we expected that cessation of agricultural management, specifically the removal of both mechanical disturbance and chemical inputs, as well as associated increases in plant community diversity, would result in restored AMF diversity and associated function (Antoninka et al., 2011). Positive management effects on the AMF community may work to increase agricultural sustainability and reduce differential effects of climate change to ecosystem services provided by agricultural systems (Oehl et al., 2004; Wu and Xia, 2006). Results from our study provide more understanding of how different agricultural management systems may affect AMF communities and related ecosystem services.

METHODS

Site Description:

The W.K. Kellogg Biological Station Long Term Ecological Research (KBS-LTER) site located in Michigan, USA (42°24'N, 85°24'W) was established in 1989 to evaluate the ecology of row-crop agricultural management typical of the north central USA grain producing region. The Main Cropping Systems Experiment (MCSE) consists of eight agricultural management systems including: conventional (tillage and chemical

inputs at recommended rates; CONV), reduced input (tillage/cultivation, but 50% reduction of all chemical inputs; LOW), no-till (conventional chemical inputs, but no tillage; NOTILL), biologically-based/organic (tillage/cultivation, but no organic or inorganic inputs; ORG), and fire maintained early succession (i.e. abandoned agricultural field; ES). All treatments are replicated five times as 1 ha fields. The full site description and experimental design is detailed in Robertson (1991). Site history prior to 1989 is well documented and consisted of mixed agricultural and horticultural cropping for 100+ years, with the most recent years dominated by conventional corn and soybean production.

Soil Samples:

Soil samples were taken from each agricultural treatment described above in June for the year 2010 (wheat), and October for the years 2011 (corn), and 2012 (soy), in accordance with the KBS-LTER soil sampling protocol available at:

lter.kbs.msu.edu/protocols/112. Briefly, five samples were taken at each replicate field sampling site using a standard soil probe (2 cm x 30 cm). Sampling times corresponded to when crops were senescing and when AMF could be expected to have highest sporulation rates (June for wheat, October for corn and soy). These samples were pooled by treatment at the field level, sieved to 4 mm, and air dried. All samples were stored at -20°C prior to processing.

AMF Spores:

We extracted spores from 50 g of soil by wet-sieving through stacked 500 µm, 212 µm, and 38 µm sieves, after Gerdemann and Nicolson (1963), and a sucrose gradient-centrifugation method described in Walker et al., (1982). Cleaned spore samples

were placed into an 85 mm petri dish and visually quantified within four days of extraction. Since many spores were able to be identified only to the family level due to degradation or damage, we chose to use a morpho-group classification for this study. Spores were enumerated and placed into one of nine morpho-groups based on AMF types described by Schussler and Walker (2010) and others (Redecker et al., 2007; Young, 2012; Redecker et al., 2013), (Table 1).

AMF Roots:

To examine the extent of AMF colonization in crop plant roots, we obtained fine root samples from 500 g of soil using a wet-sieve process (500 μm sieve). Roots were cleared with 10% KOH and stained using a 5% vinegar-ink solution using methods modified after Vierheilig et al., (1998). Visual estimation of percent root length colonization was made using 100 fields of view per sample under 200X magnification (NIKON E400), and a grid-intercept method modified after Giovanetti and Mosse (1980).

AMF Extra-radical Hyphae:

To estimate activity of AMF in the soils of each site, AMF-ERH were extracted from 20 g soil subsamples. Each subsample was mixed with 500 ml DI water in a 100 ml beaker and stirred at 80% speed for 2 min with a magnetic stirrer (Fisher Scientific 11-500-49SH). Before solid material settled, the solution was poured through 710 μm and 212 μm sieves to separate sand and large organic material from the hyphal suspension. Residue from the 212 μm filter was rinsed back into a 50 ml beaker using 10 ml of DI water. Twenty drops of 0.05% Lacto-Glycerol Trypan Blue stain were added and left to sit for 45 min. This solution was then filtered through a 38 μm sieve and rinsed with DI water until water ran clear from the sieve. The residue on the 38 μm sieve was rinsed

back into a 400 ml beaker using 200 ml of DI water and agitated for 2 min on the magnetic stirrer. A 20 ml sample was removed from ~1 cm below the water surface and drained through a 25 mm glass microanalysis vacuum filter holder fitted with a 0.45 μ m mesh nylon membrane. The membrane was then removed from the holder, dried briefly and mounted onto a slide using PVLG. Hyphal length was recorded as % of intercepts per field of view using the gridline-intercept method (McGonigle et al., 1990) under a compound microscope (Nikon E400 at 100X).

Glomalin Extraction:

Total soil glomalin was extracted from one gram of soil using the 0.8 mM sodium citrate buffer and autoclaving method described in Janos et al., (2008). We quantified the Bradford reactive fraction (Bio Rad, Hercules, CA, USA) using bovine serum albumin (0.5 mg/ml) as a standard (Koide and Peoples, 2013). Total soil glomalin has several extractible fractions (Cornejo et al., 2008), and Bradford reactive soil protein (BRSP) has been shown to consistently represent the largest fraction of total soil protein extracted using these methods (approximately 95%). Therefore we chose to use BRSP to operationally define glomalin for this work.

Analyses:

AMF diversity (Shannon's H') and AMF abundance (spores g^{-1} soil) data were calculated from pooled soil samples from each replicate field in each year and differences between different agricultural management systems were analyzed using two-factor ANOVAs with agricultural management treatment ($n = 5$) and year ($n = 3$) as the main factors. We compared AMF function and ecosystem services using % root colonization, BRSP (mg g^{-1} soil) and AMF-ERH ($m\ cm^{-2}$) data using similar 2-way ANOVAs. We

followed up all significant ANOVA models with pairwise comparisons using Fisher's LSD. AMF diversity, community structure, AMF-ERH, and root colonization data were square root transformed to better meet model assumptions for analyses. All ANOVA analyses were performed using Systat12 (SYSTAT v. 12 2008, SYSTAT Software Inc., Chicago, IL).

We used a two-factor PERMANOVA (Anderson, 2001; McArdle and Anderson, 2001), to examine overall differences in AMF community composition and structure due to the agricultural treatment and year, followed by pairwise comparisons to see whether the AMF communities were different between each agricultural treatment. To visualize differences in AMF community structure due to agricultural management, we performed a constrained ordination analysis (CAP). This procedure allowed us to look at the overall effects of agricultural management on AMF community structure while constraining the effects of a factor (year/crop in this case) (Anderson and Willis, 2003). An indicator species analysis (ISA) was performed to see if individual AMF morphospecies were associated with a particular agricultural treatment (McCune and Grace, 2002). PERMANOVA and CAP analyses were performed using Primer v. 6 (Anderson et al., 2008), and the ISA was done using PC-ORD v.6.08 (McCune and Mefford, 1999).

RESULTS

AMF Diversity:

There was a significant management by year effect on AMF richness indicated by ANOVA (Table 1). This was driven by differences among agricultural treatments in 2010 and 2012. No-till management had lower AMF richness than all other treatments in 2010,

and in 2012 richness was lowest the early succession system (Figure 1a). AMF diversity followed a similar pattern for 2010, with diversity lowest in no-till (Table 1; Figure 1b). However, in 2011 diversity was lowest in no-till, organic, and early succession, and highest under conventional and reduced input management. This pattern continued in 2012 with diversity in no-till and organic lower than conventional and reduced input, but diversity was lowest in the early succession system. AMF abundance was only affected by year and was lowest in 2010 (wheat year). There was no difference between overall abundance for 2011 and 2012 (Table 1; Figure 1c).

AMF Function:

Crop root colonization by AMF was highest during 2012, compared to 2010 and 2011. Overall root colonization was highest in in the early succession treatment and there were no differences between the four agricultural treatments (Table 1; Figure 2a/b). There was a significant interaction between management and year for AMF-ERH length, driven mainly by differences between the agricultural treatments and early succession system in 2010 and 2011. In 2010 (wheat year), conventional management had more AMF-ERH than the early succession system. This was reversed in 2011 (corn year) when there was less AMF-ERH in conventional, no-till, and reduced input as compared to early succession (Table 1; Figure 2c). AMF-ERH length in no-till was also significantly lower than organic management in 2011. Total soil glomalin – BRSP content was significantly lower in conventional compared to organic agricultural treatment, although all agriculturally managed soils had lower BRSP content overall compared with early successional soils (Table 1; Figure 2d).

AMF Community Structure:

Overall, both agricultural management and year, as well as their interaction, had significant effects on AMF community structure (PERMANOVA, treatment $F_{(\text{pseudo})4,135} = 8.0389$, $p_{(\text{perm})} = 0.001$; year $F_{(\text{pseudo})2,135} = 15.483$, $p_{(\text{perm})} = 0.001$; treatment x year $F_{(\text{pseudo})8,135} = 2.3018$, $p_{(\text{perm})} = 0.002$). AMF communities were significantly different between all agricultural management treatments and early succession as indicated by pairwise comparisons (all p -values between 0.001 – 0.034), except between the reduced input and organic systems which were not different ($p_{(\text{perm})} = 0.094$). CAP analysis showed that differences in community structure were due mostly to chemical disturbances, with no-till and conventional management overlapping, while reduced input and organic treatments clustered together. The exception to this is that early successional fields appeared more similar to NOTILL/CONV than ORG/LOW (Figure 3). Results from the indicator species analysis show that both *Glomus spp.* and *Diversisporaceae* were indicators for no-till, *Funneliformis sp.*, was significantly associated with conventional management, and *Rhizophagus sp.* was most significantly associated with biologically-based organic management as compared to the other systems (Table 2).

DISCUSSION

-- Do different row crop agricultural management systems affect AMF community structure and abundance?

We found evidence that different agricultural management systems can alter AMF community diversity and abundance, but these effects are not consistent and often change depending on the year or crop. No-till management had lower AMF richness and

diversity than all other treatments surveyed in 2010 (wheat), although both recovered to levels found for the other systems in following years. Surprisingly, conventional management had higher AMF richness and diversity than organic management in both 2011 and 2012. AMF abundance was lowest under wheat in 2010 and did not differ between any of the agricultural treatments we surveyed. This result may be due to differences in crop phenology or crop functional type influencing the relationship between spore production and root colonization as shown by Zangaro et al., (2013). Research by others has highlighted the temporal and seasonal variability of AMF diversity and abundance (Escudero and Mendoza, 2005), but variability may also be due to the indirect effects of chemical inputs, such as changes to soil pH with long-term N fertilizer addition (Liu et al., 2014).

We found consistent significant differences in AMF community structure in response to agricultural management, even given some variation due to year/crop. Results from the PERMANOVA analysis and the CAP visualization (Figure 3), indicate significant shifts in AMF community structure due to both tillage disturbance and chemical inputs. Similarity between NOTILL/CONV AMF communities in the CAP ordination are most likely due to chemical inputs. When tillage is removed, i.e. when comparing NOTILL and ES AMF communities, shifts in AMF community structure due to chemical inputs are significant. In natural ecosystems where tillage is not a consideration, chemical inputs such as fertilizer and herbicide have been shown to affect soil microbial community functioning and reduce soil organic carbon (DuPont et al., 2010). Herbicide control has been shown to affect AMF function (Lutgen and Rillig, 2004), although the effect may be indirectly due to host plant loss. Glomeraceae were the

most populous AMF family in our system, similar to what others have found in agricultural sites (Opik et al., 2010). We were able to identify members of this family to genera, allowing a split of the *Glomus* group. We are confident our morphogroup classifications are valid because although 3 of 4 ISA analysis indicators are from Glomeraceae (Table 2), functional differences at the species level have been described (Hart and Reader, 2002). In similar experimental systems, members of the AMF family Gigasporaceae have been found associated with low mechanical disturbance (Castillo et al., 2006), although this group was not an indicator for the no-till or early succession systems in our study (Table 1).

-- *Do more sustainable agricultural systems enhance AMF ecosystem services?*

One important ecosystem service that AMF provide is increased soil fertility and C sequestration through production of ERH and glomalin. Our results indicate that there were differences in these services across agricultural treatments, but not in the ways we predicted. We expected AMF-ERH to increase in length under no-till management. Surprisingly, in 2011 ERH in no-till plots was low compared to the organic treatment and early succession system. This may be due to shifts in AMF communities in response to management or crop plant host. For instance, members of the AMF family Glomeraceae are known to differ in ERH construction strategies from other families (de la Providencia et al., 2005; Voets et al., 2006). AMF in Glomeraceae often fuse extra-radicle hyphae between closely related species to form common mycorrhizal networks rather than creating networks of individual large diameter hyphae. This could explain reduced AMF-ERH in the no-till system as a shift to dominance by *Glomus* species was indicated by the results of our ISA (Table 2). AMF-ERH growth and dieback have been shown to be

responsive to changes in temperature and soil moisture (Hernandez and Allen, 2013), therefore crop cover and soil physical properties might also be affecting our result. Total soil glomalin – BRSP was higher under organic management as compared to conventional management in our study. Differences in BRSP between conventional and organic management may be due to differences in the dominant AMF species in each treatment (Wu et al., 2014) (Table 1), although difference in glomalin production by the two AMF groups indicated in our study is not specifically known. Total soil glomalin-BRSP was not affected by year, adding support to AMF’s contribution to recalcitrant soil organic carbon in these systems. We found no differences in crop root colonization among the agricultural treatments. Overall reduction of root colonization due to long-term N application has been seen in other systems (van Diepen et al., 2007), and may explain the similarity in root colonization across the agricultural management systems in our study.

-- Does cessation of agricultural management further restore AMF ecosystem services?

Root colonization and total soil glomalin were highest in the early succession system, adding support to the idea that when all agricultural inputs and management are stopped, some AMF functions can be restored. However, increases in these functions within the early succession system do not seem directly connected to changes in the AMF community. In 2010, AMF-ERH length in the early succession system was low, but recovered in 2011. This reduction could be due to edaphic conditions at the time of sampling, although burn maintenance, which has been shown to stimulate AMF-ERH activity in other systems (Treseder et al., 2004) could be the cause. However, Eom and colleagues (1999) found no effects of fire on AMF-ERH in a tallgrass prairie system.

Total soil glomalin was highest in the early succession system, adding some support to our original hypothesis that if agricultural management is stopped, AMF community structure and functions would be restored. However, AMF richness, diversity, and spore abundance all varied significantly from year to year in the early succession system, and were sometimes lower than the conventionally managed agricultural system. One explanation may be the long history (100+ years) of agricultural land use at our study site. Long term negative selection pressure from tillage and chemical inputs would have reduced original AMF diversity, potentially creating a depauperate AMF community present at the establishment of these agricultural treatments in 1989, as suggested in Verbruggen et al., (2012).

Conclusions:

For this study we surveyed AMF communities from four different agricultural management systems, which vary in both tillage and chemical input disturbance. The conventional (CONV), no-till (NOTILL), reduced input (LOW), and biologically-based organic (ORG) management systems are similar to those used in the upper mid-west and central grain producing regions of the US. The early succession (ES) system was abandoned from agriculture in 1989, at the same time the agricultural systems were established. The agricultural treatments have been under the same corn – soy – wheat rotation since 1994.

Overall, the results of our three year survey show that AMF community composition shifts in response to differences in agricultural management tillage and chemical inputs. The conventionally managed system did not have the expected effects on AMF diversity or richness, and more sustainable agricultural systems (no-till, reduced

input, and organic) did not have the positive effects on the AMF community we expected. For some years, measures of AMF community function were improved as a result of organic management. However, variations in AMF ecosystem services were not definitively associated with changes to community structure and composition or function unless agriculture was entirely abandoned.

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Table 1.

Main factors	<u>Richness</u>		<u>Diversity (H')</u>		<u>Abundance (50g)</u>		<u>Root Fraction colonized</u>		<u>AMF-ERH (m cm⁻²)</u>		<u>Glomalin – BRSP (mg g⁻¹)</u>	
	<i>F</i> -ratio	<i>p</i> -value	<i>F</i> -ratio	<i>p</i> -value	<i>F</i> -ratio	<i>p</i> -value	<i>F</i> -ratio	<i>p</i> -value	<i>F</i> -ratio	<i>p</i> -value	<i>F</i> -ratio	<i>p</i> -value
Management	2.451	0.049	11.142	0.000	1.763	0.140	3.423	0.011	1.228	0.302	15.731	0.000
Year	11.956	0.000	58.686	0.000	63.632	0.000	8.889	0.000	3.702	0.027	0.199	0.820
Management x Year	2.737	0.008	3.745	0.001	0.636	0.746	1.177	0.317	2.182	0.033	1.194	0.307

Two factor ANOVA table for all response variables ($N = 150$, *df* management = 4, *df* year = 2, *df* interaction = 8).

Significant values indicated with **bold** type.

Table 2.

AMF families	Morpho-group	Morphological Characteristics	ISA Associated Management
Glomeraceae	<i>Glomus spp.</i>	Spores ranging from light to med-dark yellow, matte to hyaline, 100-150µm diameter	No-till
	<i>Funneliformis sp.</i>		Conventional
	<i>Rhizophagus sp.</i>		Organic
	<i>Sclerocystis sp.</i>		N.S.
Claroideoglomeraceae	<i>Claroideoglomus sp.</i>	Pale yellow with "globules" inside, 100-150µm	N.S.
Gigasporaceae	<i>Gigaspora sp.</i>	Large spores (>150µm) with "bulbous" hyphal attachment	N.S.
Acaulosporaceae	<i>Acaulospora sp.</i>	Dark red-brown, hyaline <100µm	N.S.
Pacisporaceae	<i>Pacispora sp.</i>	White (clear) hyaline spores >100µm	N.S.
Diversisporaceae	<i>Diversispora sp.</i>	Yellow-orange spores, 80-120µm	No-till
Archaeosporaceae	<i>Archaeospora sp.</i>	White (clear) hyaline spores 60-100µm	N.S.

AMF morpho-group characteristics and indicator species analysis results. Significant indicator species (morpho-group) and associated management system are shown in bold-type.

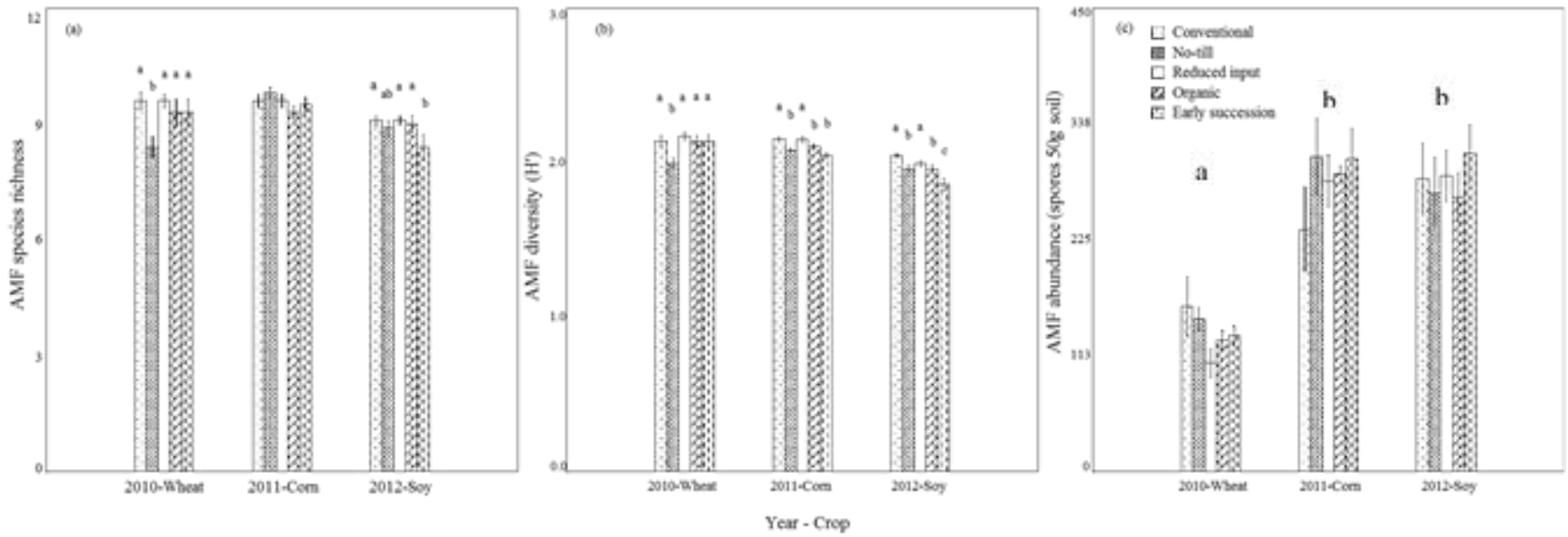


Figure 1. Results for AMF a) richness, b) diversity (H'), and c) abundance for the three year study. Different lower case letters indicate significant differences for each panel as determined by Fisher's LSD pairwise analyses. Data are mean \pm S.E.

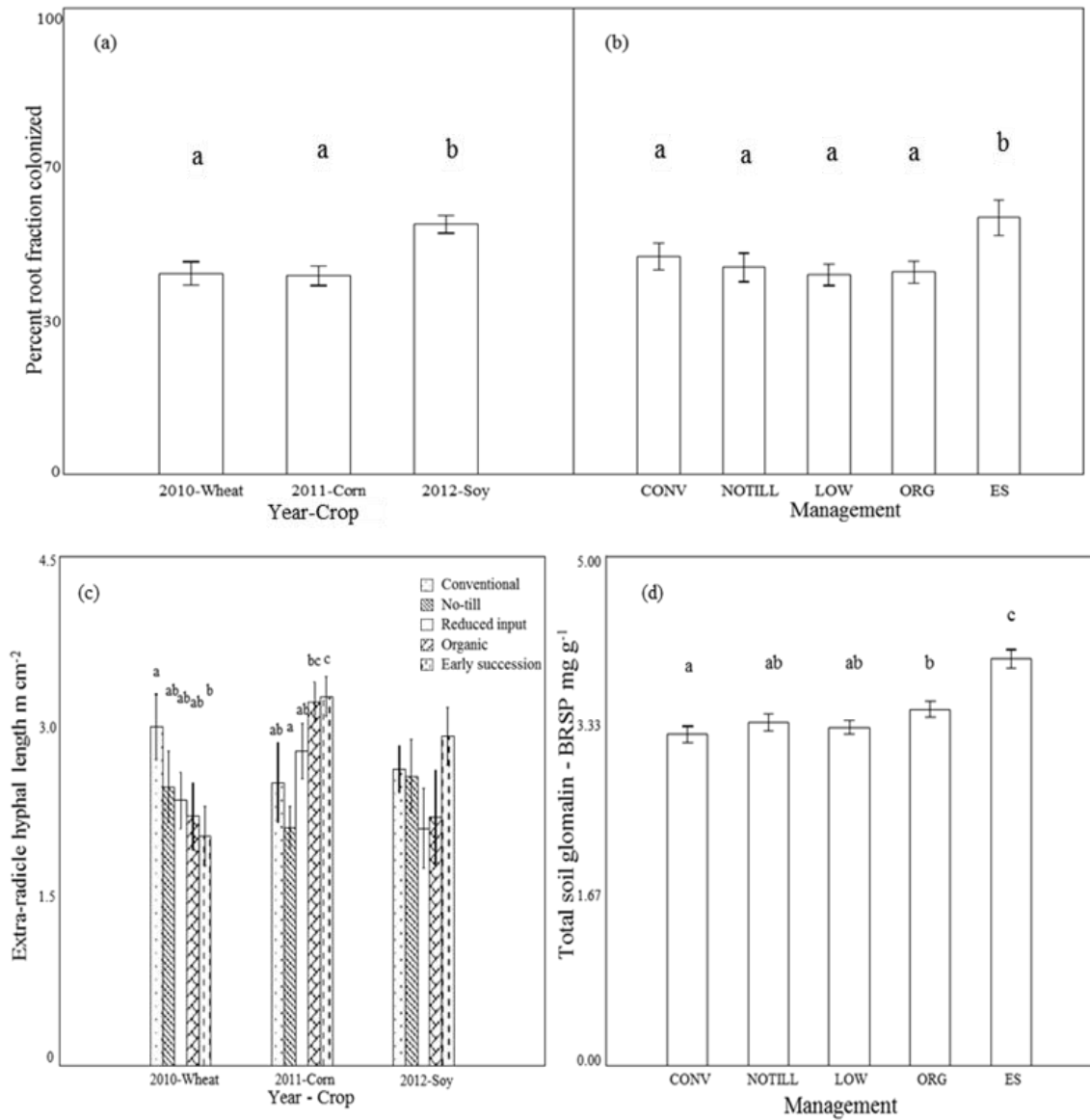


Figure 2. AMF activity measured as a-b) percent root colonized by year and agricultural management, c) ERH, and d) total soil glomalin. Different lower case letters indicate significant differences within each panel as determined by Fisher's LSD pairwise comparisons. Error bars indicate \pm S.E.

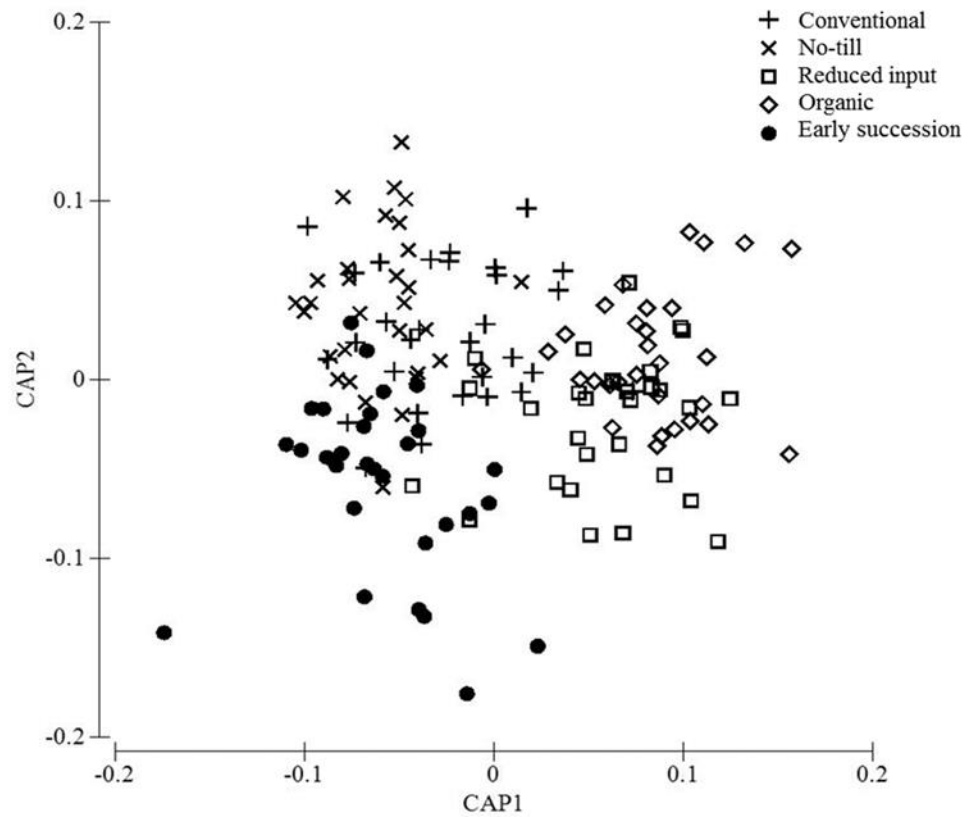


Figure 3. CAP analysis of AMF community structure associated with each agricultural treatment, controlling for differences between sampling years. CAP results based on $m = 6$, and explained 62.667% of the variation in the data with a misclassification of 37.333%, and with a trace statistic $p = 0.001$ for 999 permutations. Crossed symbols represent agricultural management with conventional chemical inputs and open symbols represent agricultural managements with reduced chemical inputs.

CHAPTER IV
SUSTAINABLE AGRICULTURAL MANAGEMENT ALTERS SOIL MICROBIAL
EFFECTS ON CROP GROWTH

SUMMARY

Native ecosystems harbor diverse soil microbial communities, which have been shown to maintain productivity and other ecosystem services. Land use change due to agriculture disturbs the soil microbial community, potentially altering crop performance and ecosystem functioning. Understanding how different agricultural management systems may affect soil microbial communities is essential to improving sustainability and ecosystem functioning of these important systems. To help address this, we conducted a greenhouse experiment to investigate changes in soil microbial community function associated with three different agricultural management systems. We measured aboveground and belowground biomass production of corn (*Zea mays* L.) and spring wheat (*Triticum aestivum* L.) grown using live soil and microbial wash inocula sourced from conventional, no-till, and biologically-based/organic agricultural management systems at the Kellogg Biological Station Long Term Ecological Research Site. We found that microbial community effects on plant growth varied across agricultural management systems. Corn aboveground biomass was greatest when grown with conventionally managed soil inocula, while corn root-to-shoot ratios were most improved by live soil communities from organically managed soil. Wheat aboveground growth was

reduced by both conventional and no-till soil inocula, but was not affected by soil inocula from organically managed systems. Overall, our results indicate that agricultural management practices can shift the functioning of soil microbial communities for crops. Conversion to more sustainable agricultural management systems could reverse some of the negative microbial effects associated with conventional agriculture, and the presence of possible positive feedbacks in conventional management systems is worth further research.

INTRODUCTION

Native ecosystems harbor diverse soil microbial communities, which have been shown to maintain productivity and other ecosystem services (de Vries et al., 2013; Eisenhauer et al., 2013). Land use change can alter soil microbial biodiversity by changing soil physiochemical properties (Baeten et al., 2010; Dunne et al., 2011; Sul et al., 2013), shifting microbial community structure (Wardle, 1995), altering food web processes (Liiri et al., 2012), and disrupting soil nutrient cycling (Bowles et al., 2014). Nearly all modern agricultural practices include tillage and chemical inputs, and these practices can negatively impact soil microbial communities, thereby indirectly reducing ecosystem nutrient cycling and primary production (Brussaard et al., 2007; van der Heijden et al., 2008). For example, in a recent study comparing bacterial functional diversity, samples from conventional agriculture had lower species richness and functional gene abundance as compared to more sustainable systems (Xue et al., 2013). In another study both bacterial and arbuscular mycorrhizal fungal (AMF) diversity were reduced due to inorganic nitrogen fertilizer application (Toljander et al., 2008). Shifts in the composition and functioning of soil microbial communities due to conventional

management could result in reduced plant growth and a cycle of increasing inputs to maintain agricultural yields.

Adoption of more sustainable agricultural practices may reverse some of the negative effects of conventional practices on soil microbial communities and improve overall soil health and sustainability. No-till agricultural practices have been reported to improve soil aggregation through both increased bacterial biomass and fungal hyphal density, specifically AMF hyphae (Helgason et al., 2010; Jansa et al., 2002), which can improve crop nutrient use (Pellegrino and Bedini, 2014). However, no-till agricultural systems rely heavily on herbicides and inorganic fertilizers, and both inputs are known to negatively impact soil biodiversity (Wright et al., 1999; Constantin et al., 2010). Similarly, organically managed agricultural systems have been shown to improve overall soil microbial biodiversity (Mäder et al., 2002) and soil structure (Elmholt et al., 2008; Gosling et al., 2006; Lee and Eom, 2009), as well as increase diversity of the AMF community (Galván et al., 2009; Gosling et al., 2006), though tillage remains a regular soil disturbance.

In this study, we compared the function of soil communities associated with conventional and more sustainable agricultural practices. To directly evaluate the effects of the soil microbial community on crop performance, we looked at how two different crop species respond to soil inocula from conventional and sustainably-managed agricultural systems under controlled conditions. We asked specifically: 1) Do soil microbial communities from different agricultural management systems vary in their effects on plant performance, and does this vary with crop species? And 2) do bacterial community effects differ from effects of the soil microbial community as a whole? We

predicted that overall plant growth would be most strongly inhibited by the microbial communities from conventionally managed soil as compared to those from more sustainable agricultural systems. We also predicted that the bacterial community would be primarily responsible for any negative effects on crop performance, with whole soil microbial community effects being more moderate due to presence of beneficial organisms such as AMF.

METHODS

Experimental Design:

We designed a 3-factor greenhouse experiment to compare microbial community function associated with conventional and sustainably-managed row-crop agricultural systems. In May 2012, we collected soil samples from the W.K. Kellogg Biological Station Long Term Ecological Research Main Cropping System Experiment (LTER-MCSE) in Hickory Corners, MI., USA. The full experimental design of the LTER-MCSE and established agricultural treatments are described in detail in Robertson (1991). Briefly, the MCSE was designed to evaluate the ecology of row-crop agricultural practices typical of the north central USA. The experiment was established in 1989 and consists of eight fully replicated and differently managed systems including conventional (CONV), no-till (NT), and zero-input organic (ORG), in an annual corn-soybean-wheat rotation. Soil samples used in our experiment were taken from replicate treatment plots in May 2012 using a 2x30 cm probe (5 samples x 5 sampling points/plot x 5 replicate plots), composited, bagged, and stored at -4°C until use.

In June 2012, we filled 120 1.6 L Mini Treepots (Model MT49; Stuewe & Sons) with approximately 1.4 L of a 2:1 mixture of general purpose sand (SAKRETE[®]) and coarse horticultural grade vermiculite (Perlite/Vermiculite Packaging Co.). Each pot received 50 g inoculum from one of three agronomic system soil sources (CONV, NT, or ORG), which were each divided into three microbial treatments: whole live soil, autoclaved soil (121°C for two, 60 minute cycles) to kill all soil organisms, or autoclaved soil plus a microbial wash isolated from 50 g of live soil. The microbial wash was made by placing 50 g live soil into a beaker with 500 ml DI H₂O and stirring for several minutes to break up any aggregates. The soil solution was then slowly decanted through a stacked set of sieves (500 µm, 212 µm, 38 µm openings) and the liquid flow-through consisting of all organisms smaller than 38 µm (i.e., bacteria) was collected (e.g., Emery and Rudgers, 2012). All inocula were gently mixed into filled pots with single-use, sterile wooden applicator sticks. Each treatment inoculum made up only 2-3% of the total pot volume.

After pots were inoculated, we planted one of two different crops into each: *Triticum aestivum* L. var. “Glenn”, a hard red spring wheat popular with farmers in the US, comprising 18.1% of 6.35 million acres planted in 2011 (Jantzi, 2011), or *Zea mays* L. var. “Nothstine”, a yellow dent field corn (Johnny’s Seeds, Maine). We placed three wheat seeds or two corn seeds near the center of each pot, covered with approximately 4-5 cm of the potting mixture. Following emergence, seedlings were thinned to one per pot by sub-surface pruning using tissue scissors cleaned with 98% EtOH between each pruning to avoid cross-contamination between microbial and soil source treatments.

Wheat plants grew from June 4 to August 5 (58 days post emergence) and corn plants grew from June 4 to August 15 (65 days post emergence), both under well-

watered, ambient greenhouse conditions (temp. range 23-42°C). We rotated trays of pots two times during the experiment to reduce edge and abiotic variability effects in the greenhouse. At the end of the experiment, we separated all aboveground biomass (ANPP), including surface litter, from the belowground biomass by clipping the stem at 1-1.5 cm above the growth media surface. ANPP samples were placed into individually labelled paper bags, dried for 48 hours at 65°C, and weighed. Belowground biomass (BNPP) in each pot was gently removed from the sand-vermiculite mixture by shaking away loose material and repeated washing in containers of standing water. We took small samples of root material from three locations on each root system and processed these for determination of AMF colonization (following methods in Vierheilig et al., 1998), as one measure of treatment contamination control (i.e., if sterile or wash treatments had evidence of AMF colonization, then those treatments may have been compromised). The remaining cleaned root systems were then placed in individually labeled paper bags, dried for 48 hours at 65°C, and weighed. Raw values for ANPP and BNPP were used to determine plant root:shoot ratios (R:S) for each pot.

Statistical Analyses:

The sterilized soil treatment associated with each MCSE soil source and crop combination was used as the baseline for calculating treatment effects on crop performance. We calculated percent differences (% Δ) between each “live” or “wash” treatment pot and the associated sterile baseline group mean, and used % Δ ANPP, % Δ BNPP, and % Δ R:S as dependent variables for analyses.

We initially conducted a 3-factor ANOVA [crop (corn, wheat) x soil source (CONV, NT, ORG) x microbial treatment (live, wash)], to examine direct and interactive

effects of the main factors on overall plant performance. As expected, due to differences in phenology and physiology between the plant species used in the experiment, “crop” and its interactions were significant in all full models (data not shown). For this reason, we chose to run all subsequent analyses as two-factor ANOVAs with data separated by crop. All analyses were performed using Systat12. (SYSTAT v. 12 2008, SYSTAT Software Inc., Chicago, IL.)

RESULTS

Experimental treatments:

To determine the effectiveness of our microbial treatment preparations, we examined roots from each treatment group for both corn and wheat plants. Overall, colonization by AMF in corn plants grown using the live soil inoculum was moderate (> 40%, data not shown) and vesicles were noted in all colonized root segments. Colonization by AMF for wheat plants grown using the live soil inoculum was relatively low regardless of the MCSE soil source (< 20%, data not shown), but large vesicles were noted in the colonized root sections. Colonization by AMF was not seen in the microbial wash or sterile treatment groups, indicating that our microbial treatments were effective.

Corn:

Corn aboveground growth was enhanced (positive $\% \Delta \text{ANPP}$) when grown in inoculum from the conventional (CONV) system, as opposed to no-till (NT) and organic (ORG) inocula (Table 1, Figure 1a). There were no differences in the effects of live and bacterial wash inocula across treatments. Corn belowground growth was reduced in all soil inocula compared to the sterile controls (negative $\% \Delta \text{BNPP}$), though this effect was

not as strong for plants grown using live soil inocula (Table 1, Figure 1b). Soil inoculum from the organic (ORG) system reduced root growth more than the conventional (CONV) and no-till (NT) systems (Figure 1c). All soil inocula made corn plants more efficient (negative $\% \Delta R:S$) compared to sterile controls. This effect was strongest for live inocula as compared to the microbial washes. Inocula from the organic (ORG) system had stronger effects on reducing R:S ratios than inocula from the no-till (NT) system (Table 1; Figure 1d, e).

Wheat:

All microbial inocula had a strong negative effect on wheat plant growth compared to sterile controls, except for plants grown using live soil inoculum from the organic (ORG) system (Table 1; Figure 2a,b). Similarly, $\% \Delta BNPP$ for wheat plants was negative for all live and soil wash inocula treatments, though plants grown using live soil from the organic (ORG) system were not as strongly affected as plants from the other treatments (Figure 2c,d). Wheat plants were overall more efficient, as indicated by a lower R:S ratio, with live soil inoculum as compared to microbial wash, and benefited most in terms of efficiency when grown using inoculum from the no-till (NT) systems (Figure 2e,f).

DISCUSSION

Our results show that the microbial communities associated with different agronomic systems have differential effects on crop performance. In general, corn showed an overall increase in plant resource use efficiency associated with all soil microbial communities, but ANPP increased the most when crops associated with microbial communities from

conventional agricultural systems. One explanation for this positive effect could be adaptation of the soil microbial community to conventional agricultural inputs associated with the KBS LTER-MCSE corn crop in 2011, the year before we collected soil for our experiment. It has been shown that plant identity can have strong effects on soil microbial communities (Ngosong et al., 2010; Becklin et al., 2012), so our results are potential evidence of positive soil feedbacks (Kulmatiski and Beard, 2011; van de Voorde et al., 2011). This type of positive soil feedback has been shown to occur with some regularity in natural AMF communities and has been implicated in some invasive species spread (Vogelsang et al., 2006; Pringle and Bever, 2008; Qin et al., 2015), but to our knowledge has yet to be documented in agricultural systems.

Soil microbes from all soil sources reduced corn belowground biomass, though this effect was strongest for inocula from organic systems. Corn is known to be a highly mycorrhizal plant (Wright et al., 2005), and the strong effects of live soil inocula on corn belowground biomass may be due to the presence of AMF (Eo and Eom, 2009), as plants that form beneficial associations with AMF can reduce root growth. Tillage and fertilization practices are known to reduce overall fungal diversity, including AMF (Wu et al., 2007), and so biologically-based/organic management may be enhancing this group of organisms and their associated benefits for crop growth.

Wheat growth was reduced by all soil microbial communities, with the exception of live soil inocula from biologically-based/organic systems, which cancelled negative effects of the bacterial community from this system. If crops select for soil microorganisms that benefit themselves, similar to what has been demonstrated with other dominant plants in natural systems, (e.g. de la Pena et al., 2010), then this type of

negative feedback may explain the reduced growth of wheat in soil inocula collected after a season of corn growth. In general, bacterial communities associated with the different agricultural treatments made wheat plants less efficient (higher R:S ratios under “wash”), as compared to live soil communities. These differences between bacterial wash and live soil communities could be driven by AMF, since our microbial wash treatment would have excluded their functional elements (Bender et al., 2015). However, wheat roots in our live soil treatments showed relatively low AMF colonization, which may indicate that an overall increase in soil biodiversity could be responsible for cancelling out the negative effects of bacteria in biologically-based/organic systems.

Overall, our results indicate that agricultural management practices can alter effects of soil microbial communities on crop above- and belowground productivity, both positively and negatively depending on crop species. Surprisingly, we have some evidence that soil microbial communities associated with conventional agricultural practices may benefit crops, possibly due to positive soil feedback mechanisms. Conversion to more sustainable agricultural management systems (i.e. biologically-based/organic) could reverse some of the negative microbial effects associated with conventional agriculture for other crop species.

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Table 1.

Plant	Main Factors	% Δ ANPP		% Δ BNPP		% Δ R:S Ratio	
		(F-ratio; p-value)	(F-ratio; p-value)	(F-ratio; p-value)	(F-ratio; p-value)	(F-ratio; p-value)	(F-ratio; p-value)
CORN	Microbial Treatment	0.742	0.3930	5.386	0.0240	9.307	0.0040
	MCSE Soil Source	13.259	<0.0001	8.423	0.0010	5.496	0.0070
	Microbial Treatment*MCSE Soil Source	0.555	0.5780	0.262	0.7700	0.789	0.4600
WHEAT	Microbial Treatment	2.035	0.1590	8.491	0.0050	67.294	<0.0001
	MCSE Soil Source	12.148	<0.0001	16.922	<0.0001	4.552	0.0150
	Microbial Treatment*MCSE Soil Source	4.886	0.0110	3.922	0.0260	0.248	0.7810

Two-way ANOVA results separated by crop (corn, wheat) for main factors microbial treatment (live, wash) and MCSE soil source (conventional (CONV), no-till (NT), and biologically-based (ORG)).

FIGURE LEGENDS

Figure 1. Corn growth in response to significant factors from ANOVA models in Table 1 as measured by (a)- percent difference in ANPP; (b, c)- percent difference in BNPP; and (d,e)- percent difference in R:S ratio. Treatments codes correspond to conventional (CONV), no-till (NT), and biologically-based (ORG) agricultural systems. Letters indicate significant Tukey pairwise comparisons within each panel. Error bars indicate \pm S.E.

Figure 2. Wheat growth in response to significant factors from ANOVA models in Table 1 as measured by (a, b) -percent difference in ANPP; (c, d) - percent difference in BNPP; and (e, f) - percent difference in R:S ratio. Treatments codes are the same as in Figure 1. Letters indicate significant Tukey pairwise comparisons within each panel. Error bars indicate \pm S.E.

Figure 1.

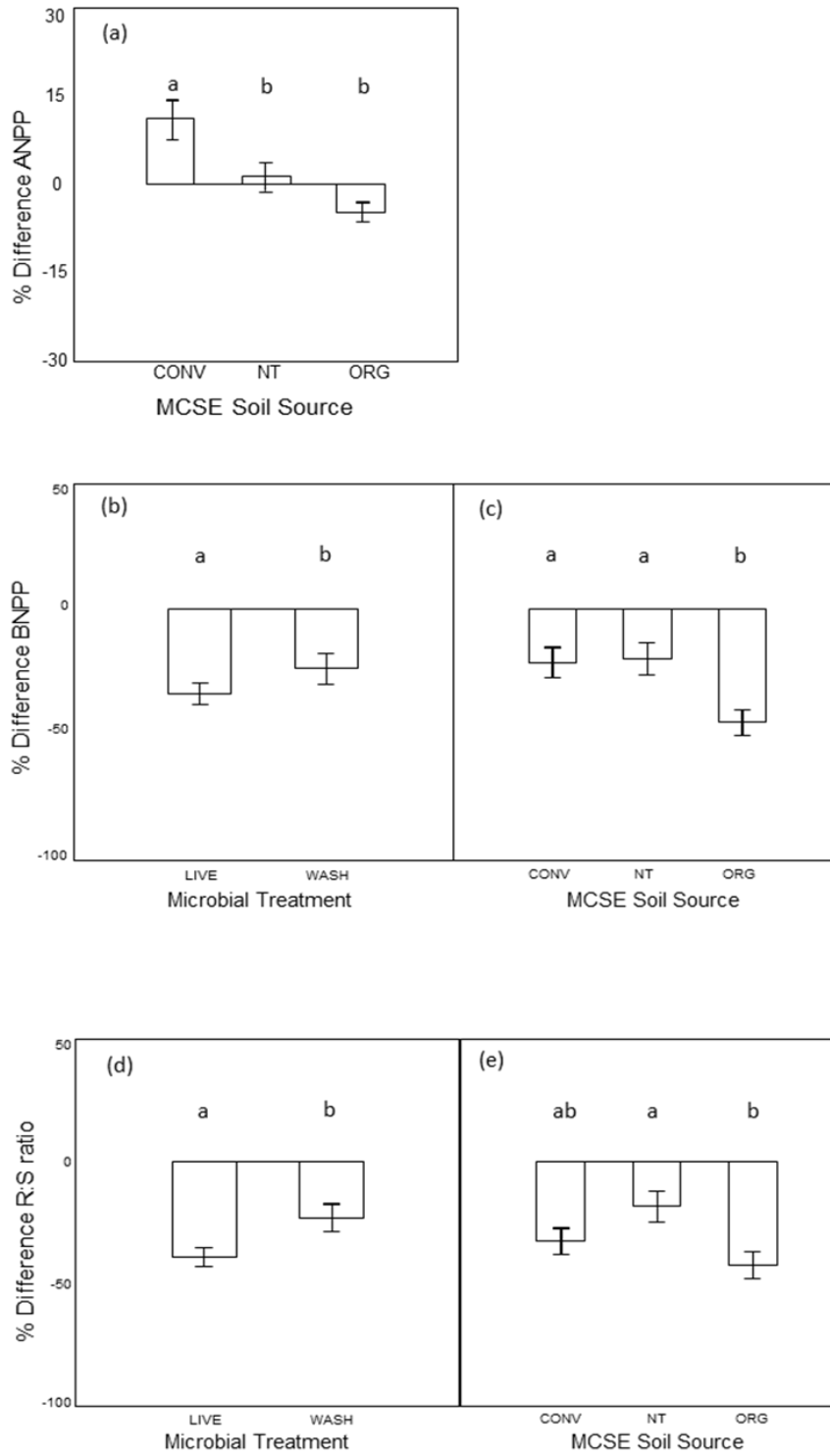
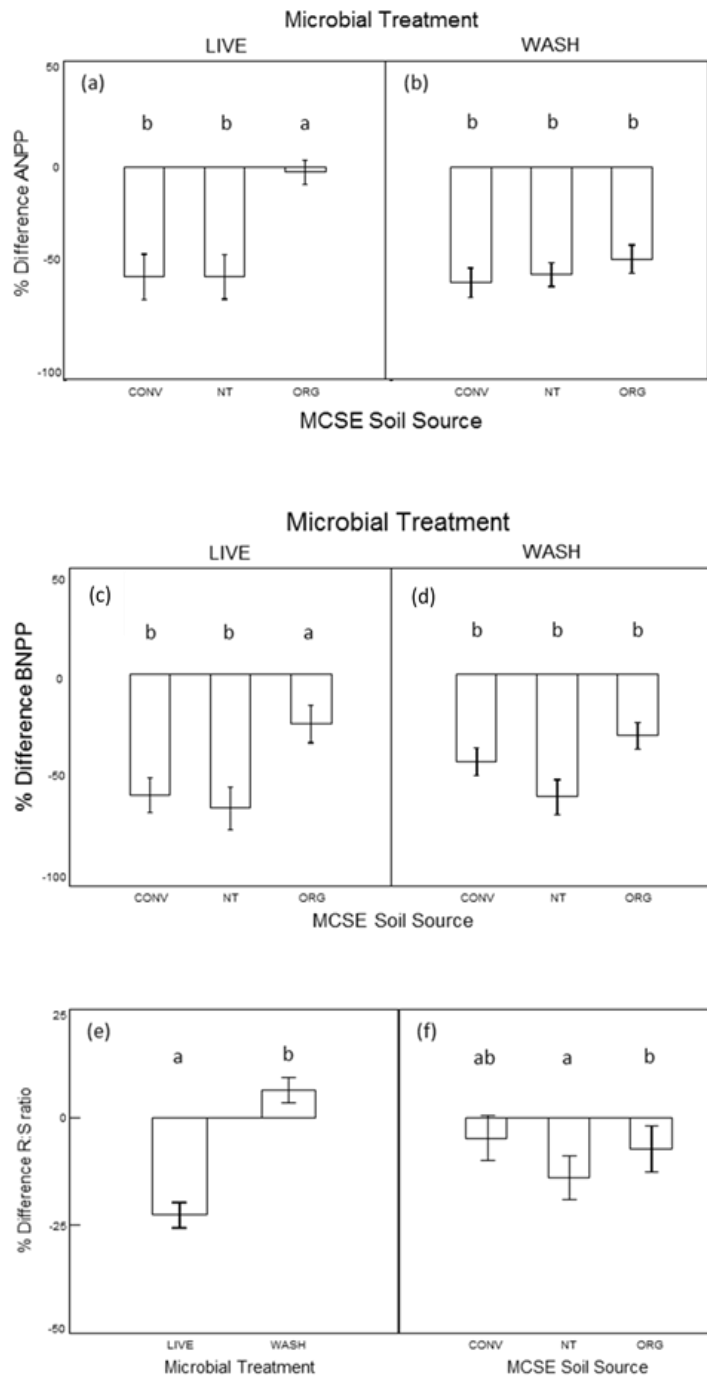


Figure 2.



CHAPTER V

RESEARCH SUMMARY

These studies represent both long term and short term effects of different row crop agricultural management systems on AMF community composition, structure and function, and contributed ecosystem services. By examining the long-term effects following conversion from conventional management, we found tillage was driving differences in AMF community composition and structure; no-till and the early succession systems harbored similar communities. No-till management increased AMF diversity and stabilized the community, but complete abandonment of agricultural management improved ecosystem services. The often reported negative effects of conventional row crop management on AMF diversity were not apparent, and organic row crop management did not improve AMF diversity, richness, or community function. Ecosystem services were limited under all row crop systems and more than 20 years of agricultural abandonment did not restore the AMF community. Overall, the more sustainable row crop management systems (no-till, reduced input, and organic) did not have the expected positive effects on the AMF community or ecosystem services.

CURRENT AND FUTURE RESEARCH INTERESTS

My current research interests include completion of the molecular analyses which complement research reported in chapter one and chapter two. Analysis of rDNA sequences from AMF spore samples taken from the archival soil samples (Ch. 1) will

provide insight as to the AMF community in this system beginning in 1989. When coupled with spore morphological data, sequence data will help provide more accurate baseline richness and diversity measures. Completing the analyses of paired-end Illumina sequencing data for the samples from 2010 – 2012 (Ch. 2) will allow comparison between the AMF community “pool” and the AMF species actively colonizing the crop plant roots. The functional roles of individual AMF species in the community are not well understood. I think being able to compare between the “active” and “non-active” AMF may help explain why some AMF communities are more effective at providing plant benefits and or ecosystem services. This type of information is important to farmers interested in managing their AMF communities with cover-crops, or optimizing crop rotations to provide maximum benefit.

My future interests include working to better understand C sequestration in agricultural soils, which has been suggested as one way to off-set carbon dioxide and methane emissions from these systems. I am also interested in gaining a better understanding of glomalin and exploring differences in production between AMF species. Also, advances in sequencing technology have simplified the complex study of temporal and spatial differences in AMF communities due to management inputs or other disturbances. This technology can be used to improve understanding of AMF functioning at the community and species level, and their relative contributions to soil C and plant growth.

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CURRICULUM VITAE

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EDUCATION:

University of Louisville	Biology (EEB)	Ph.D.	Anticipated Spring	2015
Dissertation title: Effects of Different Agricultural Management Systems on Arbuscular Mycorrhizal Fungal Diversity, Community Structure, and Ecosystem Services. Dissertation advisor: Sarah Emery				
University of Louisville	Biology	B.A.		2010

PUBLICATIONS:

Emery, S.M., J.A. Masters, S. Benanti, and C.B. Gottshall. In Press. Patterns of trophic-level diversity across a primary successional gradient. *American Midland Naturalist*.

PUBLICATIONS, submitted:

Gottshall, B. and S.M. Emery. Submitted. Sustainable agricultural management alters soil microbial effects on crop growth. *Applied Soil Ecology*

PUBLICATIONS, in process:

Gottshall, B., Monica Cooper, Phung Nyguen, and S.M. Emery, (title) Long-term responses of arbuscular mycorrhizal communities following conversion to more sustainable row crop agricultural management systems. Target journal: *Agriculture, Ecosystems and Environment*

Gottshall, B. and S.M. Emery, (title) Responses of arbuscular mycorrhizal fungi to different agricultural management systems include altered community composition and ecosystem services. Target journal: *Journal of Applied Ecology*

PRESENTATIONS:

National and International

2014: Gottshall, B. and S.M. Emery, “Legacy effects of agronomic management at the Kellogg Biological Station LTER”, Ecological Society of America, Annual Meeting, Sacramento, CA (contributed talk)

2012: Gottshall, B. and S.M. Emery, “Ecological function of microbial communities present in KBS-LTER agronomic treatment soils under low nutrient and drought conditions”, LTER Network All-Scientists Meeting, Estes Park, CO (poster)

Gottshall, B. and Christine Sprunger, “Perennialization in urban, peri-urban, and rural landscapes to enhance ecosystem services”, LTER Network All-Scientists Meeting, Estes Park, CO (working group co-organizer)

Gottshall, B. and S.M. Emery, “Legacy effects of agricultural management at the Kellogg Biological Station”, Soil, Biodiversity, and Life – Post-Graduate Course via PE-RC, SENSE, and RSEE | EU-EcoFinders Annual Meeting, Wageningen, The Netherlands (poster)

Regional

2013: Gottshall, B. and S.M. Emery, “Ecological function of microbial communities present in KBS-LTER agronomic treatment soils under low nutrient and drought conditions”, KBS LTER annual meeting, East Lansing, MI (poster)

2011: Gottshall, B. and S.M. Emery, “Arbuscular mycorrhizal fungi abundance in annual and intermediate wheat trials”, 1st Annual International Perennial Wheat Research Group Meeting, Kellogg Biological Station, MI (poster)

GRANTS:

Snapp, S. (PI), S.M. Emery (Co-PI), and C.B. Gottshall (Co-PI). 2012-14. Organic management effects on individual species of mycorrhizal fungi, and the consequences for plant performance and soil health. Ceres Trust. Funding notification Nov. 2011. (\$119,197 total; \$41,479 to UofL).

Gottshall, B (PI). 2010-2011. Effects of disturbance on mycorrhizal mutualisms, abundance and diversity in agroecosystems. KBS-LTER Small Graduate Grant. Funding notification June 2010. (\$1500)

ADDITIONAL EDUCATION/RESEARCH EXPERIENCES

2014: ESA annual meeting: Structural Equation Modeling Workshop, course participant

2013: KBS-LTER Annual meeting, group participant: “Communicating Science in Politicized Environments”

2012: LTER Network All Scientists Meeting working group participant: “Perennialization in Urban, Peri-urban, and Rural Landscapes to Enhance Ecosystem Services”

2012: “Soil, Biodiversity, and Life: The contribution of soil to sustainability of life”, Univ. Wageningen, The Netherlands, short course participant

2011: KBS/MSU Perennial Wheat Research Group, participant

2010: Soil Metagenomics Short Course, course participant, KBS/Michigan State University

TEACHING:

2010 – Present: Teaching assistant, BIOL 244: Principles of Biology Laboratory and BIOL 104: Laboratory for Introduction to Biological Systems

OUTREACH:

Breckenridge County 4H Science Day, volunteer, 2015

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Louisville Youth Science Summit, Louisville Science Center, volunteer, 2013

PROFESSIONAL SOCIETIES MEMBERSHIP:

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