Crop-Specific Management Impacts on Soil Aggregation and Microbial Communities in a Wisconsin Organic Grain Rotation

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A thesis submitted to fulfill partial requirements for the degree

Masters of Science
(Plant Pathology and Agroecology)

University of Wisconsin - Madison

2017

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Abstract

Soil aggregation, a process mediated by the soil microbial community, is a primary mechanism by which soil organic carbon (SOC) is stabilized and potentially sequestered. SOC benefits many aspects of overall soil quality, including soil nutrient availability, water holding capacity, structural stability, and crop productivity. Intensive agricultural management practices, including tillage, are known to decrease SOC content which reduces soil quality over time. Organic agriculture remains largely dependent on tillage to control weeds and optimize yields. With increasing organic acreage on the landscape in recent years, ensuring that these production methods maintain or improve SOC content, and therefore soil quality, is crucial to agricultural sustainability. As such, understanding the means by which SOC is stored and stabilized in the long-term, and how these processes respond to various agricultural management, is important. This study investigates how factors involved in SOC storage, including soil aggregation, aggregate C content, and microbial community composition, are affected by management practices specific to individual crop phases in an organic cash grain system. The cropping system we studied is a three-year organically managed rotation of corn (*Zea mays* L.), soybeans [*Glycine max* (L.) Merr.], and winter wheat (*Triticum aestivum* L.). The winter wheat phase of the rotation is followed by a mixed oat (*Avena sativa*

L.)/berseem clover (*Trifolium alexandrium* L.) cover crop. This work was performed at the Wisconsin Integrated Cropping Systems Trial (WICST) in Arlington, Wisconsin, U.S. Soil aggregate distribution was determined by calculating proportions of six aggregate fractions within the whole soil that differed by size and physical location. Aggregate fractions included macroaggregates (M; >250µm), free microaggregates (m; 53-250µm), free silt and clay (s+c; <53µm), and three fractions occluded within the M fraction: coarse particulate organic matter (cPOM; >250µm), occluded microaggregates (Mm; 53-250µm), and occluded silt and clay (Ms+c; <53µm). Of the three crop phases studied, the soil in the corn phase in one year sampled and both the corn and soybean phases in the following year was the most aggregated, as indicated by a higher proportion of the M fraction than the wheat phase in both years. This high level of aggregation was observed despite intensive tillage and cultivation practices for weed management in the corn and soybean phases, which is known to decrease soil aggregation. Aggregation in the corn phase may have benefitted from high carbon (C) inputs in the form of crop residues and animal manure relative to other crop phases, providing evidence that sufficient C input may counteract the negative impacts of tillage on the process of aggregate formation. As an additional driving factor behind the soil aggregation process, microbial community abundance and structure was also investigated. Total biomass and fungal to bacterial ratio (F:B) were both greater in the wheat phase compared with the corn and soybean phases in 2015 when sampling time across crop phases was the same. Abundance of gram-positive bacteria (Gm+), gram-negative bacteria (Gm-), and actinomycetes, however, were lower in wheat versus corn and/or soybean phases in 2015. Higher fungal abundance in the wheat phase of 2015 was mainly driven by a larger abundance of

arbuscular mycorrhizal fungi (AMF). Saprotrophic fungi (SF) were more abundant in soybean and corn versus wheat in both years. While higher F:B, AMF abundance, and total biomass in the wheat phase in 2015 may have been due to the presence of a standing cover crop at time of sampling, the larger abundance of AMF did not result in higher aggregation in this crop phase. Although fungi are known to positively impact aggregate formation, greater fungal abundance was not observed in the corn phase in 2014 where the highest level of aggregation was found that year. SF abundance was greater in corn and soybean phases in 2015 when high aggregation was observed in these phases. However, these results were not consistent with 2014 when high SF abundance in soybean did not result in increased aggregation. Thus, we concluded that microbial community composition and abundance of specific microbial ecological groups was not predictive of soil aggregation within a given year. It is possible that a residual effect of cover crops on the composition of the microbial community exists in this system, as the wheat phase transitions to corn the following season and cover crops are incorporated. However, additional sampling events throughout the early growing season are needed to assess in-season shifts in microbial community abundance and associated relationships with soil aggregation. In summary, this work demonstrates that soil aggregation and abundance of specific microbial ecological groups are dynamic with respect to crop phase within a crop rotation. While the convention in many scientific studies is to focus on a single sampling event or crop phase for the comparison of soil characteristics related to cropping systems, this approach may neglect potential variability that exists throughout a rotation, as it assumes soil properties remain constant throughout crop rotations. In order to provide management recommendations that result from accurately describing within-

rotation aggregation and potential SOC capture, more frequent sampling is recommended to provide improved characterization of complex cropping systems. Such analysis will allow targeting of specific vulnerabilities within crop rotations and improve the ability to manage SOC.

Acknowledgements

Time and hard work was contributed by numerous individuals over the course of my Master's project, and as such I would like to take the time to thank them. This thesis, and much of the work that went into my degree, would not have been possible without your effort and support. For that, I am very grateful.

Firstly, I would like to thank my advisor, Erin Silva, for providing the opportunity and support for me to obtain my degree. The Organic Research Program has truly provided an excellent learning experience and has been an inspiration in my continued interest in the agricultural realm. The strong extension focus of Erin's program has shown me that the work we do has an important impact in the farming community, an aspect I hope to bring with me in my future career.

Next, thanks to my committee, including Gregg Sanford, Gary Oates, Ann MacGuidwin, and Matt Ruark. Your willingness and availability to answer questions and stick with the project through multiple iterations of what my Master's work has ultimately turned out to be will be ever appreciated. I owe much thanks in particular to Gregg and Gary, who helped with troubleshooting of methods, interpretation of results, acted as a sounding board for ideas, and provided much-needed words of encouragement at times when I doubted my personal ability to succeed.

Thanks to members of the Silva and Jackson Lab communities, current and former: Anders Gurda, Dylan Bruce, Léa Vereecke, Anne Pfeiffer, Rachel Weil, Alex Lyon, Michelle Keller-Pearson, Charlene Grahn, Ginny Moore, Kalyn Diederich, Greg Richardson, Randy Jackson, Anna Cates, Laura Jach-Smith, David Duncan, Greta Landis, and Adam von Haden. The working environment you helped to create was

supportive, enthusiastic, and often quite fun. In particular, I thank those that have helped along the way to provide presentation feedback and instruction on complicated laboratory equipment and procedures. I am extremely grateful as well to members of the Jackson Lab field crew (and my former co-workers) who were always willing to help with intense sampling needs, and not always in the fairest of weather conditions. This work could not have been completed without the help of Jimmy Sustachek, James Tesmer, Alex Butz, Zoe Andersen, Brittany Faust, Casey Menick, Haley MeLampy, Becca Fahney, and Jessie Ferraro. Thanks are also due to Harry Read, who extracted hundreds of soil samples for microbial community analysis. This was no small task, and I appreciate the weeks' worth of time put in.

The students, faculty, and staff of the Plant Pathology and Agroecology graduate programs created wonderful environments to perform this work. I have made a great deal of lasting friendships and expanded my interests in agriculture and plant disease far beyond what I ever thought possible.

Finally, to my friends, family, and DJ, thank you all so much. The moments, both challenging and enjoyable that we have shared over the past years have given me ability to persevere through, I believe, just about anything. I especially appreciative of the games, libraries, holidays, and adventures together that served as important reminders of the need to, sometimes, relax and take stock of life beyond my role as a Master's student. Thank you all for believing in me when I wasn't always able to do so myself.

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Appendix I

Introduction

This work assesses several measures of soil quality as they are affected by agricultural management in specific crop phases of a long-term rotation. Broadly, soil quality is defined as "the ability of soil to function" with regards to agricultural productivity (crops and livestock), maintenance or improvement of air and water resources, and support of human population health (Karlen et al., 1997). Ensuring that soil quality is maintained or enhanced will be vital if agricultural production is to meet the needs of a growing human population in the years to come, though this task is not easily undertaken. Accurate measurement of soil quality depends on a combined assessment of soil physical (e.g. structure, bulk density, water conductivity), chemical (e.g. available nutrients, pH, organic matter), and biological (e.g. microbial biomass, microbial community composition) properties that can be influenced by agricultural management (Askari and Holden, 2015; Jokela et al., 2011; Karlen et al., 1997).

A major impact of agricultural activity on soil quality is the addition (or depletion) of soil organic matter (SOM) and associated soil organic carbon (SOC). SOC positively affects soil quality by increasing or improving nutrient availability, water holding capacity, soil structure, and crop productivity while reducing negative agricultural externalities such as erosion (Rasmussen et al., 1998; Blanco-Canqui and Lal, 2004; Lal, 2004b). However, intensive agricultural management including land use change and tillage practices have resulted in a net loss of SOC, enhancing erosive processes and increasing the release of CO_2 through oxidation of SOC (Lal, 2004b; Montgomery, 2007; Reicosky et al., 1997). DeLuca and Zabinski (2011) estimated that nearly 50% of SOC has been lost as a result of cultivation from surface soil horizons in

the Midwestern United States when compared with the native prairie soils that once dominated the landscape. Conservation tillage (CT) has been suggested as a means to enhance the soil's ability to sequester SOC, potentially serving as partial mitigation for climate-changing effects of greenhouse gases (GHGs) including CO_2 (Lal, 2004b). Several studies have shown, however, that soils under CT or no-tillage (NT) do not sequester SOC throughout the entire soil profile (Baker et al., 2007; Blanco-Canqui and Lal, 2008; Novak et al., 2009), casting doubts as to whether SOC sequestration may be an effective climate change mitigation tool (VandenBygaart, 2016).

Irrespective of SOC retention to effectively mitigate climate change, it must be preserved if agricultural productivity is to be maintained (Lal, 2004a). One mechanism by which SOC is protected and sequestered over time within the soil structure is through aggregation. The aggregation process begins when primary soil particles (i.e. silt and clay) are pressed together through physical forces imposed by fine plant roots and fungal hyphae. The resulting soil aggregates, termed "macroaggregates", are stabilized by both the root and hyphal physical structure as well as microbe-produced compounds that act as glues, including bacterial polysaccharides and fungal proteins (Bossuyt et al., 2001; Rillig and Mummey, 2006). Within macroaggregates, decomposing particulate organic matter (POM) serves as nucleation sites for the formation of smaller microaggregates (Angers et al., 1997; Chung et al., 2008; Kong et al., 2005). These microaggregates are also stabilized by microbial byproducts in addition to the physical protection afforded by the macroaggregate structure surrounding them (Golchin et al., 1994; Rillig and Mummey, 2006). Aggregate hierarchy theory introduced by Tisdall and Oades (1982) suggests that as soil aggregates become smaller, the binding agents contributing to their stability

become increasingly persistent. As such, microaggregates are far more stable and resistant to turnover than macroaggregates. Thus, long-term SOC storage is thought to take place within microaggregates (Jastrow 1996; Six et al. 2004), though this would not be possible without macroaggregates, which provide the formation site for stable microaggregates (Angers et al., 1997; Oades, 1984). Macroaggregates are much more vulnerable to turnover due to the transient nature of their binding agents (Tisdall and Oades, 1980, 1982), and are particularly disrupted when exposed to tillage (Grandy and Robertson, 2006).

As the main drivers of soil aggregation, and with related impacts on SOC sequestration, the soil microbial community is an important factor in soil quality. In general, increased microbial biomass and fungal abundance increases aggregate formation (Bossuyt et al., 2001; Lucas et al., 2014). While a reduction in fungal abundance produces the opposite effect, reducing bacterial abundance has not been shown to reduce aggregation (Bossuyt et al., 2001). This suggests microbial community composition is an important determinant in the formation of soil aggregates and SOC sequestration. Both agricultural management and inherent edaphic properties are known to influence abundance and composition of the soil microbial community. Soil bacterial community composition tends to be most heavily affected by soil type, while fungal community composition is often driven by nutrient availability/fertilization (Girvan et al., 2003; Lauber et al., 2008; Suzuki et al., 2009). Disturbance can also affect community abundance and composition; conventional tillage practices are known to reduce total microbial biomass as well as fungal abundance, while shifting dominance in the community to bacteria compared with CT or NT (Ghimire et al., 2014; Wang et al., 2010;

Wortman et al. 2013). While altering the microbial community can indirectly affect SOC sequestration through reduced aggregate formation, microbes may also affect SOC stocks more directly. Microbially-derived compounds likely comprise a significant portion of stable SOM (Kallenbach et al., 2015, 2016; Liang and Balser, 2008; Simpson et al., 2007), potentially reducing SOC content in agricultural soils exposed to tillage practices known to disrupt and reduce abundance of certain microbial groups.

With all of the evidence related to the negative effects of conventional tillage practices on SOC content and storage, it may seem that a switch to CT or NT practices would provide a simple solution to the SOC sequestration challenge. However, such a paradigm shift is not possible with all facets of agricultural production. For example, organic agriculture largely depends on tillage practices for weed control, with yield competitiveness strongly related to successful weed management (Cavigelli et al., 2008, 2013; Posner et al., 2008). As a growing market, organic production in the U.S. has increased dramatically since the establishment of the USDA's National Organic Program (NOP) standards in 2002. Cropland acreage under organic production in the United States grew from under 1 million acres in 1997 to over 3.5 million acres in 2013 (Greene, 2013; USDA NASS). Rising consumer demand and higher price premiums for organically-produced food have contributed to this increase. Growth in organic food sales has risen consistently as well, from around $10 billion in 2004 to over $35 billion in 2013 (Greene, 2013). As organic acreage in the U.S. continues to increase, ensuring that agricultural management in these systems maintains or improves soil quality is crucial in order for these systems to contribute to long-term SOC storage.

In organic agriculture, soil-building and SOC gains are accomplished primarily through the addition of organic materials. Organic farmers commonly incorporate organic inputs or mitigate SOC losses through use of cover-cropping, animal manures, diverse crop rotations, and judicious use of tillage (Cavigelli et al., 2013; Diacono and Montemurro, 2010; Silva, 2014). Cover crops promote SOC accrual through both above and belowground biomass inputs (Rasse et al., 2005). Animal manure additions can also increase soil SOC content (Foereid and Høgh-Jensen, 2004; Williams and Petticrew, 2009). Increasing cropping system diversity through rotation enhances soil fertility and crop yields by reducing pest and weed pressure, promoting nutrient cycling, and increasing microbial biomass and activity (Watson et al., 2002).

Despite the fact that many soil-building practices are associated with organic production, insufficient use of these practices in organic systems can lead to a decline in SOC content, and therefore, soil quality. A study by Sanford et al. (2012) at the Wisconsin Integrated Cropping Systems Trial (WICST) showed that all cropping systems involving an annual crop phase, including a long-term organic grain rotation had lost significant amounts of SOC to a depth of 90 cm over 20 years. Though this organic system includes use of cover crops, manure application, and crop rotation, it shared the largest loss of SOC observed in the study with a conventional, continuous corn system. Intensive use of tillage and insufficient belowground biomass inputs were cited as the driving factors for SOC loss in the organic grain system. Significant losses of SOC, despite the use of beneficial management practices, highlights a need for a deeper understanding of SOC cycling and storage processes in this organic system. The work

presented here attempts to contribute to that understanding by investigating the SOC storage mechanism (soil aggregation) and its driving factor (the microbial community) on a crop phase by crop phase basis in the organic grain rotation at WICST. By better understanding the nuances of SOC storage and the factors driving this process within a specific cropping system, we hope to identify management practices that will aid in the preservation of this resource in the future.

CHAPTER I

Characterization of Soil Aggregate Distribution and Aggregate Carbon Content in an Organic Cash Grain Rotation in Wisconsin, USA

Abstract

Soil organic carbon (SOC) and soil aggregate stabilization are important contributors to soil health and the maintenance of soil carbon (C) stocks. However, the impacts of specific crop management practices on these factors within a defined crop rotation remain unclear. To better understand the impact of agricultural management on these dynamic soil processes, we assessed two soil parameters: 1) soil aggregate distribution, and 2) soil aggregate C content, to determine if differences could be determined between crop phases in a three-year organic grain rotation. The study was conducted within the organic cash grain rotation at the Wisconsin Integrated Cropping Systems Trial (WICST), a 25-year trial in Arlington, WI, USA. Baseline soils were sampled after harvest of organically managed wheat, soybean and corn in 2014 and 2015. Soil aggregate proportions were characterized with wet sieving techniques, while aggregate C content was measured through flash combustion. We found that while crop phase did not affect overall the aggregate C content within the time frame of our study which we did not expect, crop phase did affect aggregate distribution as determined by the relative proportion of different size classes. Soil from the corn phase in 2014 and both corn and soybean in 2015 showed significantly greater aggregation than soil from the wheat phase of the rotation in either year, despite the intensive use of tillage in both the corn and soybean phases. Soil aggregation was positively correlated with total C inputs in the combined forms of plant biomass and manure which were highest during the

corn phase. This provides evidence that input of C may counteract some negative impacts of tillage on soil aggregation. The impact of sampling time across crop phases on aggregate distribution highlights the dynamic relationship between SOC and aggregate distribution across cropping system phases. A single sampling event may not be adequate to characterize and compare systems under diverse crop rotations, suggesting soil samples collected during each crop phase would more accurately characterize aggregate distribution dynamics. Accurate characterization of sensitive soil properties including aggregate distribution is vital to the development of management recommendations for SOC management.

Introduction

Soil organic matter (SOM) is an extremely valuable constituent of agricultural soils, with benefits for plant nutrient availability, water holding capacity, soil structure, and crop productivity while reducing erosion (Rasmussen et al., 1998; Blanco-Canqui and Lal, 2004; Lal, 2004b). Soil organic carbon (SOC), a component of SOM, is not only vital to agricultural productivity and sustainability (Lal, 2004a), but also in determining the soil's potential to serve as a carbon reservoir. SOC is preserved in the soil through chemical stabilization, biochemical recalcitrance, and, most critically, physical protection within soil aggregates, which differ in their level of persistence over time dependent on their size and nature of associated SOM binding agents (Six et al., 2000a; Tisdall and Oades, 1982).

The aggregate hierarchy theory, introduced in 1982 by Tisdall and Oades, suggests that soil structure is a result of aggregation occurring on a range of spatial

scales. The smallest soil particles, the silt and clay fraction (< 53 μm), contains the most stable SOC. The next most-stable is SOC that is bound to and physically protected as partially degraded organic matter within microaggregates (53-250 μm), held together with persistent microbial products including glomalin (Oades, 1993; Oades and Waters, 1991; Rasmussen et al., 2005). Larger macroaggregates (> 250 μm), which are bound together with more temporary binding agents such as fine roots and fungal hyphae, contain SOC that is more vulnerable to microbial decomposition, as these less persistent binding agents can more readily be degraded or disturbed (Jastrow, 1996; Oades and Waters, 1991; Tisdall and Oades, 1982).

Macroaggregates play a critical role in SOC sequestration, contributing to physical protection of soil carbon, providing a site for formation of stable, occluded microaggregates (Angers et al., 1997; Jastrow, 1996; Oades, 1984). As new C inputs enter the soil in the form of plant residues or microbial byproducts, they are stored preferentially within large soil macroaggregates, and eventually serve as nuclei for microaggregate formation (Chung et al., 2008; Kong et al., 2005). These microaggregates are less susceptible to carbon turnover induced by agricultural management and have more persistent binding agents than macroaggregates (Blanco-Canqui and Lal, 2004; Lal, 2004b; Rasmussen et al., 2005). Both occluded and freely existing microaggregates are the means by which SOC is sequestered long-term in soils.

As macroaggregation is a major influence on SOC sequestration, it follows that disturbance of these sensitive particles is a significant source of SOC losses from soil. Indeed, macroaggregates experience a rate of turnover that is greater than any other soil fraction due to the impermanent nature of their binding agents (Tisdall and Oades, 1980,

1982). As these binding agents are decomposed by soil biological activity, macroaggregates are made more vulnerable to breakdown, exposing the microaggregates contained within (Oades, 1993; Six et al., 2000a). As aggregates are disrupted, the SOM contained within is made available as a source of energy for soil microbes, and converted through respiration to CO_2 and released to the atmosphere (Rovira and Greacen, 1957; Six et al., 1998).

Agricultural management of soils, particularly disturbance with tillage, is a major contributor to macroaggregate turnover, and therefore SOC losses. Many studies have demonstrated the impacts of tillage on soil aggregation. No-till (NT) agricultural practices have been shown to preserve or enhance soil aggregation and SOC content at depths of up to 30cm, compared with conventionally-tilled (CT) soils (Baker et al., 2007; Sheehy et al., 2015; Six et al., 1998, 2000a). In addition, studies comparing previously undisturbed soils with soils under long-term agricultural use found that a single tillage event reduced the level of soil aggregation (amount of macroaggregates) to that of long-term agricultural soils, emphasizing the speed at which soils under agricultural use may become degraded (Grandy and Robertson, 2006; Tisdall and Oades, 1980). While SOC losses from land managed with reduced-till practices may be slowed through increased soil aggregation, whether a reduction in tillage can significantly increase amounts of SOC over time in the entire soil profile versus surface layers alone still remains unclear (Blanco-Canqui and Lal, 2008; Lal, 2004b; Novak et al., 2009).

While certain cropping systems practices, such as tillage, can negatively impact soil aggregation and SOC content, other practices can provide positive contributions to the increase in SOC and C sequestration. Systems with high organic matter additions

have been shown to increase structural stability (enhanced aggregation), SOC content, and improve productivity over those with lower additions (Blankinship et al., 2016; Jokela et al., 2011; Kong et al., 2005). Belowground organic matter inputs, such as plant roots and their exudates, can also contribute to the overall organic matter of a system, and are especially beneficial for enhancing SOC (Campbell et al., 1991; Rasse et al., 2005; Sanford et al., 2012). Changes in SOC content can be detected following a change in SOM inputs, as C content of the various soil aggregate fractions is sensitive to management changes (Crittenden et al., 2015; Doane et al., 2003; Whitbread et al., 2000). This sensitivity makes soil aggregates strong indicators of SOC dynamics (Denef et al., 2007).

The organic industry in the United States (U.S.), included a total of 14,093 farms in 2014 (USDA NASS; USDA ERS). The 2014 national agricultural survey of organic production conducted by the United States Department of Agriculture (USDA) reported 203,438 acres of organic corn and 98,832 acres of organic soybean among over 3.5 million organic cropland and vegetable acres in the U.S. (USDA NASS). Organic management also requires farmers to specifically incorporate soil building practices such as cover cropping and diverse crop rotations, as set forth in 7 CFR § 205.203 and 205.205 of the National Organic Program (NOP) (USDA-AMS NOP), making the use of organic inputs in these farming systems commonplace.

As organic management practices routinely incorporate organic inputs through cover cropping and animal manures, organic systems could serve as a means to achieve gains in SOC (Cavigelli et al., 2013; Diacono and Montemurro, 2010). Several studies comparing organic and conventional production methods have demonstrated the benefits

of organic systems for increasing SOC content and maintaining or improving soil structure (Foereid and Høgh-Jensen, 2004; Gerhardt, 1997; Williams and Petticrew, 2009). However, studies conducted at the Wisconsin Integrated Cropping Systems Trial (WICST) in Arlington, WI do not support these findings. Sanford et al. (2012), for example, found that an organic grain rotation at WICST lost 5 Mg SOC ha^{-1} to a depth of 91 cm over a 20-year period, comparable to the losses observed in the conventionally tilled and managed continuous corn system. In a related study, Cates et al. (2016) found that this same organic grain rotation had lower levels of aggregation when compared with other cropping systems in the trial, despite the integration of beneficial soil management practices over 20 years, which included diverse crop rotation, cover crops, and more recently, additions of a pelletized composted poultry manure for nearly a decade. Though it has been previously indicated that soils with high background levels of SOC, such as the prairie-derived soils at WICST, may not sequester C as easily as those with lower background SOC (Peichl et al., 2010), the results of these studies emphasize that SOC dynamics and soil aggregate stability as impacted by integrated cropping strategies are still poorly understood, particularly in organic cropping systems. As such, further investigation of the interactions between crop management practices, including crop rotation, fertility inputs, and intensified cover crop use, on SOC dynamics and soil aggregate stability is greatly needed, providing a better understanding of the impacts of organic agricultural management on these soil quality indicators (Lichtfouse et al., 2010; Morgan et al., 2010).

To address the need to understand the impacts of organic management on soil organic carbon and aggregation, and to develop practices and rotation strategies to enhance these factors, a multi-year experiment was begun at WICST to evaluate the impact of increased carbon inputs on SOC dynamics in the organic grain rotation. Treatments include combinations of cover crop and tillage/cultivation strategies designed to increase organic matter additions and retention across each crop phase. Ongoing work will more specifically address the impact of carbon additions due to inputs from cover crops, however, this experiment brought to light important trends in soil aggregate distribution as it relates to crop phase. As a result of these findings, the objective of the current study was developed.

The aim of this study is to assess the associations between soil aggregate distribution and the aggregate C content of differing aggregate size classes with crop phase in the long-term organic grain rotation at WICST. We hypothesized that 1) crop phases with increased organic matter inputs will exhibit a greater proportion of soil aggregates in the macroaggregate size class as compared to phases with lower organic matter inputs; and 2) observed differences in aggregate distribution by crop phase will result in reallocation of SOC among soil aggregate size fractions. Reallocation will lead to shifts in aggregate C content in aggregates of the same size resulting from short-term changes in agricultural management, such as crop phase differences within a rotation.

Materials and Methods

Site Description

The study was conducted at the Wisconsin Integrated Cropping Systems Trial

(WICST), located at the University of Wisconsin Arlington Agricultural Research Station in Columbia County, WI (43˚18'18"N, 89˚19'48"W). The soil at WICST is classified as a Plano silt-loam, a Fine-silty, Mixed, Superactive, Mesic Typic Argiudolls (USDA NRCS Soil Taxonomy) formed over a parent material of alluvial loess deposits. Average annual temperature at the Arlington Agricultural Research Station is 6.9˚C, with an average minimum of 0.5˚C and an average maximum of 13.3˚C. Annual precipitation averages 869 mm, with the majority (64%) occurring in the spring and summer (1981-2010, National Climatic Data Center). WICST, established in 1990, is a long-term cropping systems trial designed to assess the productivity, profitability, and environmental impacts of two different agricultural enterprise types under varying crop diversity levels (Posner et al., 1995). These two enterprise types represent cash-grain as well as dairy forage production strategies commonly found in Wisconsin and the upper Midwest. Prior to cultivation (ca 1850), deep-rooted tallgrass prairie vegetation was dominant in the region. In the mid-1800s, the predominant agricultural use was continuous wheat production which later shifted to livestock feed as the dairy industry of Wisconsin grew. From the 1960s until its establishment, the WICST site had been cultivated with an alfalfa (*Medicago sativa* L.)-corn (*Zea mays* L.) rotation, using dairy manure as a nutrient source (Posner et al., 1995). In 1989, the trial was planted to corn to achieve soil homogenization and improve blocking accuracy based on yield variability.

WICST is a randomized complete block design consisting of four replications of each of six cropping systems. All phases of each cropping system are present in a year. Of the four total replicates present at WICST, three were sampled in this study as one

replicate (block two of four) has consistently demonstrated soil properties inconsistent with those of the other three replicates and is susceptible to frequent flooding, potentially adding additional compounding factors to soil properties. Therefore, block two is not a good representation of the site as a whole (G.R. Sanford, personal communication, 2016). The six cropping systems at WICST represent cash-grain and dairy forage enterprises, with conventional and organic representatives of each. Plots are 0.3 ha in size. Our study was carried out in just one of these six cropping systems, an organically-managed cash-grain rotation. This rotation is three years in length, with a crop sequence of corn, soybean [*Glycine max* (L.) Merr], and winter wheat (*Triticum aestivum* L.). The winter wheat phase includes a mixed oat (*Avena sativa* L.) and berseem clover (*Trifolium alexandrium* L.) cover crop planted after wheat is harvested. This gave us three replicates with three crop phases per replicate, or nine experimental plots total.

Annually, the organic cash grain cropping system receives an application of composted pelletized poultry manure in spring of both the corn and wheat phases. The corn phase receives poultry manure at a rate of 4.48 Mg ha^{-1} while the wheat phase receives 2.24 Mg ha^{-1}. A combination of tillage and cultivation methods are used for field preparation, weed control, and post-harvest field management. Specific tillage and cultivation activities, and the timing of activities, can be found in Figure 1.1. All crop phases are field cultivated prior to planting. Corn and soybean phases receive several rotary hoe or tine-weeding events for weed control, as well as several in row cultivations. Plots are chisel plowed in fall after corn and soybean harvest, and in summer after wheat harvest prior to cover crop planting. Cover crops are terminated during the field cultivation event preceding corn planting in spring. At minimum, corn and soybean

phases receive six tillage and/or cultivation events during the year, and the wheat phase receives two to three.

Tillage and Cover Crop Split-Plot Treatments

This study was focused on the impact of specific crop phases on soil aggregate distribution and associated aggregate C content. However, during the years we sampled, an experiment designed to evaluate the impact of increased cover crop intensity and decreased tillage intensity on organic row crop systems' ability to store soil C and microbial community composition was overlaid on our sampling area. Six treatments of varying cover crop and tillage intensities were randomly applied in a split-plot design with 4.6 m x 4.6 m split plots overlaid on each of the nine main plots. The experimental treatments consisted of varying combinations of biomass additions to the soil through intensified use of cover crops at specific points in the rotation, as well as reduced tillage in certain portions compared with control treatments which did not include additional cover crops or reduction of tillage. Specifics of split-plot treatments applied can be found in Table 1.1. Intensification of cover crop usage included planting a cereal rye (*Secale cereale* L.) after harvest of the corn phase in place of a no cover crop control, and using a high-biomass sorghum (*Sorghum bicolor* L.) after the wheat phase instead of the control oat cover crop. Both the sorghum and oat cover crops following the wheat phase were interseeded with berseem clover. Oat and sorghum cover crops were terminated with tillage prior to corn planting. Tillage reduction using rolled-crimped rye was implemented prior to the soybean phase, with experimental subplots receiving no tillage and being planted to soybean with a no-till drill. In treatments that did not include no-till

soybean and a standing rye cover crop remained, rye was terminated with tillage prior to soybean planting. For a visual representation, see Figure 1.2.

Soil Sampling

Baseline soils were sampled after harvest of wheat, soybean and corn in 2014 (July, September, and November respectively). In 2015, soil samples from each treatment were collected in late October at the end of the growing season, following tillage and planting of the rye cover crop in the corn phase, planting of wheat in the soybean phase, and standing cover crop senescence in the wheat phase. At each sampling event, five soil cores 15 cm deep and 1.9 cm in diameter were composited per sub plot, and placed immediately in a cooler until they could be stored at 4˚C. Within two weeks of sampling, each sample was picked free of visible plant residues and rocks prior to further processing which included sieving to 2 mm. Samples were stored frozen at -20˚C to retain field moisture as well as viability of microbial residues to be used in community analysis, the applications of which are covered in Chapter II.

Aggregate Fractionation

Wet-Sieving (Macroaggregates, microaggregates, and silt and clay)

Samples were removed from the freezer and placed in a refrigerator at 4˚C to thaw for a minimum of 24 hr before aggregate fractionation. A small amount (15-20 g) of each sample was removed and dried in a 60˚C oven until the soil weight stabilized to determine volumetric moisture content. The aggregate fractionation technique used is a simplified version of that employed by Six et al. in 1998 (Figure 1.3, step A), and similar to the method in Cates et al. (2016). A 250 µm mesh sieve was set within a large metal

basin. Deionized water (DI) was added to the basin until the water level was approximately 2 cm above the sieve's mesh. An 80 g subsample was carefully sprinkled onto the 250 µm mesh sieve. The soil was allowed to soak for 5 min, then the sieve was moved up and down in the water-filled basin approximately 3 cm at a slight angle at a rate of 25 times per min for 2 min. Material that washed through the sieve was collected in the large metal basin, and any particles stuck to the outside of the sieve were carefully washed into the basin with a squeeze bottle containing DI. Macroaggregate (M) soil particles (>250 µm) remaining in the sieve were washed into a pre-weighed aluminum pan for drying at 60˚C. The soil particles and water that were retained in the first basin were poured into a 53 µm mesh sieve nested within a second large metal basin. If the water level was not sufficient, DI was added until the water level was 2 cm above the sieve's mesh. The 53 µm sieve was then moved up and down approximately 3 cm at a slight angle 25 times per min for 2 min. Again, material that had washed through the 53 µm mesh was collected in the second metal basin and fine soil particles stuck to the outside of the sieve were carefully washed into the basin. Microaggregate (m) soil particles (53-250 µm) remaining in the sieve were rinsed into a pre-weighed aluminum pan for drying at 60˚C. The smallest silt and clay (s+c) soil particles (<53 µm) that had collected in the second basin were also rinsed into their own pre-weighed aluminum pan for drying at 60˚C.

Microaggregate Isolation (Coarse particulate OM, occluded microaggregates, and occluded silt and clay)

The M fraction was further separated into coarse particulate organic matter (cPOM), occluded microaggregates (Mm) and occluded silt and clay (Ms+c) (Six et al.,

2002; Figure 1.3, step B). From each dried M sample, 15 g of soil were placed in a beaker containing approximately 50 mL of DI for 20 min. After soaking, the slurry was poured into a microaggregate isolator (as described in Six et al., 2002; built by David Sloan, UW-Madison Department of Soil Science) containing a 250 µm mesh circle with 50 metal beads at the bottom. The isolator, with a constant but slow flow of DI, was shaken for 5 min to disrupt the macroaggregate structure, allowing fine soil particles to move through the mesh and collect in a (third) metal basin. Once shaken, the 250 µm mesh was rinsed, and cPOM (>250 µm) was collected in a pre-weighed aluminum pan for drying at 60˚C. The soil particles and water that passed through the 250 µm mesh circle and into the third metal basin were poured into a 53 µm mesh sieve nested within a final (fourth) metal basin. If the water level was not sufficient, DI was added until the water level was 2 cm above the sieve's mesh. This sieve was moved up and down at a slight angle 25 times per min for 2 min. Soil particles passing through the sieve but remaining on the outside of the sieve were rinsed carefully into the fourth basin. The 53-250 µm Mm fraction remaining in the 53 µm sieve were washed into a pre-weighed aluminum pan for drying at 60˚C. The Ms+c fraction (<53 µm) and water that collected in the fourth basin were rinsed into a pre-weighed aluminum pan for drying at 60˚C. For a visual representation of fractionation setup and equipment, see Appendix 1.

Aggregate proportion calculations

Once all fractions were separated and dried in a 60˚C oven until their weights were stable, they were each weighed in the aluminum pan and placed in Whirl-Pak™ bags for short-term storage. Dry soil weight was calculated by subtracting the known

weight of the pan from the total weight after drying. Aggregate distribution was determined by calculating the summed total weight of all dried fractions from one subplot sample, and dividing each fraction's weight by the sum total to obtain the proportions. Proportions of soil aggregate fractions were not independent of one another, as proportions in each sample were calculated from the same total dry soil weight and must total that dry weight. A larger proportion of one soil fraction would lead to lower proportions of other fractions. Free aggregate fraction proportions (M, m, s+c) were calculated as follows:

$$free\ aggregate\ prop. = \frac{free\ aggregate\ dry\ wt.}{total\ step\ A\ soil\ dry\ wt.}$$

Proportions of fractions occluded within M (cPOM, Mm, Ms+c) were calculated as follows:

$$occluded\ aggregate\ prop. = \frac{occluded\ aggregate\ dry\ wt.}{total\ step\ B\ soil\ dry\ wt.} \times M\ prop.$$

Aggregate SOC Content

Small (~100 mg) samples of each dried soil fraction were collected into 2 mL microcentrifuge tubes containing a 4 mm stainless steel ball bearing. The tubes were ball-milled for 15 min until all samples were ground to a fine flour like powder. After shaking, 8-10 mg of each sample was measured into a 9 x 5 mm tin capsule. Tins were analyzed by flash combustion using a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy) to assess C content of each sample. As there is not an appreciable amount of inorganic C present in the soil at WICST, the entire concentration of C determined through this analysis is assumed to be SOC (Paul et al., 2001).

Estimation of C Inputs

Estimates of C inputs for 2014 and 2015 were calculated using methods employed by Jokela et al. (2011), Sanford et al. (2012), and Cates et al. (2016). Aboveground C inputs were calculated based on existing harvest indices and belowground inputs from existing root:shoot ratios and rhizodeposition estimates (Bolinder et al., 2007). Green manure inputs in the wheat phase and manure moisture content were not collected in 2014 and 2015. For these years, green manure inputs and manure moisture content were estimated using average values of data collected between 2009-2013 from Jokela et al. (2011), Sanford et al. (2012), and Cates et al. (2016). Aboveground C inputs were classified as post-harvest crop residues and any green or animal manures used, while belowground C inputs consisted of root residues as well as root exudates.

Statistical Analysis

Aggregate distribution and aggregate carbon content data were analyzed separately by soil fraction using an RCBD in PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc., Version 9.4, Cary, NC). For the purpose of this study, we were primarily concerned with the main effect of crop phase on soil aggregate distribution and aggregate carbon content. This study was carried out over a period of two years instead of a single year, therefore we tested the potential effects of year. Our full model included crop, year, and the crop × year interaction as fixed effects. Initial statistical analyses that included the six split-plot treatments of the overlaid experiment described in Table 1.1 did not significantly affect aggregate distribution and had minimal effects on aggregate C content that may also be explained by spatial variability. However, removing these split-plot treatment effects from our models entirely resulted in residual heteroscedasticity.

Therefore, split-plot treatments were included as covariates in the general analysis of the main effect of crop phase. These treatments will be henceforth referred to as covariates. Inclusion of covariates increased residual homoscedasticity relative to the model excluding this covariate. Block and the block × crop interaction were included as random effects. Ultimately, two response variables in the GLIMMIX models were considered: soil aggregate distribution based on aggregate dry weight proportions and soil aggregate C content.

As the year × crop phase interaction was significant in many cases, the SLICE and SLICEDIFF statements were included in the LSMEANS line of code to separate out effects of crop phase for each individual level of year (2014 and 2015). Slicing by year was done as the sampling time differences between the two years was a likely source of the significant year × crop phase interaction and comparing the two years directly with these sampling time differences was not possible. Slicing by year allowed for an in-depth investigation as to why crop phase interactions with year may have been significant. Transformation of the response variables was only used where necessary to fit assumptions of normality. It was determined that for all models of aggregate distribution, a normal (Gaussian) distribution provided the best fit. The log-normal distribution (log-transformation of experimental data) provided the best fit for all models analyzing aggregate carbon content. Means obtained through the log-normal distribution cannot be back transformed in PROC GLIMMIX to meaningful estimates of actual experimental means. Interpretation of the aggregate carbon content data is therefore limited to relative comparisons between crop phases. A Tukey adjustment was performed to more accurately separate means and limit type I error at the $\alpha = 0.05$ level.

To determine the strength of the relationship between crop phase C inputs and macroaggregation, PROC CORR and PROC REG were used in SAS version 9.4. Correlation coefficients were calculated using proportion of macroaggregates and crop phase C inputs as variables. A linear regression was performed with proportion of macroaggregates as the dependent variable and total crop phase C inputs as the independent variable. Data was checked for influential points using Cook's distance and residuals vs. fitted plots.

Results

Growing Conditions

Average annual temperature at Arlington, WI in 2014 was 7.1˚C, with a minimum of 1.7˚C and a maximum of 12.4˚C (NOAA Online Weather Data, National Weather Service). Overall, temperatures during the growing season (April-October) were within the normal observed range while winter temperatures (January-March) were below the long-term average from 1981-2010. Annual precipitation in 2014 totaled 897 mm, just above the long-term average of 869 mm (NOAA Online Weather Data, National Weather Service). Accumulation of precipitation during the mid-late growing season (June-October) was above normal. In 2015, the average annual temperature was 8.9˚C, with a minimum of 3.6˚C and a maximum of 14.3˚C (NOAA Online Weather Data, National Weather Service). Like 2014, the majority of the growing season (April-October) fell within the normal temperature range, while winter temperatures (January-February) were cooler than the long-term average. However, the maximum daily temperature rose above the long-term average in many instances. Annual precipitation in 2015 totaled 1006 mm,

well above the long-term average (NOAA Online Weather Data, National Weather Service) but for the majority of the growing season, accumulation totals were below normal. The above-average precipitation in 2015 occurred predominantly after the growing season, in late October-December.

Cropping System Yields and Estimated Above and Belowground C Inputs

Grain yield in the corn phase averaged 10.5 Mg ha^{-1}, the same as the previous 5-year average (2009-2013) of 10.5 Mg ha^{-1}. Soybean yield averaged 3.6 Mg ha^{-1}, slightly larger than the previous 5-year average of 3.2 Mg ha^{-1}. Grain yield for wheat was only available in 2015 due to a crop failure in 2014 resulting in the crop harvested for silage. Silage yield from the wheat plots in 2014 averaged 4.34 Mg ha^{-1}. The average wheat grain yield in 2015 was 4.8 Mg ha^{-1}, slightly larger than the previous 5-year average of 4.3 Mg ha^{-1}. Wheat straw yield in 2015 averaged 3.83 Mg ha^{-1}, approximately 30% greater than the previous five-year average of 2.75 Mg ha^{-1}. Yield and additional cropping system information including soil properties can be found in Table 1.2.

Average annual C inputs for 2014-2015 were 7931 kg ha^{-1} total in the corn phase, approximately 2.5 times the total C input the soybean and wheat phases received. Total C inputs in the soybean phase averaged 3211 kg ha^{-1}, while inputs in the wheat phase averaged 3131 kg ha^{-1} annually. Aboveground (AG) C inputs were 2.5 times greater in the corn phase versus soybean and wheat, while belowground (BG) C inputs were approximately 2 times greater for corn over soybean and 3 times greater for corn over wheat. The values of above- and belowground C inputs can be found in Table 1.3.

Soil Aggregate Distribution Across Crop Phases

Crop phase had a significant effect on aggregate distribution in both 2014 and 2015 (Table 1.4, Figures 1.5 and 1.6). The year × crop interaction was also significant in all six soil aggregate fractions. In order to examine the nature of the year × crop interaction, models separated the effect of crop phase over each level of year (2014 and 2015). The proportions of the various aggregate fractions were not independent. Specifically, the abundance of M was correlated to the free and occluded fractions, with increased macroaggregation leading to smaller proportions of other free fractions, and larger proportion of fractions occluded within these large particles.

In 2014, aggregate distribution was affected by crop phase for all fractions except cPOM. Soil in the corn phase consisted of higher proportions of M than either the soybean or wheat phases by over 25% ($p < 0.0001$). Concomitantly, significantly higher proportions of the Mm and Ms+c fractions were seen in the corn phase versus the soybean or wheat phases along with the larger proportion of M. The proportion of Mm in the corn phase was also approximately 25% higher versus soybean and wheat ($p < 0.0001$), and Ms+c was present in a proportion over 30% greater in corn versus soybean and wheat ($p < 0.0001$). Significantly lower proportions of the free m and s+c fractions were observed in the corn phase versus soybean and wheat. The soybean and wheat phases contained four times the free m of the corn phase ($p < 0.0001$), and nearly two times the free s+c ($p < 0.0001$). The aggregate distribution of the soybean and wheat phases did not differ significantly from each other in 2014.

In 2015, significant differences in aggregate distribution among crop phases were present, but were different from those observed in 2014. All six aggregate fractions

showed a significant effect of crop phase, however in this year the corn and soybean aggregate distributions did not differ with the exception of the cPOM fraciton. For the cPOM fraction, the corn phase had an approximately 15% greater proportion than the soybean phase ($p = 0.016$), while wheat did not differ from either of the other two crop phases. Both corn and soybean contained a greater proportion of M than the wheat phase by about 10% ($p < 0.0001$). To again emphasize the dependence of other soil fractions on the proportion of M, approximately 25% less free m was observed in corn and soybean versus the wheat phase ($p < 0.0001$), while occluded fraction proportions (Mm and Ms+c) were significantly increased by approximately 10% in corn and soybean versus wheat ($p = 0.013$ and 0.004 respectively for Mm; $p = 0.0002$ and 0.008 respectively for Ms+c). The free s+c proportion was increased by over 15% in wheat versus corn and soybean ($p = 0.003$ and 0.006 respectively), in contrast with 2014 findings where lower proportions of this fraction were observed alongside increased M proportions.

Aggregation Relationship with C Inputs

The relationship between total annual C inputs and macroaggregation was positive. The correlation coefficient was 0.6941 ($p = 0.001$), indicating that macroaggregation increased with total C inputs. A positive relationship between AG and BG C inputs with macroaggregation was also observed (correlation coefficients = 0.6831 and 0.6826 respectively, $p = 0.0018$ in both cases), though the relationship was not as strong as with total C input. Though the relationship between BG C input and macroaggregation was not as strong as with total C, BG inputs represent a more accurate estimation of within-year C inputs that may affect soil properties of a given crop phase.

AG inputs are commonly incorporated at the end of a growing season taking additional time to decompose, and as such their effects might not have an impact on soil properties until subsequent years (and crop phases in a rotation). Thus, BG C input was used as the metric to determine a relationship between C inputs and macroaggregation.

Soil Aggregate Carbon Content Across Crop Phases

Crop phase effect alone (when considered over both years) on aggregate C content was not significant for any of the six aggregate fractions investigated, but the year × crop interaction was always highly significant (Table 1.5; $p \leq 0.0001$). Models separated the effect of crop phase over each level of year (2014 and 2015) to determine the nature of this interaction. Crop phase effects on aggregate C content were inconsistent between 2014 and 2015. Aggregate C content data was log-transformed for improved model fit, and as such, mean comparisons among crop phases are only applicable within the framework of this study.

In 2014, all soil aggregate fractions were significantly impacted by crop phase. C content in cPOM was between 20-30% greater in corn and soybean versus wheat ($p = 0.002$ and < 0.0001 respectively), while corn and soybean were not different from one another. In the M fraction, corn and soybean were significantly different at the 0.05 level with soybean having a larger aggregate C content by approximately 15% ($p = 0.046$). Wheat did not differ from either of the other two phases. The aggregate C content in the occluded Mm fraction was significantly greater in soybean versus corn and wheat but corn and wheat did not differ significantly. For Mm, C content was 15-20% greater in soybean versus corn and wheat ($p = 0.049$ and 0.004 respectively). Aggregate C content in the occluded Ms+c was significantly different between soybean and wheat, with

soybean being greater by approximately 20% ($p = 0.017$) while corn did not differ from either of the other two phases. In the free m fraction, corn and soybean did not differ in their aggregate C content while wheat differed from both, having a lower aggregate C content by over 15% ($p = 0.005$ vs. corn and 0.030 vs. soybean). In the final soil aggregate fraction, free s+c, C content differed between corn and wheat, with corn having a greater C content by nearly 25% ($p = 0.0004$). Soybean did not differ from the other two phases.

Crop phase also had significant effects on aggregate C content in 2015, though the effects were not the same as observed in 2014. In the cPOM fraction, both soybean and wheat phases had a greater C content by over 35% versus corn ($p < 0.0001$). Soybean and wheat phases did not differ from one another. For the M fraction, corn had a reduced C content by over 20% versus wheat ($p = 0.004$) while soybean did not differ from the other two phases. The aggregate C content in the occluded Mm fraction was greater by over 15% in wheat versus corn ($p = 0.009$) and marginally greater ($p = 0.046$) in wheat versus soybean. Free m had a significant effect of crop phase on C content between wheat and corn, with wheat being greater by over 15% ($p = 0.019$) while soybean did not differ from the other two phases. Finally, aggregate C content of the occluded Ms+c and the free s+c fractions did not differ significantly among crop phases.

Discussion

This study provides additional evidence that soil aggregation, particularly on the macroaggregate level, is sensitive to agricultural management and therefore can vary among crop phases with different annual management practices. Despite studies

corroborating this finding (e.g. Bach and Hofmockel, 2016; Bossuyt et al., 2001; Chivenge et al., 2011), many published studies investigating SOC sequestration and soil aggregate dynamics do not incorporate strategies for data collection on a year-by-year (or crop-by-crop) basis. A review of the literature suggests that experiments carried out in systems focused around crop rotations often involve a common practice of collecting data at a single time point, or within only one crop phase (Andruschkewitsh et al., 2013; Birkhofer et al., 2008; Kong et al., 2005; Padbhushan et al., 2016; Pulleman et al., 2003; Jokela et al., 2011; Cates et al., 2016). Though less time- and resource-consuming, this limited sampling strategy encourages the assumption that soil properties, including SOM/SOC content and soil aggregation, are relatively constant throughout each crop rotational phase, ignoring the variability associated with management strategies within different crop phases. Sampling during each crop phase may provide an improved assessment of the sensitivity of the soil properties across the cropping system, with the increased frequency of sampling improving our understanding of variability in aggregate distribution and C cycling over both short and long-term time frames.

Soils collected from the corn phase alone in 2014 and both the corn and soybean phases of the crop rotation in 2015 demonstrated the greatest degree of aggregation (as measured by the proportion of soil in macroaggregates), containing nearly 90% macroaggregates in 2014 and over 70% in 2015. Jokela et al. (2011) found macroaggregation values of 66-86%, depending on soil depth, in the corn phase at the WICST, and Six et al. (2000b) found average macroaggregation was 85% among four long-term agricultural experiments in the central US, supporting our findings. This value is double the proportion of macroaggregation observed by Cates et al. (2016), who found

that the organic grain rotation contained ~40% macroaggregates. However, soil sampling in Cates et al. was performed after wheat harvest, which more closely aligns with the wheat phase where aggregation values were markedly lower in this study.

Both the corn and soybean phases in the organic grain rotation at the WICST receive multiple and frequent tillage and cultivation passes (~6 annually) to prepare the seedbed for planting and manage weeds without synthetic herbicides (Posner et al., 2008). With the high degree of soil disturbance during these cropping phases, the large proportion of macroaggregates observed was unexpected. The high level of aggregation observed in these heavily-disturbed crop phases at the WICST indicates that tillage is not the only factor controlling soil structural stability and aggregate formation in the organic rotation. Other factors, such as the quantity of C inputs, likely influence the formation and stability of soil aggregates. In the organic cash grain rotation at WICST, the amount of C input to the soil is largely dependent on crop phase. The 2014 and 2015 estimates of C input to each phase, calculated based on methods previously employed by Jokela et al. (2011), Sanford et al. (2012), and Cates et al. (2016), indicate that corn received up to 2.5 times the totalC input of soybean and wheat. Reasons for this include increased biomass accumulation during the growing season as well as greater animal manure inputs. Compared with soybean and wheat, the corn phase produces a great deal more biomass, both above and belowground, which translates to a greater amount of residues (and therefore C) left behind once the main crop is harvested.

Of even greater importance in terms of C input effect on soil aggregation is the amount of belowground C input each crop phase receives. Corn receives, on average, twice the belowground C that soybean receives, and three times that of wheat.

Belowground plant biomass is particularly influential in formation and maintenance of soil aggregate structure. Blankinship et al. (2016) showed that decaying plant roots played a key role in stabilizing macroaggregates, especially in dry soil conditions. Additionally, Cates et al. (2016) demonstrated a positive relationship between belowground biomass C content and the C content of macroaggregates, indicating that the binding of macroaggregates is affected by recent belowground biomass additions.

Along with increased belowground biomass contributions, the corn phase receives twice the composted poultry manure received by the wheat phase, while soybean receives no manure. Applications of manures (both animal- and plant-based) are known to increase SOC content of soils (Birkhofer et al., 2008; Diacono and Montemurro, 2010). Organic cropping systems receive additional benefits from the more routine use of plant- or animal-based amendments over soils in conventional systems, as the use of synthetic fertilizers in conventional agriculture can reduce aggregate stability (Williams and Petticrew, 2009). Farmyard manure and plant composts have been shown to increase aggregate stability in agricultural soils after several years of use (Dorado et al., 2003; Tejada et al., 2009). Elevated C inputs, through increased biomass accumulation and manure usage, are a potential cause of enhanced soil aggregation observed in the corn phase. Additional factors aside from C input quantity must also play a role in soil aggregation in this rotation, however, as high aggregation values were observed in the soybean phase in 2015 which receives greatly reduced C input compared to corn.

Spatial separation of soil samples in each crop phase between 2014 and 2015 is likely a contributing factor to the significant year and year × crop effects in both the aggregate distribution and aggregate C content data. As a 3-year corn-soybean-wheat

crop rotation, experimental plots progress through their rotation within the corresponding plot in each replication. As such, the physical location of the plots sampled for one crop phase in 2014 would not be the same plots sampled in 2015; for example, plots in which corn was grown in 2014 would have soybean growing in 2015, therefore the 2015 corn phase samples would be taken from another location in the field (the plots where wheat was grown in 2014). This impact of spatial variation on soil physical properties has been documented in previous studies (Reza et al., 2016; Strudley et al., 2008).

Though evidence exists that SOC dynamics are sensitive to many management and environmental factors, we did not observe many consistent effects of crop phase on aggregate C content in our study. While effects of crop phase on aggregate C content were observed within each year individually, these results often did not hold between the two years. Instead, patterns of aggregate C content were observed in some cases from 2014 to 2015 in successional crop phases. For example, in the cPOM fraction, corn and soybean had the highest C content in 2014 followed in 2015 with the soybean and wheat phases (the phases that follow corn and soybean in rotation respectively) having the highest C content. Additional examples include both the m and Mm fractions, where soybean was observed to have the greatest C content in 2014, followed by wheat having the greatest C content in 2015. The only consistent pattern observed was in the M fraction, with corn having the lowest C content in both years though it did not differ significantly from wheat in 2014 or soybean in 2015. Such patterns make it difficult to attribute changes in aggregate C content to crop phase differences alone, and instead suggest that spatial variation (i.e. differences between experimental plots) has a greater impact on this soil property. Thus, these results did not support our second hypothesis

that observed differences in aggregate distribution would result in reallocation of SOC among soil aggregate size fractions, and therefore shifts in aggregate C content in aggregates of the same size. This lack of findings can be attributed to the long-term and well-established nature of this crop rotation at WICST that has undergone relatively few management changes in the past 26 years. It is likely that the organic grain rotation at WICST has reached equilibrium among its three crop phases, without noticeable C content changes from year to year (and therefore crop to crop) within plots, though long-term (20 years) losses in SOC have been previously studied at this site (Sanford et al., 2012). The nature of cPOM may have also contributed to the observed variability, as this fraction is mainly composed of plant materials at varying levels of decay (and therefore varying chemical composition) that was occluded within macroaggregates (Six et al., 2002). When analyzing cPOM for C content, the composition can vary depending on whether a sample contained fresher or slightly older plant material.

Conclusions

This study provides evidence that aggregate distribution differs within a crop rotation dependent on crop phase. The corn phase in 2014 and both corn and soybean phases in 2015 had the highest composition of macroaggregates, despite intense tillage practices. The corn phase also had the largest quantity of C input to the soil, suggesting that C inputs may counteract the negative impacts of tillage, improving aggregate formation and stability in a given cropping system with intensive tillage events. No consistent effect of crop phase between the two years on aggregate C content was seen.

It is likely this system reached an equilibrium among the three crop phases, and aggregate C content would not change dramatically from year to year.

The dynamic nature of soil aggregation observed among crop phases within this crop rotation emphasizes the impact of agricultural management practices on sensitive soil properties such as soil aggregation and SOC content. Studies that compare cropping systems based on sampling at a single time point or during a single crop phase may overlook these nuances in aggregate and SOC dynamics, leading to over-generalizations or inaccurate conclusions. Such studies limit their frame of inference by ignoring potential differences that might exist in soil structural and chemical makeup between crop phases within rotations, or by assuming cropping system soil structure and chemistry are constant. Reliance on a single phase of a rotation to represent an entire cropping system may lead to inaccurate characterizations, and therefore inaccurate management recommendations. Increased sampling frequency during multiple crop phases of a cropping system may provide an improved characterization of that system's sensitive soil properties, allowing for more accurate understanding of SOC fluxes and soil aggregate stabilization.

Tables and Figures

Table 1.1 Detailed description of cover crop and tillage split-plot treatments in the organic grain rotation at the WICST, indicating cover crop usage, type and tillage regime. Control treatment (1) is representative of previous management practices used in the organic grain rotation at WICST. Shaded cells indicate where a treatment differs from the control.

	Crop Phase I				**Crop Phase II**			**Crop Phase III**		
Trtmt.	**Till**	**Crop**	**Till**	**Cover**	**Till**	**Crop**	**Till**	**Crop**	**Till**	**Cover**
1	Yes	Corn	Yes	None	N/A	Soybean	Yes	Wheat	Yes	Berseem clover/ Oat
2	Yes	Corn	Yes	None	N/A	Soybean	Yes	Wheat	Yes	Berseem clover/ Sorghum
3	Yes	Corn	Yes	Rye	Yes	Soybean	Yes	Wheat	Yes	Berseem clover/ Oat
4	Yes	Corn	Yes	Rye	No	Soybean	Yes	Wheat	Yes	Berseem clover/ Oat
5	Yes	Corn	Yes	Rye	Yes	Soybean	Yes	Wheat	Yes	Berseem clover/ Sorghum
6	Yes	Corn	Yes	Rye	No	Soybean	Yes	Wheat	Yes	Berseem clover/ Sorghum

Table 1.2 Various yield and soil parameters by crop phase, averaged from 2014-2015 measurements. Numbers in parentheses indicate standard errors of displayed means.

Crop Phase	Phase parameter					
	Grain Yield (Mg ha^{-1})	Forage Yield (Mg ha^{-1})	Soil P (ppm)	Soil K (ppm)	SOM (ppm)	Soil pH
Corn	10.46(0.49)	-	41(3.2)	112(9.8)	4.6(0.17)	6.9(0.07)
Soybean	3.58(0.07)	-	58(9.0)	147(15.2)	4.9(0.14)	6.7(0.11)
Wheat	4.83(0.08)†	4.09(0.30)‡	69(8.9)	157(21.4)	5.3(0.28)	6.7(0.12)

†Wheat grain yield based on 2015 average only; grain crop failure in 2014 resulted in all wheat being harvested for silage.
‡Wheat forage yield averaged between silage harvest in 2014 and straw harvest in 2015.

Table 1.3 Average (2014-2015) C inputs by crop phase in the organic grain rotation at WICST. Where data was missing, average of values from 2009-2013 (Jokela et al., 2011; Sanford et al., 2012; Cates et al., 2016) were used in calculations of 2014 and 2015 C inputs.

Crop Phase	Aboveground C Inputs (kg ha^{-1})	Belowground C Inputs (kg ha^{-1})	Total C Inputs (kg ha^{-1})
Corn	5588(202)†	2343(110)	7931(312)
Soybean	2101(41)	1111(22)	3211(62)
Wheat	2472(22)	659(87)	3131(88)

†Numbers in parentheses indicate standard errors of displayed means.

Table 1.4 Aggregate distribution by crop phase and year and associated p-values in the organic grain rotation at WICST, 2014-2015.

Crop Phase and Year	Soil Aggregate Fraction†					
	M	m	s+c	cPOM	Mm	Ms+c
	Proportion of dry soil weight, mg g^{-1}					
Corn 2014	869(7.2)‡	49(4.2)	81(6.1)	11.5(0.44)	484(6.6)	374(5.1)
Corn 2015	720(12.0)	159(8.5)	121(4.1)	14.0(0.91)	412(9.2)	294(4.9)
Soybean 2014	623(13.1)	218(8.7)	159(6.1)	12.0(0.50)	361(9.8)	249(5.6)
Soybean 2015	714(8.9)	163(5.5)	123(3.7)	11.6(0.47)	419(9.6)	284(6.3)
Wheat 2014	649(13.5)	200(8.9)	152(8.3)	13.3(0.89)	368(9.4)	267(6.0)
Wheat 2015	640(12.2)	217(7.6)	144(4.9)	13.5(0.86)	370(12.4)	256(6.6)
ANOVA	p-values					
Crop	0.0002	0.0002	0.0004	0.1378	0.0054	0.0011
Year	0.0168	0.0002	0.7423	0.1006	0.5933	<0.0001
Year × Crop	<0.0001	<0.0001	<0.0001	0.0310	<0.0001	<0.0001

†M = macroaggregates; m = microaggregates; s+c = silt and clay; cPOM = coarse particulate organic matter; Mm = occluded microaggregates; Ms+c = occluded silt and clay.

‡ Numbers in parentheses indicate standard errors of the displayed means.

Table 1.5 Aggregate carbon content by crop phase and year and associated p-values in the organic grain rotation at WICST, 2014-2015.

Crop Phase and Year	Soil Aggregate Fraction†					
	M	m	s+c	cPOM	Mm	Ms+c
	SOC content of aggregates, g 100g soil^{-1}					
Corn 2014	2.25(0.12)‡	2.84(0.10)	2.19(0.10)	7.63(0.26)	2.45(0.13)	1.68(0.09)
Corn 2015	2.38(0.07)	2.38(0.06)	1.69(0.06)	5.88(0.50)	2.41(0.06)	1.60(0.03)
Soybean 2014	2.66(0.15)	2.77(0.16)	2.00(0.18)	8.50(0.37)	2.86(0.16)	1.98(0.12)
Soybean 2015	2.59(0.15)	2.55(0.13)	1.76(0.11)	9.34(0.54)	2.54(0.13)	1.70(0.09)
Wheat 2014	2.25(0.09)	2.30(0.06)	1.65(0.08)	5.80(0.69)	2.30(0.07)	1.58(0.04)
Wheat 2015	3.04(0.19)	2.93(0.21)	2.05(0.16)	9.31(0.38)	2.95(0.15)	1.95(0.12)

ANOVA	p-values					
Crop	0.1797	0.9259	0.4495	0.0591	0.3080	0.3822
Year	0.0003	0.6535	0.1896	0.0240	0.1200	0.8793
Year × Crop	0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001

† M = macroaggregates; m = microaggregates; s+c = silt and clay; cPOM = coarse particulate organic matter; Mm = occluded microaggregates; Ms+c = occluded silt and clay.

‡Numbers in parentheses indicate standard errors of the displayed means.

Figure 1.1 Tillage practices by crop phase in the organic grain rotation at WICST. Arrows are color-coded indicating type of tillage practice.

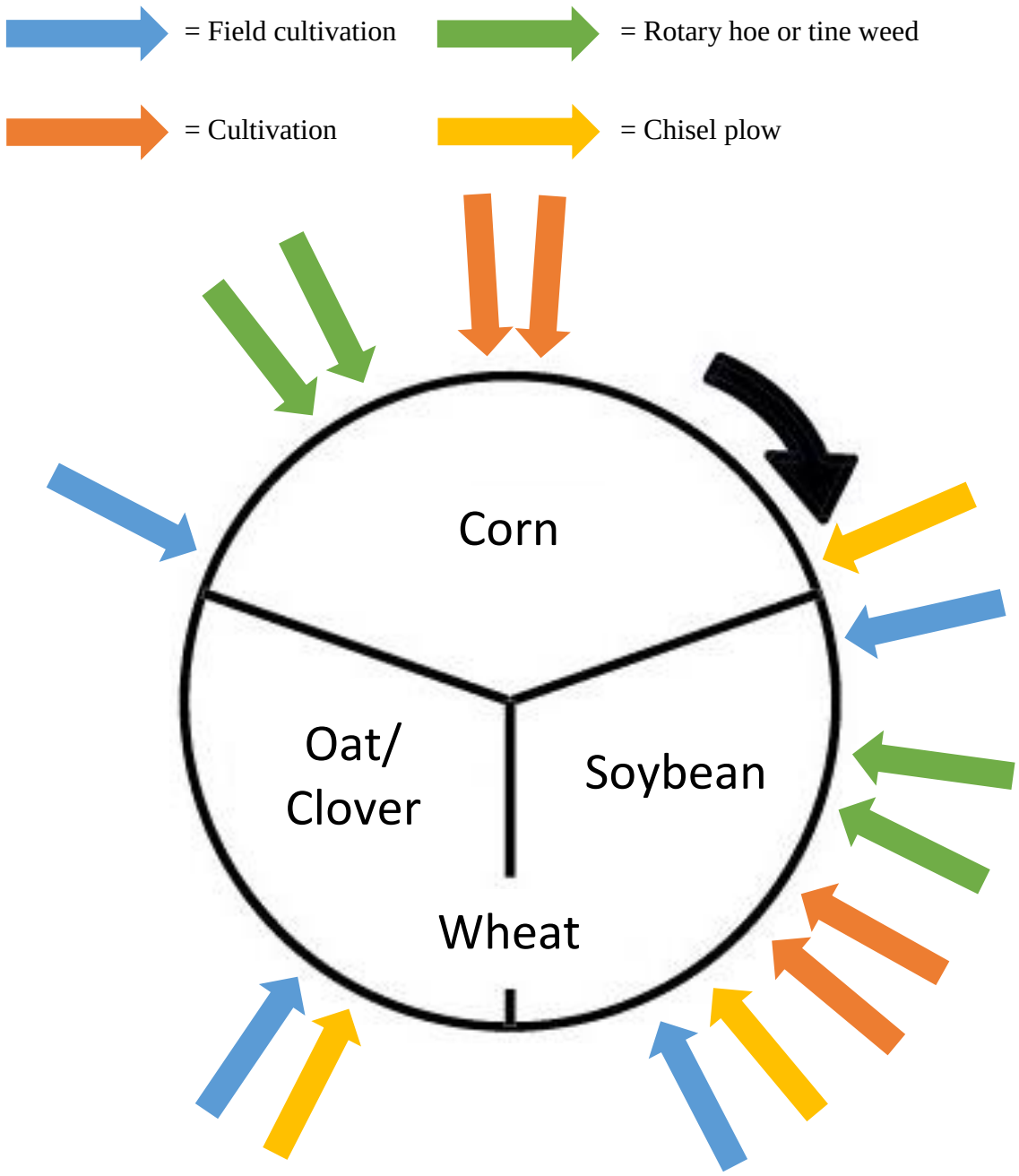
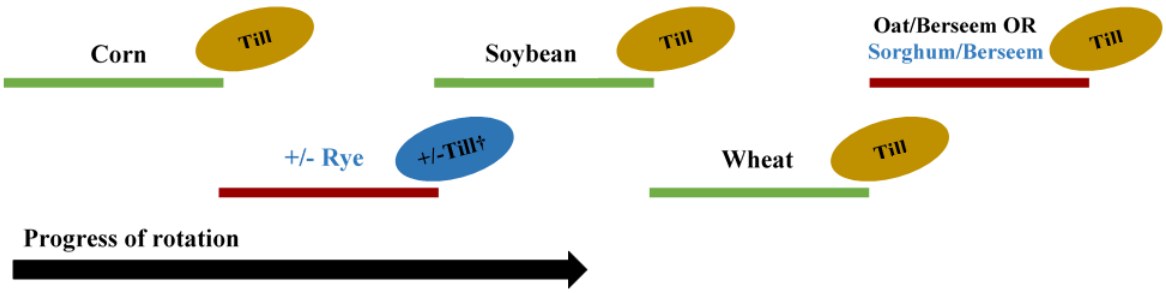


Figure 1.2 Cover crop and tillage treatments applied to organic corn-soybean-wheat rotation at WICST. Crops in the upper row are planted in spring/summer, while crops in the bottom row are fall-planted. Crops marked with a green line indicate main crops, and crops in red indicate cover crops. Tillage is indicated with ovals. A blue color indicates management changes associated with the 'sustainable intensification' study.



†With no-till soybean treatments, a roller-crimper was the method of termination for the rye cover crop.

Figure 1.3 Soil aggregate fractionation technique, adapted from Six et al. (1998). Figure adapted from G.R. Sanford.

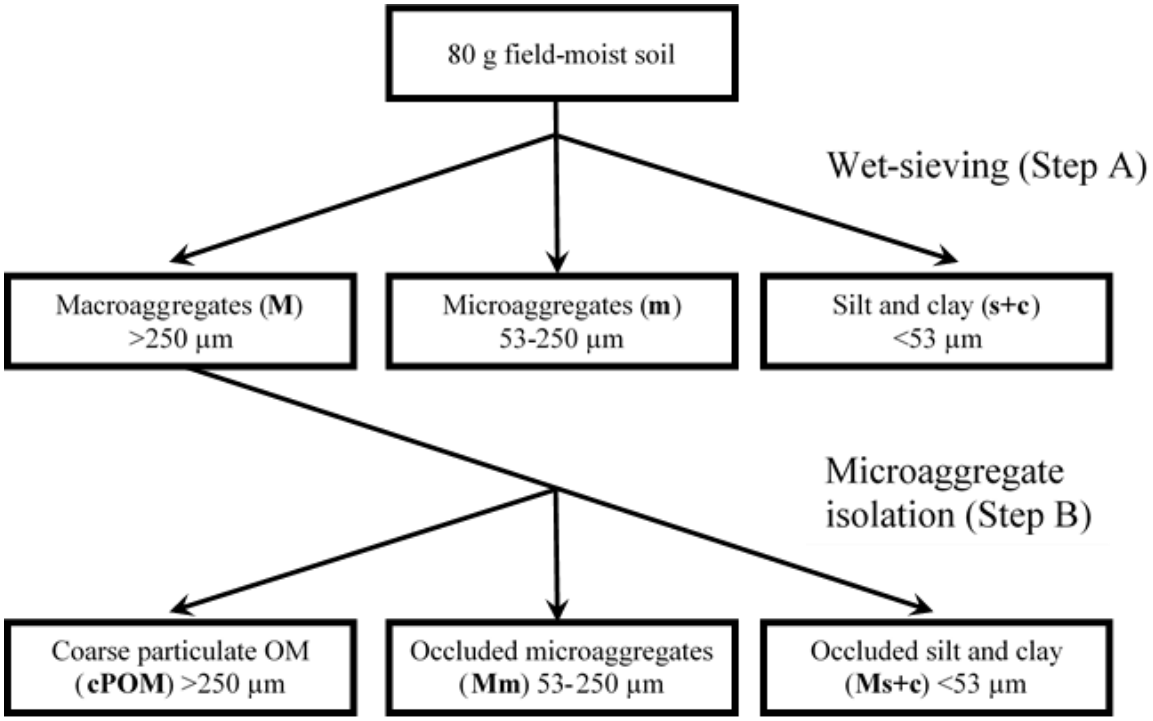


Figure 1.4 Macroaggregation as affected by belowground C inputs in the organic grain rotation at WICST. Belowground C inputs represent 2014 and 2015 averages, calculated based on methods used in Jokela et al. (2011), Sanford et al. (2012), and Cates et al. (2016). Macroaggregation is measured in g macroaggregates g dry soil^{-1}. Filled shapes represent 2014 means, while unfilled shaped represent 2015 means.

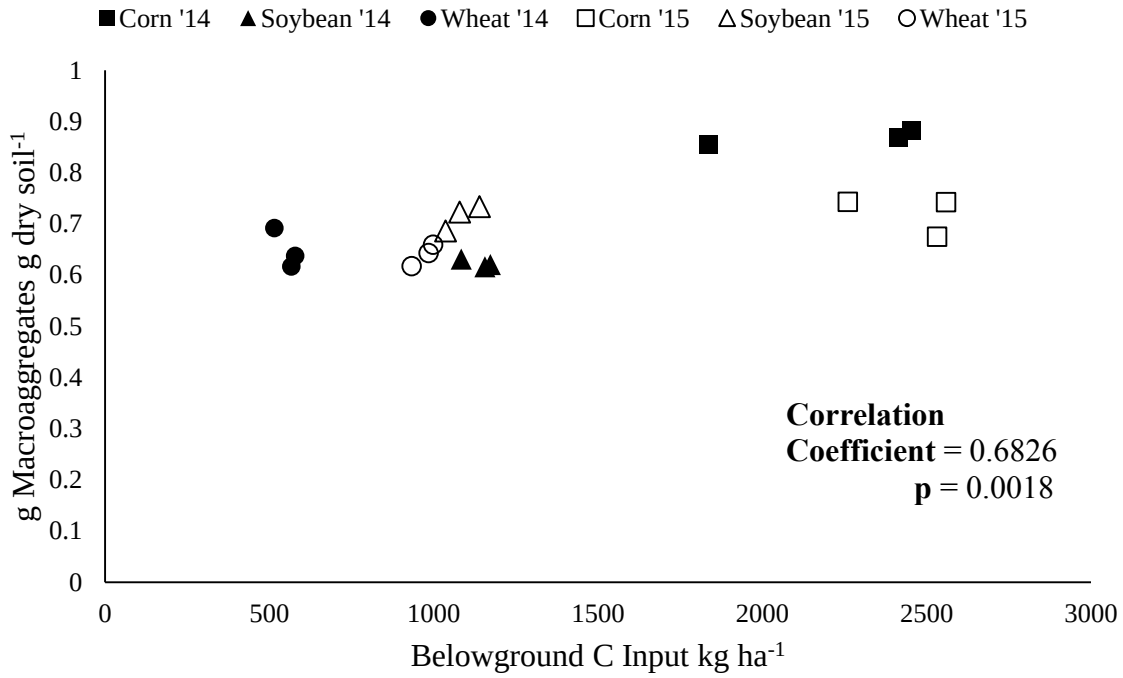


Figure 1.5 Soil aggregate distribution of the organic grain rotation at WICST, 2014. Vertical bars indicate aggregate fractions that are occluded within macroaggregates; macroaggregate proportions can be determined by adding the 3 sections with vertical bars together. Uppercase letters indicate significant differences in macroaggregate proportions (sum of sections with vertical bars) between crop phases, lowercase letters indicate significant differences in all other fractions between crop phases. In 2014, no significant differences existed among crop phases in proportion of cPOM, therefore no lowercase letters are displayed for this fraction. s+c = silt and clay, m = microaggregates, cPOM = coarse particulate organic matter, Ms+c = occluded silt and clay, Mm = occluded microaggregates.

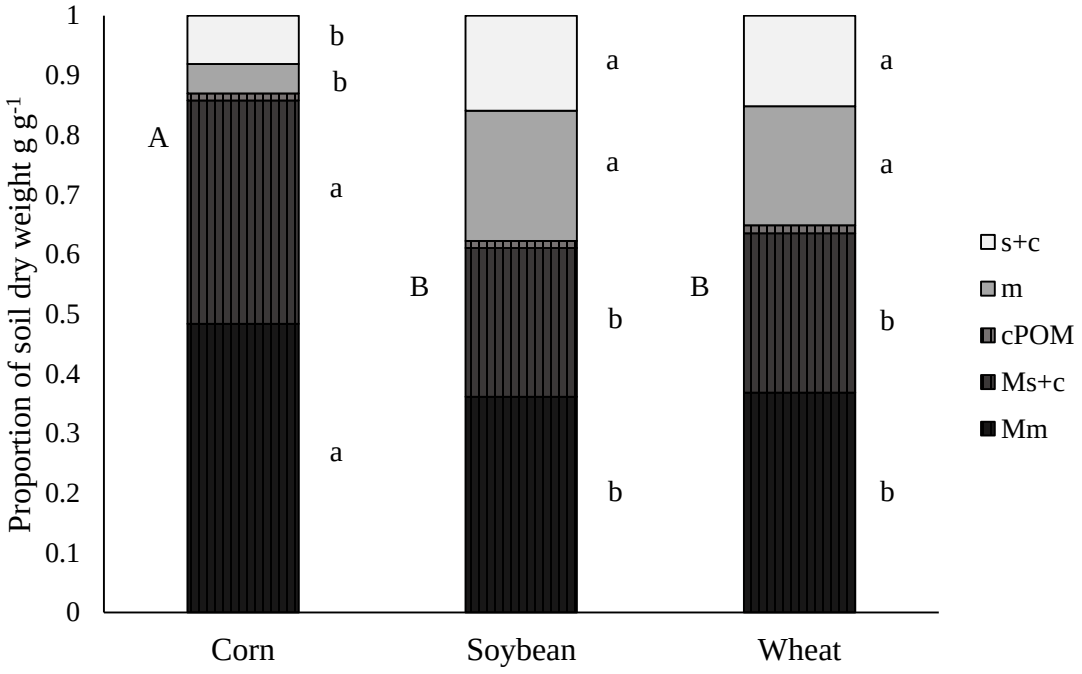


Figure 1.6 Soil aggregate distribution of the organic grain rotation at WICST, 2015. Vertical bars indicate aggregate fractions that are occluded within macroaggregates; macroaggregate proportions can be determined by adding the 3 sections with vertical bars together. Uppercase letters indicate significant differences in macroaggregate proportions (sum of sections with vertical bars) between crop phases, lowercase letters indicate significant differences in all other fractions between crop phases. s+c = silt and clay, m = microaggregates, cPOM = coarse particulate organic matter, Ms+c = occluded silt and clay, Mm = occluded microaggregates.

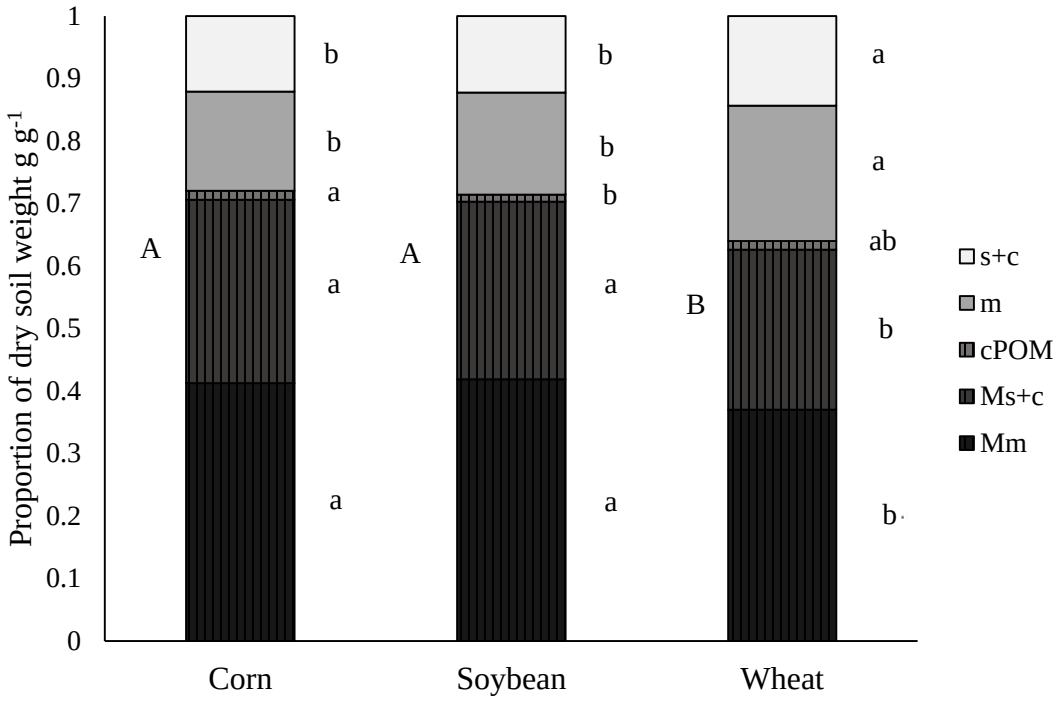


Figure 1.7 Aggregate C content of soil fractions in the organic grain rotation at WICST, 2014. Lowercase letters indicate significant differences within fractions between crop phases. M = macroaggregates, m = microaggregates, s+c = silt and clay, cPOM = coarse particulate organic matter, Mm = occluded microaggregates, Ms+c = occluded silt and clay.

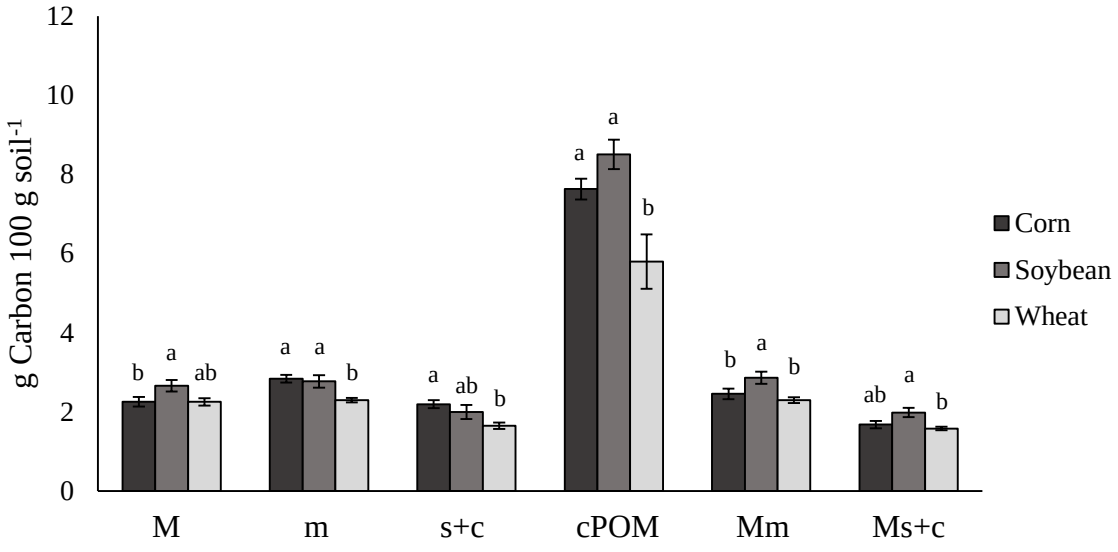
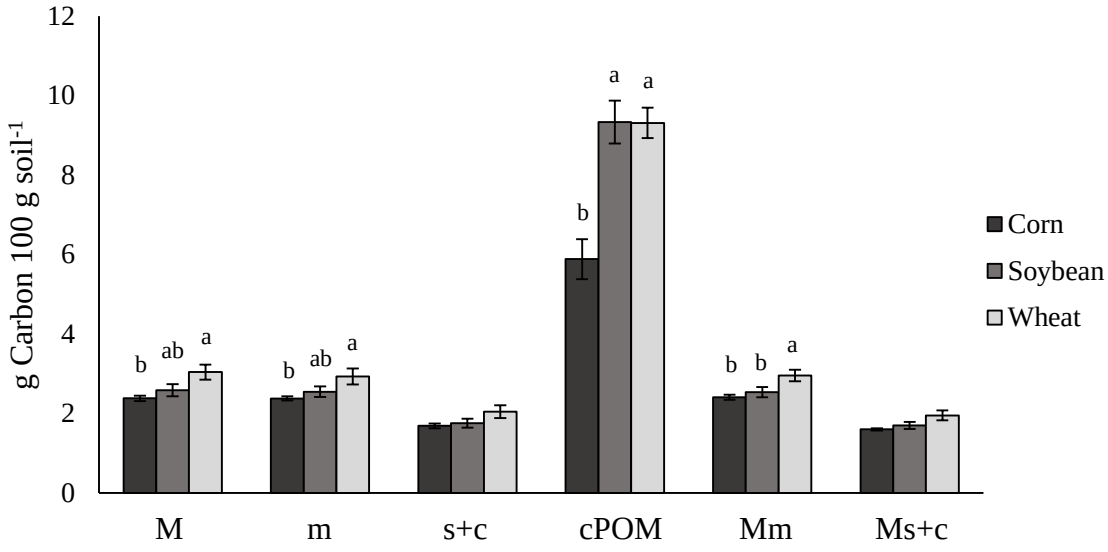


Figure 1.8 Aggregate C content of soil fractions in the organic grain rotation at WICST, 2015. Lowercase letters indicate significant differences within fractions between crop phases. M = macroaggregates, m = microaggregates, s+c = silt and clay, cPOM = coarse particulate organic matter, Mm = occluded microaggregates, Ms+c = occluded silt and clay.



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CHAPTER II

Impacts of Crop Phase on Microbial Community Composition in an Organic Cash Grain Rotation in Wisconsin, USA

Abstract

Microbial community composition can affect soil aggregation and therefore soil organic carbon (SOC) stabilization. Our previous work has shown that crop phase within an organic corn-soybean-wheat rotation significantly affected soil aggregate distribution, with heavily disturbed corn and soybean being significantly more aggregated than the less-disturbed wheat phase. Here, we investigate whether microbial community composition is an underlying factor in the observed crop phase effect on soil aggregation. We assessed total lipid biomass, relative abundance of five separate microbial ecological groups, including gram-positive bacteria (Gm+), gram-negative bacteria (Gm-), arbuscular mycorrhizal fungi (AMF), saprotrophic fungi (SF), and actinomycetes, as well as microbial community composition through a principal component analysis (PCA) in three soil aggregate fractions in two years, 2014 and 2015. These fractions included macroaggregates (> 250µm), microaggregates (53-250 µm), and silt and clay (< 53µm). We found significant differences among crop phases in total lipid biomass, ecological group relative abundance, and community composition, though all significant effects decreased with soil aggregate size. In 2015 when sampling time did was the same across all three crop phases, wheat had the largest amount of lipid biomass, AMF relative abundance, and F:B ratio, while corn and soybean tended towards a larger relative abundance of Gm+, actinomycetes, and SF. Results from 2014 where sampling time differed were not as consistent and varied with soil aggregate size. While our data

provides evidence that crop phase and its associated management can impact microbial community composition, we did not see a direct link between fungal abundance and soil aggregation within crop phase as we had predicted. It is possible that crop phase management effects may carry over into the following season, influencing microbial community composition in the next phase of the crop rotation. Additional sampling events to assess microbial community composition throughout the growing season would provide additional insight into microbial community shifts and impacts this may have on the soil aggregation process.

Introduction

Improving the soil organic matter (SOC) content of agricultural soils is critical for continued productivity and sustainability of our food systems, particularly in light of increasing global populations and the threat of climate change (Lal, 2004). Soil aggregation is a key factor contributing to long-term soil organic carbon (SOC) accumulation and related agricultural benefits through improved water infiltration, decreased erosion, and increased nutrient and water holding capacity. Physical protection within aggregates allows SOC to become stabilized as it becomes inaccessible for microbial metabolic processes, therefore preventing release into the atmosphere as CO_2 (Rovira and Greacen, 1957; Six et al., 1998). SOC is stored and stabilized long-term within microaggregates (Jastrow 1996; Six et al. 2004), defined as soil particles 53-250 μm in diameter. Stable microaggregates are formed within larger macroaggregates (soil particles defined as >250 μm in diameter) (Angers et al., 1997; Jastrow 1996; Oades, 1984), using plant residues contained within as nucleation sites (Chung et al., 2008; Kong

et al., 2005). Increasing macroaggregate stability and decreasing their turnover rates results in increased microaggregate protection and increased availability of nucleation sites (Six et al. 2000, 2004).

Biological processes that determine soil aggregate formation and stability are largely mediated by the soil microbial community. The stability and formation of aggregates depends on the persistence of fungal and bacterial byproducts that bind them (Six et al., 2000; Tisdall and Oades, 1982). Macroaggregate formation is mainly dependent on physical forces applied by fine plant roots and fungal hyphae, which enmesh and bind small soil particles (Bossuyt et al., 2001; Rillig and Mummey, 2006). Fungal and bacterial byproducts released within macroaggregates cause fine soil particles to be attached to plant residues, forming microaggregates (Golchin et al., 1994; Rillig and Mummey, 2006). Microbial byproducts that serve as aggregate binding agents include both polysaccharides and proteins, including the arbuscular mycorrhizal fungal (AMF) product glomalin (Rillig and Mummey, 2006; Six et al., 2004). While studies have shown that bacterial byproducts contribute to aggregate stability (Carrasco et al., 2009; Oades, 1993), fungal byproducts are generally believed to enhance stability to an even greater degree, due to their more complex structure (Six et al., 2006).

Shifts in microbial abundance and community structure can influence soil aggregation processes (Bossuyt et al., 2001). Both agricultural management practices (e.g. fertilization, tillage, crop type) as well as environmental factors (e.g. soil type, temperature, and soil properties such as pH) can affect microbial community abundance and structure in soils (Girvan et al., 2003; Lauber et al., 2008; Ngosong et al., 2010). Soil bacterial community composition tends to be most heavily impacted by soil type, while

fungal community composition is often driven by nutrient availability and fertilization (Girvan et al., 2003; Lauber et al., 2008; Suzuki et al., 2009). Where soil type is held constant, however, agricultural management tends to have a larger effect on community structure than some environmental parameters such as temperature, moisture, and pH (Buyer et al., 2010).

Among the agricultural management practices that have the potential to influence microbial communities, tillage is among the most disruptive. Tillage, while directly reducing soil aggregation through physical disturbance (Baker et al., 2007; Grandy and Robertson, 2006; Sheehy et al., 2015), can also indirectly and negatively affect the aggregation process by disrupting the microbial communities which drive aggregation. This reduces the soil's ability to regain structure after disturbance efficiently as microbial communities vary in their ability to recover from disturbance (Allison and Martiny, 2008). Compared with reduced or no-tillage practices, conventional tillage can decrease total microbial biomass (Ghimire et al., 2014; Wortman et al. 2013), and is especially disruptive for the fungal portion of the community while favoring bacteria (Beare et al., 1997; Wang et al., 2010). Disruption or suppression of the fungal portion of the microbial community reduces formation of macroaggregates, while reducing bacterial populations does not have the same effect (Bossuyt et al., 2001). While both fungi and bacteria play a role in the stabilization of soil aggregates, increased effectiveness in macroaggregate formation and long-term stability of fungal byproducts may make fungi the more beneficial component of the microbial community in terms of SOC stabilization and storage.

Not all farming practices that employ tillage as a management strategy necessarily affect the microbial community in a negative manner. Organic farming, which often relies on tillage as a means to reduce weed pressure, can positively impact the microbial community as compared to conventional agricultural production (Birkhofer et al., 2008; Ghimire et al., 2014; Ullrich et al., 2011). These increases are associated with the use of organic amendments including green and animal manures (Lejon et al., 2007), as opposed to synthetic fertilizers which reduce microbial biomass and substrate use efficiency (Birkhofer et al., 2008). Cover-cropping, a practice commonly employed in organic agriculture, benefits total microbial biomass by enhancing fungal abundance (Buyer et al., 2010; Wortman et al., 2013).

By supporting the microbial communities driving the processes of soil aggregation (and therefore SOC storage), organic farming methods tend to provide enhanced SOC content or improved soil structure over conventional methods (Foereid and Høgh-Jensen, 2004; Gerhardt, 1997; Williams and Petticrew, 2009). Despite evidence to support the positive impacts of organic management on soil microbial communities, this does not always translate into improved soil aggregation and increased carbon storage in these systems, particularly when background levels of SOC are high (Peichl et al., 2010). A study by Sanford et al. (2012) found that an organic grain rotation at the Wisconsin Integrated Cropping Systems Trial (WICST) in Arlington, WI lost 5 Mg SOC ha^{-1} to a depth of 91 cm over a 20-year period, among the highest of any cropping system in the trial, with the exception of a conventionally-tilled and managed continuous corn system. In a related study, Cates et al. (2016) found that this same organic grain

rotation at WICST had lower levels of aggregation compared with other cropping systems in the trial. The results of these WICST studies emphasize the fact that SOC dynamics and soil aggregate stability are still not well-understood, particularly in organic cropping systems. As the main drivers of soil aggregation (and therefore SOC storage), additional study is required to determine the impacts the microbial community and its functional groups have on these processes under organic management (Balser et al., 2006; Lichtfouse et al., 2010).

To address this gap in knowledge, we investigated the relationship between soil microbial community and soil aggregate structures within the organic cash-grain rotation at WICST. Previous work (Chapter I) showed that soil aggregation differed significantly with crop phase in this rotation, with the heavily-tilled corn phase having a higher proportion of macroaggregates (and associated occluded soil aggregate fractions) and lower proportions of smaller free soil aggregate fractions than either the soybean (which is also heavily-tilled) or wheat phases. To determine whether microbial community composition is associated with the observed variation in soil aggregation within the WICST organic grain rotation, we compared the soil microbial communities among crop phases within three soil aggregate size classes separated from the whole soil: macroaggregates (>250 μm), microaggregates (53-250 μm), and silt and clay (<53 μm). We used a modified Bligh and Dyer (1959) lipid extraction to isolate organic material which was saponified and converted to fatty acid methyl esters (FAMEs) as the FAME procedure introduced by Microbial ID, Inc. (Newark, DE). FAMEs were used for gas chromatography (GC) profiling. GC profiles given by the FAMEs allowed characterization of community composition based on abundances of microbial ecological

groups (e.g. broad groupings of fungi and bacteria). We hypothesized that 1) overall microbial community composition will differ with crop phase; 2) crop phases incorporating fewer tillage operations would have higher abundance of fungi and fungal to bacterial ratios (F:B), while crop phases with a greater number of tillage operations would favor higher abundances of bacteria; and 3) abundance of fungal groups will be associated with differences in soil aggregation across crop phases.

Materials and Methods

Site Description

The study was conducted at the Wisconsin Integrated Cropping Systems Trial (WICST), located at the University of Wisconsin Arlington Agricultural Research Station in Columbia County, WI (43˚18'18"N, 89˚19'48"W). The soil at WICST is classified as a Plano silt-loam, a Fine-silty, Mixed, Superactive, Mesic Typic Argiudolls (USDA NRCS Soil Taxonomy) formed over a parent material of alluvial loess deposits. Average annual temperature at the Arlington Agricultural Research Station is 6.9˚C, with an average minimum of 0.5˚C and an average maximum of 13.3˚C. Annual precipitation averages 869 mm, with the majority (64%) occurring in the spring and summer (1981-2010, National Climatic Data Center). WICST, established in 1990, is a long-term cropping systems trial designed to assess the productivity, profitability, and environmental impacts of two different agricultural enterprise types under varying crop diversity levels (Posner et al., 1995). These two enterprise types represent cash-grain as well as dairy forage production strategies commonly found in Wisconsin and the upper Midwest. Prior to cultivation (ca 1850), deep-rooted tallgrass prairie vegetation was

dominant in the region. In the mid-1800s, the predominant agricultural use was continuous wheat production which later shifted to livestock feed as the dairy industry of Wisconsin grew. From the 1960s until its establishment, the WICST site had been cultivated with an alfalfa (*Medicago sativa* L.)-corn (*Zea mays* L.) rotation, using dairy manure as a nutrient source (Posner et al., 1995). In 1989, the trial was planted to corn to achieve soil homogenization and improve blocking accuracy based on yield variability.

WICST is a randomized complete block design consisting of four replications of each of six cropping systems. All phases of each cropping system are present in a year. Of the four total replicates present at WICST, three were sampled in this study as one replicate (block two of four) has consistently demonstrated soil properties inconsistent with those of the other three replicates and is susceptible to frequent flooding, potentially adding additional compounding factors to soil properties. Therefore, block two is not a good representation of the site as a whole (G.R. Sanford, personal communication, 2016). The six cropping systems at WICST represent cash-grain and dairy forage enterprises, with conventional and organic representatives of each. Plots are 0.3 ha in size. Our study was carried out in just one of these six cropping systems, an organically-managed cash-grain rotation. This rotation is three years in length, with a crop sequence of corn, soybean [*Glycine max* (L.) Merr], and winter wheat (*Triticum aestivum* L.). The winter wheat phase includes a mixed oat (*Avena sativa* L.) and berseem clover (*Trifolium alexandrium* L.) cover crop planted after wheat is harvested. This gave us three replicates with three crop phases per replicate, or nine experimental plots total.

Annually, the organic cash grain cropping system receives an application of composted pelletized poultry manure in spring of both the corn and wheat phases. The

corn phase receives poultry manure at a rate of 4.48 Mg ha^{-1} while the wheat phase receives 2.24 Mg ha^{-1}. A combination of tillage and cultivation methods are used for field preparation, weed control, and post-harvest field management. Specific tillage and cultivation activities, and the timing of activities, can be found in Figure 1.1 (Chapter I). All crop phases are field cultivated prior to planting. Corn and soybean phases receive several rotary hoe or tine-weeding events for weed control, as well as several in row cultivations. Plots are chisel plowed in fall after corn and soybean harvest, and in summer after wheat harvest prior to cover crop planting. Cover crops are terminated during the field cultivation event preceding corn planting in spring. At minimum, corn and soybean phases receive six tillage and/or cultivation events during the year, and the wheat phase receives two to three.

Soil Sampling

Baseline soils were sampled after harvest of wheat, soybean and corn in 2014 (July, September, and November respectively). In 2015, soil samples from each treatment were collected in late October at the end of the growing season, following tillage and planting of the rye cover crop in the corn phase, planting of wheat in the soybean phase, and standing cover crop senescence in the wheat phase. At each sampling event, five soil cores 15 cm deep and 1.9 cm in diameter were composited per sub plot, and placed immediately in a cooler until they could be stored at 4˚C. Within two weeks of sampling, each sample was picked free of visible plant residues and rocks prior to further processing which included sieving to 2 mm. After sieving, samples were placed in a freezer at -20˚C for storage before they were processed further. Samples were stored

cold and/or frozen to retain field moisture as well as viability of microbial residues to be used in community analysis.

Aggregate Fractionation

Samples were removed from the freezer and placed in a refrigerator at 4˚C to thaw for a minimum of 24 hr before aggregate fractionation. A small amount (15-20 g) of each sample was removed and dried in a 60˚C oven until the soil weight stabilized to determine volumetric moisture content. The aggregate fractionation method we used is a simplified version of that employed by Six et al. in 1998 (Chapter I Figure 1.3, step A), and similar to the method in Cates et al. (2016). A 250 µm mesh sieve was set within a large metal basin. Deionized water (DI) was added to the basin until the water level was approximately 2 cm above the sieve's mesh. An 80 g portion of each soil sample was carefully sprinkled onto the 250 µm mesh sieve. The soil was allowed to soak for 5 min, then the sieve was moved up and down in the water-filled basin approximately 3 cm at a slight angle at a rate of 25 times per min for 2 min. Material that washed through the sieve was collected in the large metal basin, and any particles stuck to the outside of the sieve were carefully washed into the basin with a squeeze bottle containing DI. Macroaggregate (M) soil particles (>250 µm) remaining in the sieve were washed into a pre-weighed aluminum pan. Approximately 10g of the M/DI slurry in the aluminum pan was collected into a specimen cup and then frozen at -20˚C.

The soil and DI slurry that was retained in the first basin was then poured into a 53 µm mesh sieve nested within a second large metal basin. If the water level was not sufficient, DI was added until the water level was 2 cm above the sieve's mesh. The 53

µm sieve was then moved up and down approximately 3 cm at a slight angle 25 times per min for 2 min. Again, material that had washed through the 53 µm mesh was collected in the second metal basin and fine soil particles stuck to the outside of the sieve were carefully washed into the basin with a squeeze bottle containing DI. Microaggregate (m) soil particles (53-250 µm) remaining in the sieve were rinsed into a pre-weighed aluminum pan. Approximately 10g of m/DI slurry from the pan was collected in a specimen cup and frozen at -20˚C. A slurry of silt and clay (s+c) particles (<53 µm) and DI remained in the second basin. About 10g of the slurry was added to a specimen cup and frozen at -20˚C while remaining s+c/DI slurry was rinsed into a pre-weighed aluminum pan. Samples that had been frozen in specimen cups following aggregate fractionation were lyophilized for approximately 24 h to remove moisture prior to the lipid extraction process.

Determination of Microbial Community Composition of Aggregate Fractions

Lipids were extracted from approximately 3g of lyophilized soil samples as per the protocol described in White and Ringelberg (1998) using the initial extraction steps of the modified method from Bligh and Dyer (1959). The resulting fatty acids were then saponified and converted to fatty acid methyl esters (FAMEs) and analyzed according to the methods of Microbial ID Inc. (Newark, DE). Lipids were obtained from the soil samples using a chloroform-methanol extraction with a phosphate buffer. Samples were shaken for approximately 1 h and centrifuged to separate phases. The supernatant was removed with a pipette, and the samples were placed in a dark environment at room temperature to separate overnight. The following day, the top layer was removed with a

vacuum aspirator and the tubes containing the remaining $CHCl_3$ layer were placed in a Rapid-Vap at 33˚C for drying. Fatty acids were saponified with the FAME method from Microbial ID Inc., adding sodium hydroxide and heating in a water bath for 30 minutes. Methanolysis was then performed by adding a strong acid.

2 µl of extracted FAMEs per sample were analyzed with a Hewlett-Packard 6890 gas chromatograph, equipped with a flame ionization detector and an Ultra 2 capillary column (Agilent Technologies, Santa Clara, CA). Gas chromatography conditions were controlled by the MIDI Sherlock program and lipid peaks were identified using bacterial fatty acid standards and the Sherlock peak identification software (MIDI Inc., Newark, DE). Peak areas of two internal standards, 9:0 (nonanoic methyl ester) and 19:0 (nonadecanoic methyl ester), were used to measure quantities of fatty acids. Fatty acids were converted to µmol lipid g soil^{-1}. Total microbial biomass was estimated from total µmol lipid g soil^{-1}, and absolute abundances of microbial ecological groups (including gram-positive bacteria [Gm+], gram-negative bacteria [Gm-], arbuscular mycorrhizal fungi [AMF], saprotrophic fungi [SF], and actinomycetes) were calculated from average µmol lipid g soil^{-1} of all lipids in a sample corresponding to those groups (see Table 2.1). Relative ecological group abundances (mol%) were calculated by taking the average µmol lipid g soil^{-1} corresponding to that group/total µmol lipid g soil^{-1} for a sample. Fungal to bacterial ratios (F:B) were calculated using total µmol fungal lipid g soil^{-1} /total µmol bacterial lipid g soil^{-1}. Fatty acids that were present in quantities 0.5 mol% or greater in both 2014 and 2015 samples were included in our group analysis.

Statistical Analysis

Total microbial biomass, F:B, and ecological group relative abundance analysis

Microbial biomass, F:B, and ecological group data were analyzed separately by soil aggregate fraction (M, m, and s+c) using an RCBD in PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc., Cary, NC). As sampling occurred across two production seasons, the potential effects of year and crop phase were tested using crop, year, and the crop × year interaction as fixed effects. Split-plot treatments (as described in Chapter I) were included in the model as a covariate to provide improved fit and random distribution of the residuals. Block and the block × crop interaction were treated as random effects. Our response variables of interest included total microbial biomass (henceforth referred to as total biomass), F:B, and the relative abundance (mol %) of five microbial ecological groups: gram-positive bacteria (Gm+), gram-negative bacteria (Gm-), arbuscular mycorrhizal fungi (AMF), saprotrophic fungi (SF), and actinomycetes.

Where year and the year × crop phase interaction was significant, the SLICE and SLICEDIFF statements were included in the LSMEANS line of code to separate out effects of crop phase for each individual level of year (2014 and 2015).. After first running the GLIMMIX models, residual plots for certain response variables indicated a few violations of equal variance. Several alternative distributions were tried with the GLIMMIX models, and it was determined that for models analyzing total biomass, F:B, and mol % of Gm+, Gm-, and actinomycetes as response variables, a normal (Gaussian) distribution was the best fit. The best-fitting models for analysis of mol % of AMF and SF were GLIMMIX models run within the log-normal distribution, thus interpretation of the AMF and SF data will be in terms relative to other crop phases within this experiment

only. Outliers in all models with residuals well outside of two standard deviations from the mean were removed from analysis. Specific data points removed can be found in Appendix II. A Tukey adjustment was performed to more accurately separate means and limit type I error at the $\alpha = 0.05$ level.

Microbial community analysis

Multivariate analysis of principal components (PCA) was performed on negative arcsine-transformed mol % of the lipid biomarkers listed in Table 2.1 in order to determine microbial community structure within each aggregate fraction and year in JMP Pro version 12.2.0 (SAS Institute Inc., Cary, NC). Variability contributed by effect of subsamples (split-plot treatments used as covariates) within crop phase within block within year (year/block/crop/subsample) was tested for first two principal components (PC1 and PC2) using linear mixed-effect (LME) models in RStudio version 3.2.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Variability contributed to the principal components by each variable was standardized and calculated as described in Oates et al. (2012). Effect of crop phase on PC1 and PC2 was determined separately by aggregate fraction using PROC GLIMMIX in SAS version 9.4. In these models, a Tukey adjustment was performed to more accurately separate means and limit type I error at the $\alpha = 0.05$ level. These data were analyzed using the lognormal distribution to provide an improved model fit.

Results

Crop Phase Effects on Total Biomass and F:B

Crop phase significantly affected total lipid biomass associated with the largest soil particles, M ($p = 0.013$), but did not significantly affect biomass associated with the

m or s+c soil fractions ($p = 0.43$ and $p = 0.80$, respectively; Table 2.2). A significant year × crop phase interaction ($p < 0.0001$) also existed in the M fraction, but did not for m or s+c ($p = 0.27$ in both cases). Thus, the effect of crop phase on total biomass in M was analyzed over each year separately. This analysis revealed that the effect of crop phase on total lipid biomass was significant only in 2015 ($p < 0.0001$). Total lipid biomass in the wheat phase averaged over 50% higher than corn or soybean (0.348, 0.230, and 0.223 μmol g soil^{-1}, respectively, $p < 0.0001$) and the corn and soybean phases were not different from one another. In 2014, the effect of crop phase on total biomass associated with M was not significant at the 0.05 level ($p = 0.053$). Lipid biomass totaled 0.195, 0.226, and 0.187 μmol g soil^{-1} for corn, soybean, and wheat respectively. Total lipid biomass associated with M in all three phases was generally larger in 2015 than 2014 overall, most markedly in the wheat phase between the two years by nearly 50% ($p < 0.0001$).

Though the year × crop phase interaction was not significant, a significant year effect ($p < 0.0001$) in the m fraction was observed, thus this fraction was also analyzed separately over the two levels of year. Average total biomass associated with m in 2014 was 0.175, 0.205, and 0.198 μmol g soil^{-1} for the corn, soybean, and wheat phases respectively. The 2015 lipid biomass totaled 0.227, 0.247, and 0.276 μmol g soil^{-1} for corn, soybean, and wheat respectively. Though 2015 m-associated lipid biomass was observed to be larger than 2014 ($p < 0.0001$), this was not explained by crop phase differences.

The full statistical model showed that crop phase had a significant effect on F:B associated with M and m soil fractions ($p = 0.0005$ and $p = 0.013$, and was not significant

at the 0.05 level ($p = 0.056$) in the s+c fraction. All three soil fractions had significant year × crop phase interactions ($p < 0.0001$ in all cases); as such, each fraction was analyzed separately by year. In 2015, F:B in the M fraction of wheat and corn averaged > 1, indicating total fungal lipid biomarkers were greater than the total of bacterial lipid biomarkers. Bacterial biomarkers dominated M fractions in the soybean phase in 2015, and all three crop phases in 2014. The two smaller soil fractions (m and s+c) always had a F:B < 1 indicating bacterial dominance.

As with the total biomass, no significant effect of crop phase at the 0.05 level was observed on F:B in 2014 for either the M or m soil fractions ($p = 0.22$ and 0.10 respectively). However, differences were observed within these fractions in the 2015 soil samples ($p = 0.002$ in M; $p = 0.02$ in m). In 2015, M F:B of the wheat phase was approximately 1.5 times that of corn or soybean ($p < 0.0001$), and m F:B was over 25% greater in wheat versus corn or soybean ($p < 0.0001$). The s+c soil fraction showed significant crop effects in both 2014 and 2015 ($p = 0.02$ and $p = 0.04$ respectively). In 2014, s+c F:B was greatest in corn (0.296), while soybean and wheat F:B was 0.247 and 0.258 respectively (LSD = 0.033). In 2015, the s+c F:B was greatest in wheat (0.542), with corn and soybean at 0.433 and 0.429 respectively (LSD = 0.085). Along with the total biomass, F:B increased in all soil fractions between 2014 and 2015 ($p < 0.0001$). Potential influences on these factors, such as increases in specific microbial ecological groups, are investigated further through analysis of ecological group relative abundance.

Crop Phase Effects on Relative Abundance of Microbial Ecological Groups

For each ecological group (Gm+, Gm-, AMF, SF, and Actinomycetes) and soil aggregate fraction (M, m, s+c), the interaction of year × crop phase on relative abundance

(mol %) was significant ($p < 0.05$; Figures 2.1 and 2.2 a-e), with the exception of Gm- biomarkers associated with m ($p = 0.32$) and SF biomarkers associated with s+c ($p = 0.24$). In order to determine differences resulting from this interaction, all groups were analyzed separately by year as well as soil aggregate fraction.

Crop phase effects differed in 2014 versus 2015. In 2014, the effect of crop phase on relative abundance of groups in M was significant for all groups except for Gm+ and actinomycetes ($p = 0.95$ and 0.73 respectively). The Gm- group was approximately 10% more abundant in M of the corn phase versus soybean and wheat ($p = 0.0001$ and 0.004 respectively). AMF relative abundance of both the corn and wheat phases was over 1.5 times greater than the soybean phase ($p < 0.0001$ and $= 0.008$ respectively). The other fungal ecological group we investigated, SF, had greater relative abundance by approximately 1.5 times in soybean versus corn and wheat ($p = 0.0007$ and 0.002 respectively). In 2015, relative abundance of all groups associated with M fractions showed significant effects of crop phase except Gm- ($p = 0.23$). For Gm+, wheat had about 25% lower relative abundance than the corn or soybean phases ($p = 0.0003$ and < 0.0001 respectively). AMF relative abundance in wheat was more than twice that in corn or soybean ($p < 0.0001$), while actinomycete relative abundance was approximately 30% higher in corn and soybean than wheat ($p < 0.0001$). The effect of crop phase on M SF mol % was also significant in 2015, responding similarly but to a lesser degree than 2014. Relative abundance of SF in corn and soybean phases was greater by about 30% than the wheat phase ($p = 0.0006$ and 0.002 respectively).

Relative abundance of ecological groups in m was slightly less affected by crop phase than those in the M fraction, and effects were reduced in 2014 versus 2015. In

2014, only the Gm+ and actinomycete groups were significantly affected by crop phase. The proportion of Gm+ lipids (calculated as mol %) was approximately 10% lower in the corn phase than soybean or wheat ($p < 0.0001$ and $= 0.0005$ respectively). The proportion of actinomycete lipids was also significantly lower by about 10% in corn versus soybean or wheat ($p = 0.0001$ and 0.002 respectively). In 2015, the relative abundances of Gm+, actinomycetes, AMF, and SF in m were all significantly affected by crop phase. The proportion (mol %) of Gm- lipids were not different with respect to crop phase ($p = 0.44$). Proportions of Gm+ lipids were slightly reduced in wheat compared with corn and soybean ($p = 0.0006$ and 0.006 respectively), while AMF relative abundance showed the strongest crop effect with the relative proportion of AMF in wheat nearly twice that of corn or soybean ($p < 0.0001$). In the actinomycete group, corn had approximately 10% greater relative abundance than wheat ($p = 0.007$), while soybean did not differ from either of the other two crop phases. Similarly for SF, corn had a higher relative abundance than wheat by over 10% ($p = 0.014$) while soybean did not differ from the other two crop phases.

In the smallest soil fraction, s+c, crop phase affected the relative abundance of all ecological groups with the exception of SF ($p = 0.22$). Relative abundance of Gm+, Gm-, and actinomycetes was increased in the soybean and wheat phases over the corn phase. Gm+ relative abundance was higher in soybean and wheat versus corn by nearly 1.5 times ($p = 0.0001$ and 0.002 respectively). Similarly, Gm- and actinomycete relative abundance was increased in soybean and wheat phases compared with the corn phase by approximately 30% ($p = 0.0002$ and 0.044 respectively for Gm-; $p = 0.0002$ and 0.025 respectively for actinomycetes). For AMF, wheat had a higher relative abundance than

corn by approximately 1.5 times ($p = 0.007$) while soybean did not differ from the other crop phases. In 2015, AMF was the only group in the s+c fraction affected by crop phase with over twice the relative abundance in the wheat phase versus corn or soybean ($p < 0.0001$).

Several trends emerged with respect to relative abundance of microbial ecological groups. Between 2014 and 2015, the proportion of AMF lipids increased in all soil fractions ($p \leq 0.0004$), though this increase was primarily observed in the wheat phase. This distinct difference in relative abundance between the two sampling years was not consistent among the three soil fractions for any other group.

Crop Phase Effects on Microbial Community Composition

Six separate principal component analyses (PCAs) were run to assess microbial community composition, one for each of the three soil fractions and each year investigated, due to the significant year × crop phase interactions observed in ecological group relative abundances (Figures 2.3 and 2.4 a-c). This section will describe each of these analyses by soil fraction separately.

Microbial community composition of the M fraction

In the M soil fraction, variability in microbial community composition was explained most by year (21%) and crop phase (34%). In 2014, principal component 1 (PC1) accounted for 50.4% of variability in microbial composition. Lipid biomarkers correlated most positively with PC1 included five Gm+ lipids: i15:0, a15:0, i16:0, i17:0, and a17:0, one Gm- lipid, cy19:0, as well as the actinomycete biomarkers 10Me16:0 and 10Me18:0. Biomarkers that were negatively correlated with PC1 included the AMF

biomarker *cis*16:1ω5 as well as two SF biomarkers *cis*18:2ω6,9 and *cis*18:1ω9. Effect of crop phase was not significant for PC1 ($p = 0.83$).

In 2015, principal component 1 (PC1) accounted for 71.1% of variability in microbial composition. Lipid biomarkers correlated most positively with PC1 in this year included all six Gm+ lipids: i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0, one Gm- lipid, cy19:0, as well as the actinomycete biomarkers 10Me17:0 and 10Me18:0. Biomarkers that were negatively correlated with PC1 included the AMF biomarker *cis*16:1ω5, one Gm- biomarker, *cis*18:1ω7, and one SF biomarker, *cis*18:2ω6,9. Effect of crop phase was significant at the 0.05 level for PC1, though marginally so ($p = 0.049$). Soybean and wheat were the only two crop phases that differed significantly ($p = 0.049$), with PC1 values for soybean being more negative and more positive for wheat. This indicates that lipid biomarkers (and associated ecological groups) with negative values (AMF, the *cis*18:1ω7 Gm- biomarker, and one SF biomarker) drove variability in community composition in the soybean phase. Lipid biomarkers with positive values (the six Gm+ lipids, the cy19:0 Gm- biomarker, and two actinomycete biomarkers) drove community composition variability in wheat.

Variability in principal component 2 (PC2) was explained by year (16%) and spatial variation (subsample 37%) along with crop phase (24%). In 2014, PC2 accounted for an additional 16.4% of total microbial community variability. Biomarkers that were most positively correlated with PC2 included the two SF lipids (*cis*18:1ω9 and *cis*18:2ω6,9). Strong negative correlations were seen with PC2 and the lipid biomarker associated with AMF, *cis*16:1ω5, as well as one Gm- lipid, *cis*18:1ω7. The effect of crop phase was significant for PC2 ($p = 0.021$), though only corn and soybean phases were

significantly different from one another ($p = 0.019$). Soybean was associated with negative values of PC2, which correlated with the AMF biomarker and one Gm- biomarker, the lipid *cis*18:1ω7. Corn was associated with positive PC2 values. These values were correlated with the two SF biomarkers.

PC2 in 2015 accounted for an additional 13.4% of community variability. Biomarkers that were most positively correlated with PC2 included the two SF lipids (*cis*18:1ω9 and *cis*18:2ω6,9) and one actinomycete biomarker, 10Me16:0. Negative correlations were seen with PC2 and the AMF biomarker as well as several Gm- lipid biomarkers, *cis*18:1ω7, cy19:0, and *cis*16:1ω7. Effect of crop phase was stronger on PC2 in 2015 ($p = 0.002$), with the wheat phase separating from both corn and soybean significantly ($p = 0.004$ and 0.003 respectively). Corn and soybean were associated with negative values of PC2, and therefore the AMF and Gm- groups tended to drive variability for PC2 in these phases. The wheat phase was associated with positive values of PC2; SF drove this phase's variability for PC2.

Microbial community composition of the m fraction

PC1 of the m PCA was explained by year (11%) and spatial variation (block 5%; subsample 48%), as well as crop phase (21%). In 2014, PC1 accounted for 52.6% of microbial community variability, with the highest positive correlations between PC1 and lipid biomarkers observed in several Gm+ lipids (i15:0, a15:0, i16:0, and a17:0) as well as a single Gm- lipid (cy19:0) and one actinomycete lipid (10Me16:0). While no biomarkers were negatively correlated with PC1, the smallest positive correlation was between PC1 and the AMF biomarker *cis*16:1ω5. Statistical models showed that the crop phase effect was significant at the 0.05 level for PC1 ($p = 0.047$), with only corn and

soybean phases separating significantly ($p = 0.048$). The highest positive values of PC1 were associated with the corn phase, indicating that four of the Gm+ biomarkers as well as one Gm- (cy19:0) and one actinomycete lipid (10Me16:0) drove variability in this phase. Negative values were associated with the soybean phase, and these correlated somewhat (though not closely, as no biomarkers were negatively correlated with PC1) with the AMF biomarker.

In 2015, PC1 accounted for 40.9% of microbial community variability. Five Gm+ lipid biomarkers (i15:0, a15:0, i16:0, i17:0, and a17:0) were most positively correlated with PC1. Negative values of PC1 were most strongly correlated with the AMF biomarker *cis*16:1ω5 and more weakly with one Gm- biomarker, *cis*18:1ω7. Statistical models indicated that the three crop phases did not separate significantly at the 0.05 level along PC1 ($p = 0.062$).

PC2 variation was explained by year (41%), spatial variation (subsample 25%), and crop phase (31%). In 2014, PC2 accounted for 14.4% of community variation. Positive correlations between PC2 and lipid biomarkers occurred with AMF and SF lipids and one Gm+ lipid, i14:0. Negative correlations were observed most strongly with three of the six Gm+ biomarkers (a15:0, i17:0, and a17:0) and one Gm- biomarker, cy19:0. Crop phase did not significantly affect community composition along PC2 of microaggregates in 2014 ($p = 0.66$). Several sources list the biomarker i14:0 as Gm+ specific, so its association apart from the others in this case is interesting (Mentzer et al., 2006; Rinnan et al., 2008; Zelles et al., 1992).

An additional 14.0% of community variability was explained by PC2 in 2015. The two SF lipid biomarkers were most strongly correlated with positive PC2 values,

while two Gm- biomarkers (cy 19:0 and cy 17:0) as well as three Gm+ biomarkers (a15:0, i16:0, and i17:0) were correlated with negative PC2 values. Again, crop phase did not have a significant effect on PC2 in 2015 ($p = 0.13$).

Microbial community composition of the s+c fraction

For the s+c soil fraction, variation in PC1 was explained by year (39%) and spatial variability (subsample 35%) along with crop phase (23%). In 2014, PC1 accounted for 51.8% of microbial community variability. Lipid biomarkers with the highest positive correlation with PC1 included both Gm+ and Gm- biomarkers i15:0, cy19:0, *cis*18:1ω7, i17:0, *cis*16:1ω7, a15:0, and a17:0. No biomarkers were negatively correlated with PC1, but the SF biomarker *cis*18:2ω6,9 had the lowest positive correlation. The effect of crop phase was not significant on microbial community composition for PC1 ($p = 0.27$).

In 2015, PC1 in the s+c fraction accounted for 58.2% of microbial community variability. The actinomycete biomarker 10Me16:0, five Gm+ biomarkers (i15:0, a15:0, i16:0, i17:0, and a17:0), and one Gm- biomarker (*cis*16:1ω7) were correlated with positive values of PC1. No biomarkers were negatively correlated with PC1, but the actinomycete biomarker 10Me17:0 had the lowest positive correlation. The effect of crop phase was not significant on microbial community composition for PC1 ($p = 0.24$).

PC2 of the s+c fraction was explained by year (29%), spatial variation (subsample 39%), and crop phase (29%). In 2014 PC2 accounted for 11.9% of microbial community variation. The lipid biomarkers most positively correlated with PC2 were one Gm+ lipid (i14:0), one actinomycete lipid (10Me17:0), one SF lipid (*cis*18:2ω6,9). Negative correlations between PC2 and lipid biomarkers occurred most strongly with four

remaining Gm+ biomarkers (a15:0, i16:0, i17:0, and a17:0) and one Gm- biomarker (cy19:0). Crop phase effect was not significant for community composition along PC2 in 2014 ($p = 0.17$).

An additional 11.3% of community variability was accounted for by PC2 in 2015. Positive values of PC2 were associated most strongly with the AMF biomarker and one actinomycete biomarker (10Me17:0). Three Gm+ biomarkers (a15:0, i17:0, and a17:0), along with one Gm- biomarker (cy19:0) and one actinomycete biomarker (10Me16:0) were correlated most strongly with negative values of PC2. Statistical models indicated crop phase had a significant effect at the 0.05 level on PC2 in 2015 ($p = 0.045$), though once adjusted with the Tukey means separation procedure, no crop phases were significantly different at $\alpha = 0.05$.

Discussion

Previous research has shown that crop rotation can positively impact microbial biomass, including the overall diversity and types of crops included in the rotation (Ngosong et al., 2010; Roberts et al., 2011). Our results indicated that the M soil fraction was the only aggregate class within which total lipid biomass was affected by crop phase. Similarly, Zhang et al. (2014) found that effect of management on microbial biomass depends on aggregate size, and that effects were only seen in aggregates >250 μm. Of the three crop phases, wheat had the most lipid biomass as compared to corn and soybean, largely due to increases in fungal abundance and therefore higher F:B within the wheat crop phases. These effects were only observed in 2015 when sampling time was the same for the three crop phases, however, when differences in ground cover existed among the

three crop phases. Crop effects on abundance of specific microbial groups have been noted previously (Larkin and Honeycutt, 2006; Wortman et al., 2013). In our study, AMF was the microbial ecological group with abundance most significantly affected by crop phase. Increases in AMF relative abundance in wheat versus corn and soybean in 2015 provides some explanation for the increase in total biomass and F:B described earlier. Indeed, higher F:B values in M and m fractions of wheat indicate the microbial community composition in this phase is shifted towards a greater abundance of fungi compared with corn and soybean. Higher AMF relative abundance and F:B in the wheat phase in 2015, which is less-intensely tilled and had been disturbed less recently than the corn and soybean phases, supports our first hypothesis that crop phases with reduced tillage pressure would have increased abundance of fungi.

The observed differences in total biomass, F:B, and microbial ecological group relative abundance may also be affected by soil sampling time in addition to crop phase management. Baseline (2014) soil samples were collected post-harvest in July for wheat, September for soybean, and November for corn. At this time, all crop phases had been recently tilled and had no plants actively growing. 2015 samples were all taken in October, after corn and soybean harvest/tillage, and with a standing cover crop (oat/berseem clover) in the wheat phase. This plant material likely played a role in the increased AMF abundance observed in the wheat phase compared with corn and soybean in 2015. With roots for AMF to colonize, this group could proliferate to a greater degree as opposed to times during the production season when no roots were present as in the corn and soybean phases. The result is increased AMF abundance and total lipid biomass in wheat compared with corn and soybean, which we observed in 2015 when cover crops

were being grown but not 2014 without cover crops. Cover cropping can increase fungal abundance (Buyer et al., 2010; Lienhard et al., 2013; Wortman et al., 2013), supporting our results. As crop rotational phase shifts in plots from year to year, sampling within different physical locations for each crop phase in each year may have also contributed to the observed differences between the two years.

Lesser crop effects on microbial ecological group abundance were observed for SF, Gm+, actinomycetes, and finally Gm- (with almost no notable crop effect) as compared with AMF, particularly as soil fraction size decreased (Figures 2.2 and 2.3). Slightly increased relative abundance of Gm+, SF, and actinomycetes was seen in corn and soybean compared with wheat in 2015, though crop phase effects were less consistent in 2014. This finding is supported by Buyer et al. (2010), who found that Gm+ abundance was higher in non-cover cropped treatments. Denef et al. (2009) showed that Gm+ and actinomycete groups are less involved in processing rhizodeposited carbon. It is possible that a lack of readily available rhizodeposited carbon in the corn phase due to lack of cover crops favored groups that obtain carbon from other sources, such as more recalcitrant materials (Linn and Doran, 1984). The corn and soybean phases were each sampled close to harvest in both seasons and therefore close to plant maturity and senescence, where bacterial abundances have been observed to be highest (Ngosong et al., 2010). A senescing plant community may have decreased labile carbon sources available to microbes; increased recalcitrance of carbon sources has been linked to increased Gm+ and actinomycete abundance (Linn and Doran, 1984). Decreased SF abundance in wheat compared with corn and soybean in 2015 is not as intuitive, however, as high intensity of tillage in corn and soybean phases would be thought to disrupt fungal

hyphal networks as described in Lienhard et al. (2013). Buyer et al. (2010) found increased proportions of fungi (AMF and otherwise) in cover cropped treatments; we did not see this trend with SF in our cover-cropped wheat. Conversely, Mbuthia et al. (2015) noted increased SF abundance in tilled soils compared with no-till, supporting our results as corn and soybean receive much heavier tillage than wheat. This increase was attributed to certain groups of fungi being known to adapt well to environmental stresses.

Soil fungi, including AMF, are known to be important in the formation of M, encouraging formation of m thereby stabilizing soil carbon (Bossuyt et al., 2001; Rillig and Mummey, 2006). In Chapter I, we found differences among crop phases in soil aggregate distribution, with the corn phase in 2014 and both corn and soybean in 2015 having a higher proportion of M than wheat in either year. Higher aggregation in the corn and soybean phases would suggest that factors known to induce M formation (such as increased abundance of fungi) would be enhanced in this phase as has been observed elsewhere (Bossuyt et al., 2001). However, the data presented here do not always support this. We found that the wheat phase had the highest relative abundance of AMF (along with corn in 2014 and alone in 2015) and highest F:B in most soil fractions, while soybean had the highest relative abundance of SF in M in 2014 alone. For AMF, differences in relative abundance arose from 2015 samples, in which wheat was sampled during a standing cover crop while corn and soybean had minimal to no plant growth. This lead to increased AMF abundance in the wheat phase in 2015 in part because AMF were able to form mycorrhizal associations with plant roots later in the season compared with corn and soybean. Despite high AMF abundance, the wheat phase did not exhibit increased aggregation in either year. SF have also been suggested as important for soil

aggregation processes (Lehmann and Rillig, 2015). Higher relative abundance in corn and soybean in 2015 where aggregation was highest may support this, but is inconsistent with 2014 where reduced aggregation was observed in soybean compared with corn when soybean had increased SF abundance.

High AMF relative abundance and F:B in wheat in 2015, and higher SF relative abundance in soybean, where we saw lower soil aggregation compared with corn in 2014, did not support our hypothesis that fungal abundance could be associated with observed differences in aggregate distribution among crop phases, at least within the crop phase the fungal abundance was measured. However, effects of management in one crop phase may have lag time and carry over into the next phase of a rotation. Cover crops in wheat are incorporated the following spring prior to corn planting. Schutter et al. (2001) showed that spring-incorporated cover crops can enhance fungal abundance in the crop phase in which they were incorporated. If this is the case in our study, corn (as the crop phase following wheat/cover crop) should have increased fungal abundance during the early growing season, potentially influencing soil aggregation during this phase. Additional sampling events during the early growing season would be necessary to confirm this hypothesis.

Composition of the microbial community as determined by PCA was only minimally affected by crop phase, and these effects diminished with decreasing soil fraction size. Reduction in community sensitivity with decreasing aggregate size to different cropping regimes has been observed previously (Trivedi et al., 2015). In our study, differences in community composition were only detectable in M and m soil fractions. The observed differences in groups which drive variability in community

composition did not correlate with our relative abundance results. In M, the community separated marginally along PC1 in 2015 alone and significantly along PC2 in both years. PC1 and PC2 in 2015 accounted for 71.1% and 13.4% of community variation respectively, while PC2 in 2014 accounted for 16.4% of variability. In both years, the PCA indicated that the soybean phase was driven mainly by the Gm- biomarkers and the AMF biomarker *cis*16:1ω5. The wheat phase was driven by SF biomarkers *cis*18:1ω9 and *cis*18:2ω6,9 or Gm+ biomarkers, which contrasts with our result that SF relative abundance was lower in wheat than corn and soybean, and AMF abundance in M of wheat was higher than soybean in both years. Though AMF relative abundance was lower in soybean compared with wheat, it is possible that this group still drives the community composition if its mol % is overall greater as compared with other groups. As organic fertilizer amendments (poultry manure) are not used in the soybean phase, perhaps soybeans rely on AMF to obtain limiting nutrients such as phosphorous (P). AMF is generally limited in its ability to colonize plant roots if nutrients are readily available, including those provided by organic fertilizers (Gosling et al., 2006). Where corn separated from the other two crop phases along PC2, community variability was driven by SF biomarkers in 2014 and AMF and Gm- biomarkers in 2015. This is again in contrast with our ecological group relative abundance results as SF abundance in M of corn was lower than soybean in 2014 and AMF abundance was lower than wheat in 2015.

For m community composition, separation based on crop phase was only observed along PC1 in 2014 between corn and soybean, accounting for 52.6% of community variation. The corn phase community variability was apparently driven by Gm+, Gm-, and actinomycete biomarkers. This finding was supported by Buyer et al.

(2010), as non-cover cropped treatments (such as the corn phase in our rotation) were shown to increase Gm+ abundance. Again the soybean community composition appeared to be driven by the AMF biomarker. This relationship was weaker in m, however, as soybean was correlated with negative values of PC1 for which no biomarkers were associated. The AMF biomarker came closest with the smallest positive PC1 values. Perhaps at the m scale, carbon sources are different from larger aggregates, influencing microbial ecological group abundance.

These results support our hypothesis that microbial community composition would vary to some degree by crop phase, but emphasize that a great deal of unexplained variability exists in regard to the results of our analysis. Variability was only partially explained by temporal (year), spatial (block, subsample) and management (crop phase) effects. There are likely many other factors including environmental variables (e.g. temperature, moisture) and soil properties (e.g. pH, nutrient composition) that we did not consider within the context of this study which impact community composition. Temperature, moisture and pH are important predictors of bacterial community composition (Buyer et al., 2010; Lauber et al., 2008; Ngosong et al., 2010), while soil nutrients are better predictors of fungal composition (Lauber et al., 2008; Suzuki et al., 2009).

Conclusions

This study investigated whether microbial community composition is an underlying factor in the observed crop phase effect on soil aggregation. Total biomass and relative abundance of the five microbial ecological groups investigated were affected by crop phase, though these effects differed with year and decreased with decreasing soil

aggregate size. In 2015, when sampling time was the same across all three crop phases, the wheat phase with a standing cover crop had a greater total biomass, higher relative abundance of AMF, and higher F:B than corn and soybean with none, emphasizing the important impacts of cover crops on community composition and abundance of specific microbial ecological groups. Corn and soybean tended to have higher relative abundance of Gm+ and actinomycetes in 2015 indicating increased disturbance in these phases. Differences in microbial community composition were also observed, though these were mainly restricted to larger soil aggregate fractions and did not reflect which ecological groups were most abundant. SF was a driver of community variability in M of the wheat phase, while AMF was a primary driver of microbial community variability in soybean. We did not find evidence for a direct link between fungal abundance and level of soil aggregation observed in crop phases within the year the phase was sampled. There is evidence that effects of management may carry over into the following season, affecting community composition in the next crop phase of the rotation. We hypothesize that this may be the case in our rotation, as the cover-cropped wheat phase may induce increased AMF abundance during the early growing season of the corn phase in the following year. Moreover, microbial community composition is affected by a wide range of factors beyond agricultural management that we did not account for (e.g. environmental, physical). These variables likely impact community composition in ways that are not yet well-understood. Additional sampling events throughout the season would provide more insight into the effects of time within the growing season, management events, and environmental factors such as soil temperature and moisture on microbial communities.

Tables and Figures

Table 2.1 Lipid biomarkers used in analysis of microbial ecological groups. Lipids used were present in amounts higher than 0.5 mol %.

Microbial ecological group	Lipid biomarkers corresponding to group
Gram-positive bacteria (Gm+)[a,b]	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0
Gram-negative bacteria (Gm-)[b]	*cis*16:1ω7, cy17:0, *cis*18:1ω7c, cy19:0
Arbuscular mycorrhizal fungi (AMF)[c]	*cis*16:1ω5
Saprotrophic fungi (SF)[c,d]	*cis*18:1ω9, *cis*18:2ω6,9
Actinomycetes[d]	10Me16:0, 10Me17:0, 10Me18:0

References:
[a] Zelles et al.(1992)
[b] Frostegard et al. (1993)
[c] Balser et al. (2005)
[d] Federle et al. (1986)

Table 2.2 Total lipid biomass, F:B, and associated p-values in the organic grain rotation at WICST, 2014-2015.

Crop phase, year, and soil fraction†	Total Biomass (μmol g soil^{-1})	F:B
Corn 2014		
M	0.195(0.005)‡	0.697(0.022)
m	0.175(0.018)	0.399(0.024)
s+c	0.108(0.014)	0.295(0.016)
Corn 2015		
M	0.228(0.012)	1.006(0.077)
m	0.227(0.007)	0.600(0.010)
s+c	0.105(0.004)	0.433(0.007)
Soybean 2014		
M	0.226(0.018)	0.756(0.085)
m	0.205(0.011)	0.343(0.013)
s+c	0.098(0.007)	0.249(0.009)
Soybean 2015		
M	0.223(0.011)	0.872(0.058)
m	0.247(0.009)	0.616(0.022)
s+c	0.106(0.005)	0.429(0.009)
Wheat 2014		
M	0.187(0.011)	0.641(0.039)
m	0.198(0.009)	0.409(0.021)
s+c	0.097(0.011)	0.258(0.016)
Wheat 2015		
M	0.348(0.016)	1.512(0.070)
m	0.276(0.014)	0.821(0.028)
s+c	0.121(0.005)	0.542(0.017)

	ANOVA p-values					
	Total Biomass			F:B		
Soil Fraction	Crop	Year	Year × Crop	Crop	Year	Year × Crop
M	0.0134	<0.0001	<0.0001	0.0005	<0.0001	<0.0001
m	0.4305	<0.0001	0.2713	0.0130	<0.0001	<0.0001
s+c	0.8026	0.1558	0.2652	0.0564	<0.0001	<0.0001

†M = macroaggregates; m = microaggregates; s+c = silt and clay.
‡ Numbers in parentheses indicate standard errors of displayed means.

Table 2.3 P-values of fixed effects for statistical models determining significant differences among crop phases for relative abundance of microbial ecological groups by soil fraction, 2014-2015.

Ecological group and soil fraction†	ANOVA p-values		
	Crop	Year	Year × Crop
Gm+			
M	0.0606	<0.0001	0.0005
m	0.0203	0.0426	<0.0001
s+c	0.1415	<0.0001	0.0002
Gm-			
M	0.0518	0.1156	0.0077
m	0.6856	0.0002	0.3190
s+c	0.1338	<0.0001	0.0003
AMF			
M	0.0119	0.0004	<0.0001
m	0.0331	<0.0001	<0.0001
s+c	0.0127	<0.0001	0.0037
SF			
M	0.0047	0.8716	0.0244
m	0.3315	<0.0001	0.0020
s+c	0.5402	<0.0001	0.2435
Actinomycetes			
M	0.0325	0.0626	0.0009
m	0.0123	0.0018	0.0018
s+c	0.1493	<0.0001	0.0017

†Gm+ = gram positive bacteria; Gm- = gram negative bacteria; AMF = arbuscular mycorrhizal fungi; SF = saprotrophic fungi; M = macroaggregates; m = microaggregates; s+c = silt and clay.

Figure 2.1 Average mol% of five microbial ecological groups separated by soil fraction for 2014. Error bars represent standard error of displayed means. Where statistical differences exist within soil fractions lowercase letters are shown. a) Gram-positive bacterial biomarkers (Gm+) b) Gram-negative bacterial biomarkers (Gm-) c) Arbuscular mycorrhizal fungi biomarker (AMF) d) Saprotrophic fungi biomarkers (SF) e) Actinomycete biomarkers. M = macroaggregates; m = microaggregates; s+c = silt and clay.

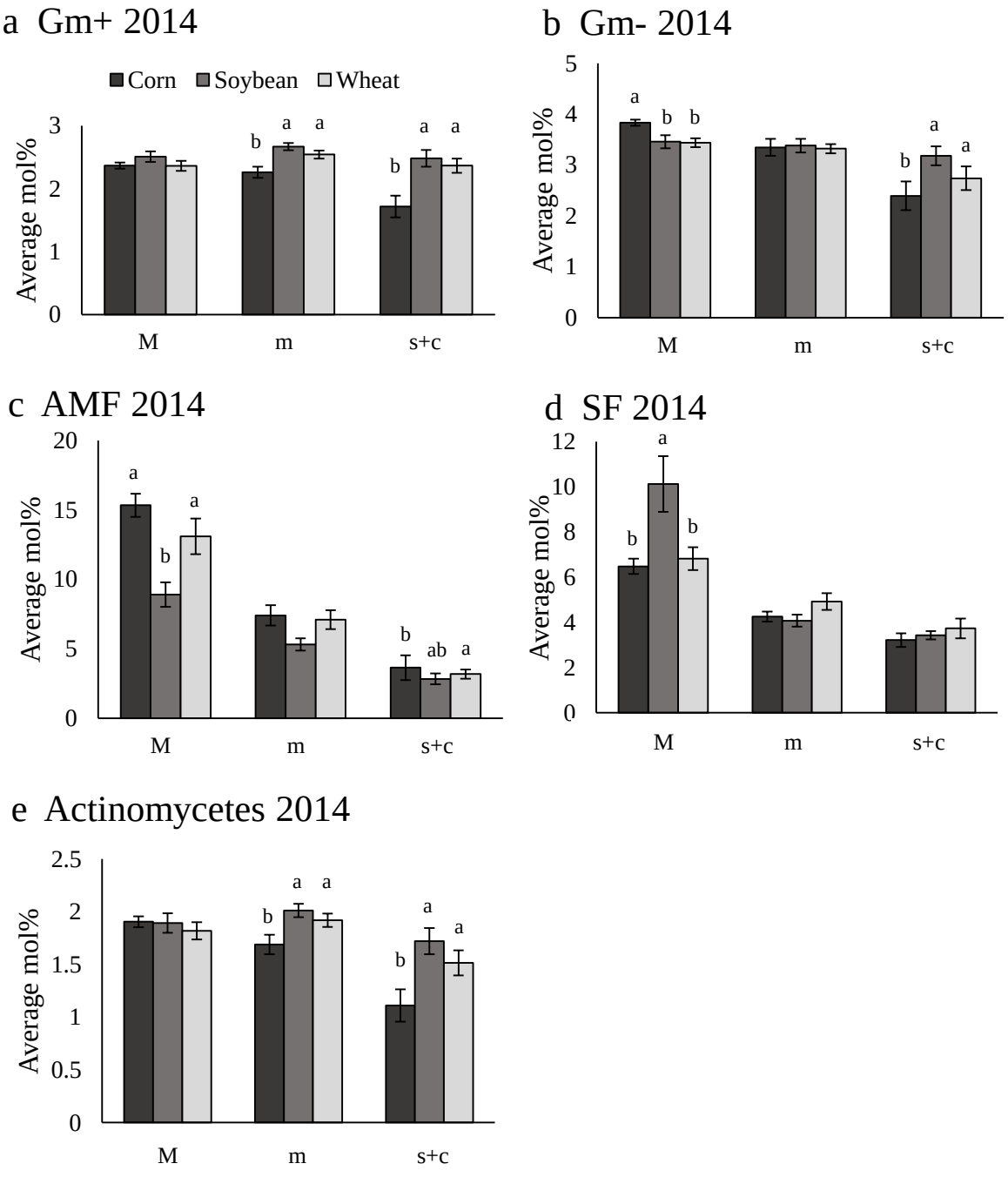


Figure 2.2 Average mol% of five microbial ecological groups separated by soil fraction for 2015. Error bars represent standard error of displayed means. Where statistical differences exist within soil fractions lowercase letters are shown. a) Gram-positive bacterial biomarkers (Gm+) b) Gram-negative bacterial biomarkers (Gm-) c) Arbuscular mycorrhizal fungi biomarker (AMF) d) Saprotrophic fungi biomarkers (SF) e) Actinomycete biomarkers. M = macroaggregates; m = microaggregates; s+c = silt and clay.

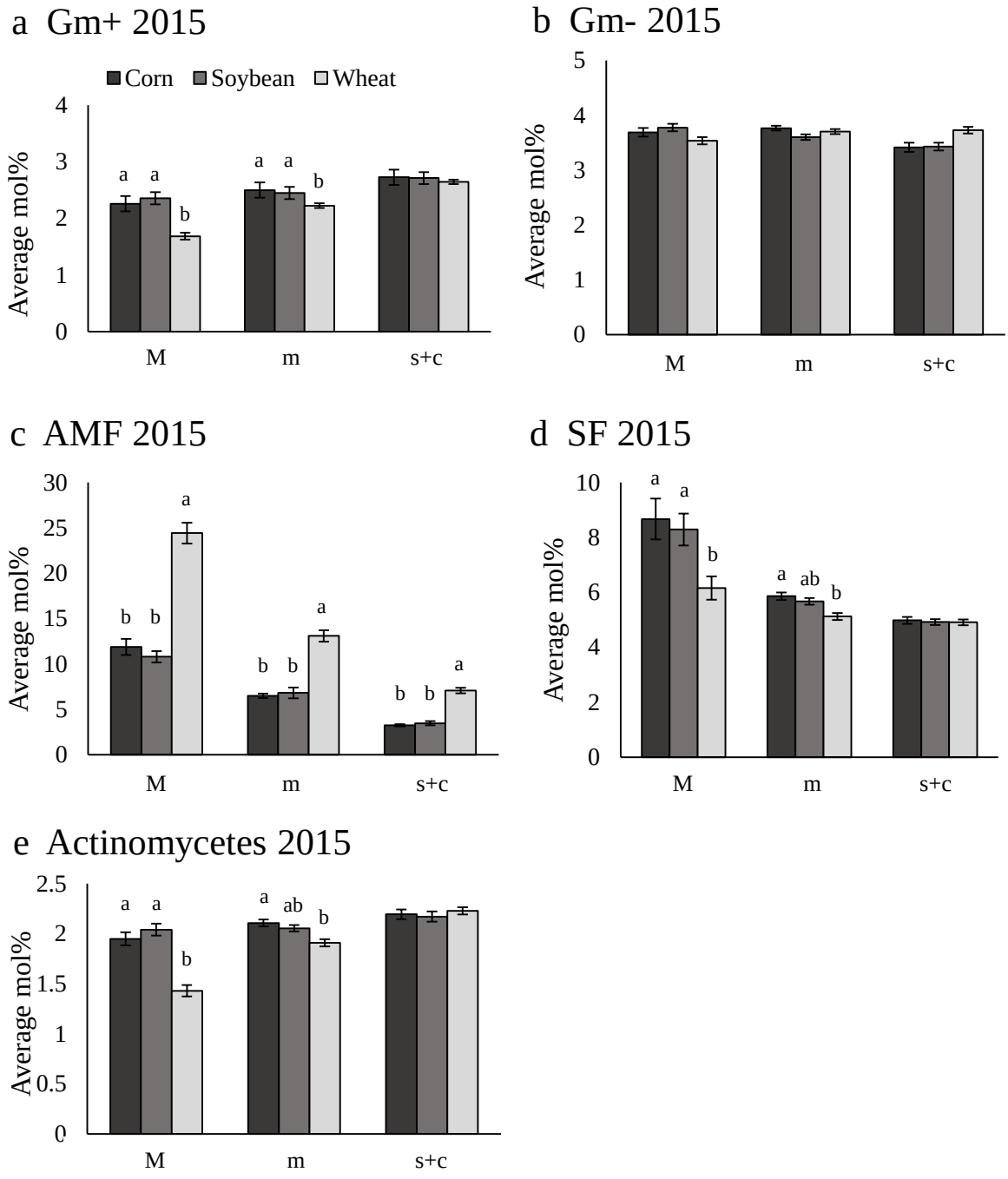


Figure 2.3 Plots of PC1 versus PC2 from multivariate PCA of lipid biomarkers of the corn, soybean, and wheat phases, separated by soil fraction, 2014. Significant separation among crop phases along PC1 and PC2 was determined through a general linear mixed effects model in SAS version 9.4. Error bars represent standard errors of the displayed PC means. a) Macroaggregates b) Microaggregates c) Silt and Clay.

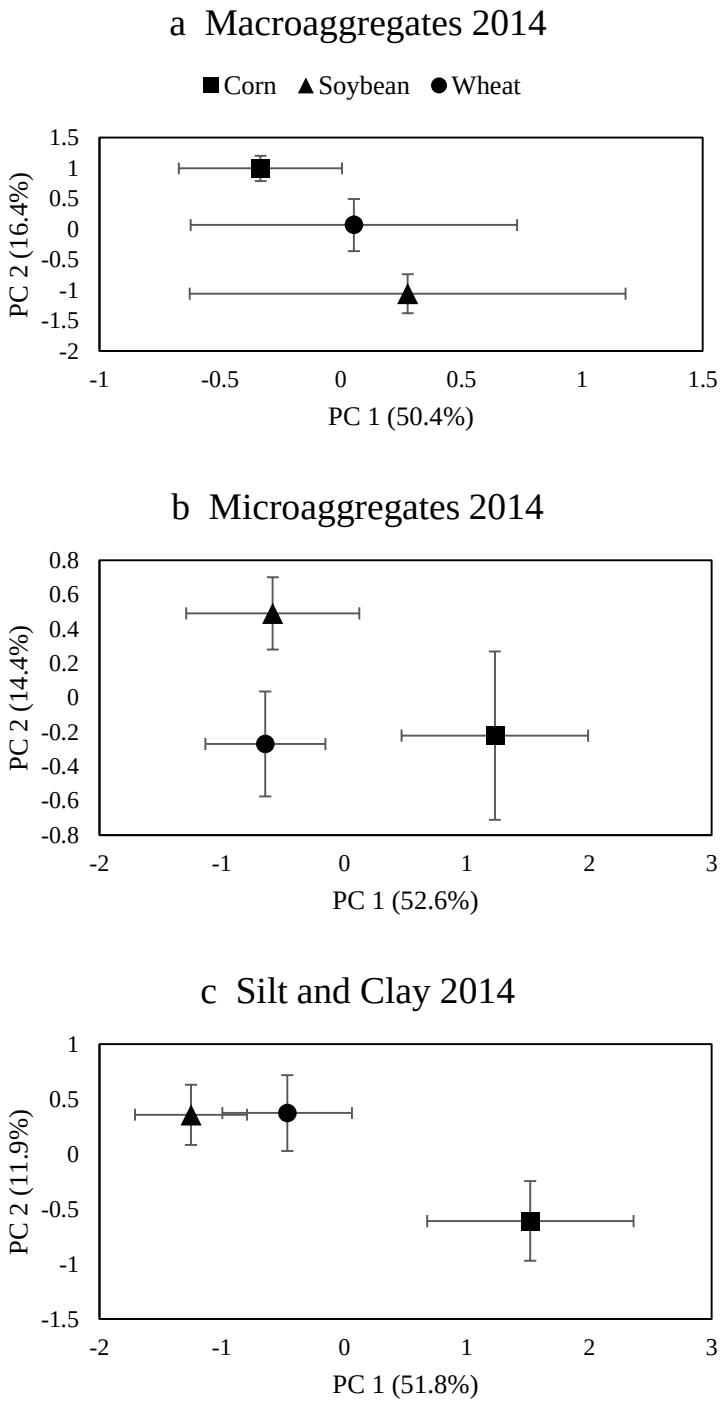
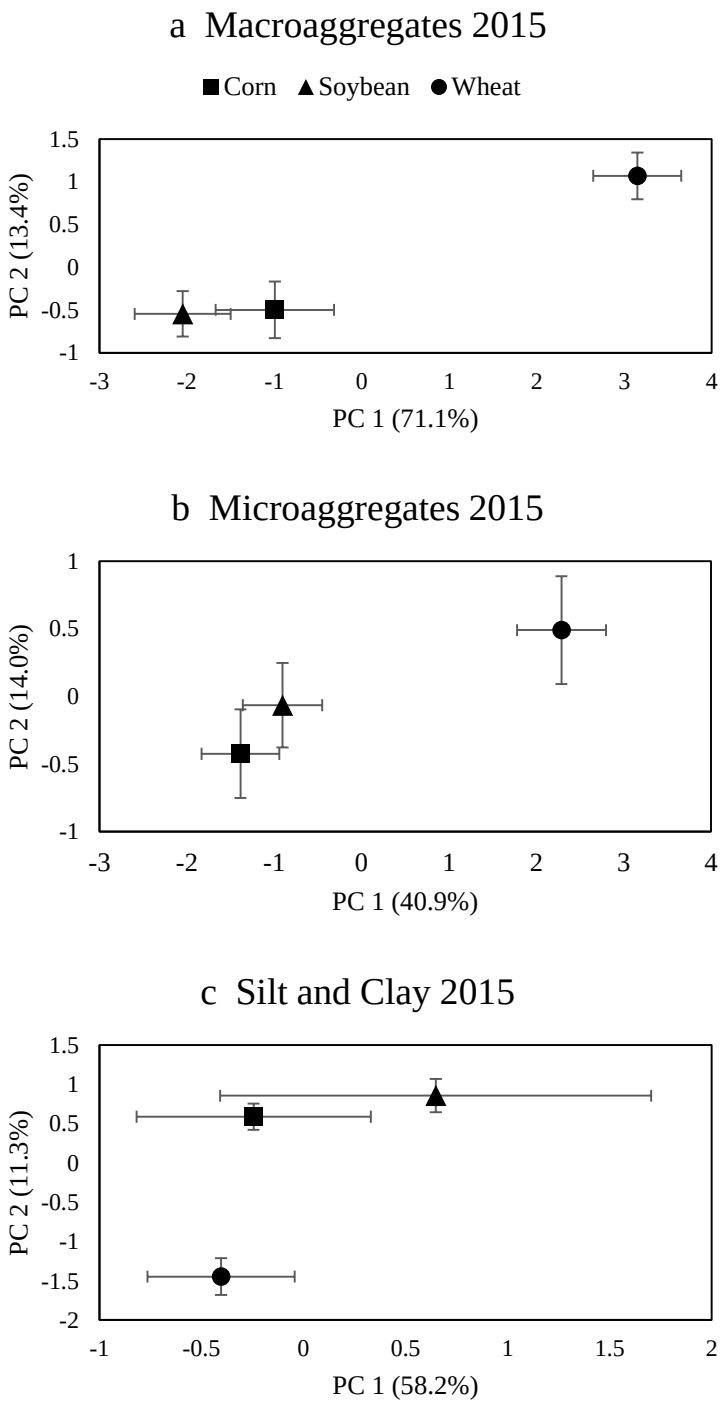


Figure 2.4 Plots of PC1 versus PC2 from multivariate PCA of lipid biomarkers of the corn, soybean, and wheat phases, separated by soil fraction, 2015. Significant separation among crop phases along PC1 and PC2 was determined through a general linear mixed effects model in SAS version 9.4. Error bars represent standard errors of the displayed PC means. a) Macroaggregates b) Microaggregates c) Silt and Clay.



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Conclusions

Our findings indicate that aggregate distribution differed significantly among crop phases, with the corn phase of 2014 and both corn and soybean in 2015 having a higher proportion of large soil aggregates (macroaggregates) and associated occluded aggregate fractions than the wheat phase in either year. The high level of aggregation in corn and soybean occurred in spite of an intensive tillage and cultivation regime. High soil C inputs also occur in the corn phase relative to both the soybean and wheat phases. While tillage is known to reduce the level of aggregation in soils compared with no-till (Baker et al., 2007; Sheehy et al., 2015; Six et al., 1998, 2000a), our findings and the findings of others (Andruschkewitsch et al., 2014; Williams and Petticrew, 2009) suggest that sufficient C input may be able to counteract negative effects of tillage on aggregate formation. Though we saw significant differences in aggregate distribution among crop phases, these differences did not result in a reallocation of aggregate C content in aggregate fractions from crop phase to crop phase. Aggregate C content was largely unaffected by crop phase, though spatial variation (differences between experimental plots) did appear to play a role as crop phases in 2015 often had similar levels of aggregate C content as their previous rotational phase in 2014. Other studies have shown that aggregate C content is sensitive to management changes (Crittenden et al., 2015; Doane et al., 2003; Whitbread et al., 2000), and that aggregates can serve as ideal indicators of early SOC dynamics (Denef et al., 2007), however, we did not observe this in our study. The lack of observable differences in C content due to crop phase is not surprising given the age (26 years) of this well-established rotation, which has likely

reached an equilibrium within experimental plots with respect to SOC content among the three crop phases.

With respect to the microbial community, we found that the wheat phase had a larger total biomass and higher fungal abundance (particularly of AMF) than either the corn or soybean phases in 2015 when sampling time was the same for all three phases. This finding was somewhat expected, as the wheat phase had an established oat/berseem clover cover crop at the time of sampling in 2015 as well as reduced tillage pressure, while corn and soybean had little to no vegetation and increased tillage pressure. Cover crops are known to increase microbial biomass as well as fungal abundance (Buyer et al., 2010; Wortman et al., 2013). Conversely, SF abundance was reduced in wheat versus corn and soybean in 2015. This result was surprising in that intensely-tilled soils, such as those in the corn and soybean phases, are known to reduce fungal abundance as hyphal networks are disrupted (Lienhard et al., 2013). Findings of Mbuthia et al. (2015), however, support our results in that increased abundance of SF was noted in tilled soils compared with no-till. Increased fungal abundance overall in the wheat phase in 2015, as indicated by increased F:B over corn and soybean, was inconsistent with our observation of increased aggregation in the corn phase. We would have expected that increased fungal abundance and higher aggregation would be observed simultaneously within the same crop phase, as fungi are known to be major drivers of the aggregation process (Bossuyt et al., 2001; Rillig and Mummey, 2006). These findings indicate that perhaps microbial community composition and abundance of specific microbial ecological groups within a specific crop phase is not an ideal indicator of soil aggregation. Additionally, limited significant differences observed with respect to which ecological groups describe

the majority of variability in community composition emphasize that perhaps the community is not extremely different among the crop phases in our study, at least at the time of year that we sampled.

Overall, our findings suggest that soil aggregation and abundance of particular microbial ecological groups are affected by specific management practices imposed in individual crop phases in organic grain rotations. This finding is important because much of the existing body of literature on SOC and soil aggregate dynamics, both at WICST and elsewhere, focuses on the differences between entire crop rotations instead of singular phases or between conventional and organic cropping systems (Andruschkewitsh et al., 2013; Birkhofer et al., 2008; Padbhushan et al., 2016). This is especially true in experiments conducted in long-term trials, where conclusions have been routinely drawn based on a single sampling event or samples taken within one crop phase instead of sampling multiple or all phases of a rotation (Cates et al., 2016; Jokela et al., 2011). Such studies limit their frame of inference by ignoring potential differences that might exist in soil structural and chemical makeup between crop phases within rotations, or by assuming cropping system soil structure and chemistry are constant. Reliance on a single phase of a rotation to represent an entire cropping system, and then using this information to compare to other entire cropping systems, may lead to inaccurate characterizations and therefore inaccurate management and policy recommendations. Additional sampling events, which account for variability of individual crop, should provide an improved ability to describe our soils in complex rotations, thus improving management recommendations for SOC retention developed from scientific findings. Future work should be directed at understanding seasonal variability in soil aggregate and microbial

community dynamics. By gaining an expanded picture of processes behind SOC storage and stabilization, we will enhance our ability to ensure that management strategies are appropriate to meet sustainability and soil health goals.

Previous literature has documented that crop management regimes affect soil aggregation, SOC content, and microbial biomass. Specifically, the positive impacts of organic production practices on soil structure, SOC content (Foereid and Høgh-Jensen, 2004; Gerhardt, 1997), and microbial biomass as compared to conventionally-managed soils have been shown (Birkhofer et al., 2008; Ullrich et al., 2011). However, within the organic grain rotation at WICST, SOC declined over 20 years and lower total aggregation was observed versus other cropping systems (Cates et al., 2016; Sanford et al., 2012) despite use of practices beneficial to SOC storage and soil quality including cover-cropping and manure application, thus demonstrating continued lack of clarity as to the more nuanced impacts of organic management in specific soil environments, particularly where background levels of SOC are high. While our findings are specific to organically-managed grain rotations in the C-rich Mollisols of the North Central U.S. corn belt, they are no less important as organic production methods gain popularity and acreage continues to expand in the U.S. Understanding SOC dynamics and provisioning of ecosystem services such as SOC stabilization or sequestration are key objectives in the organic community.

Appendix I: Photographs of aggregate fractionation equipment and setup

Figure A.1 Sieves and metal basins used in wet-sieving (Step A) of aggregate fractionation.

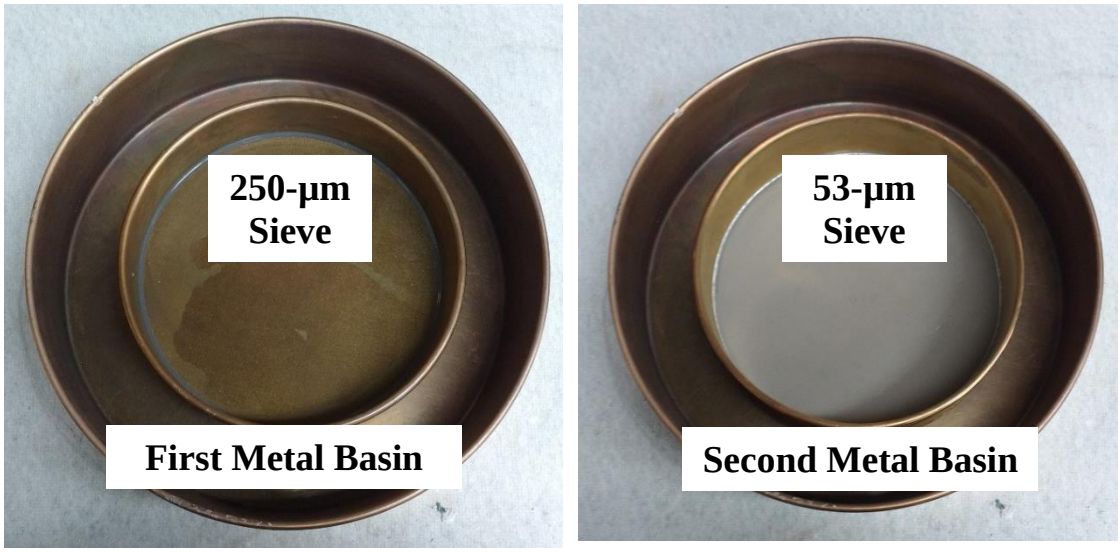


Figure A.2 Microaggregate isolator used in Step B of aggregate fractionation.

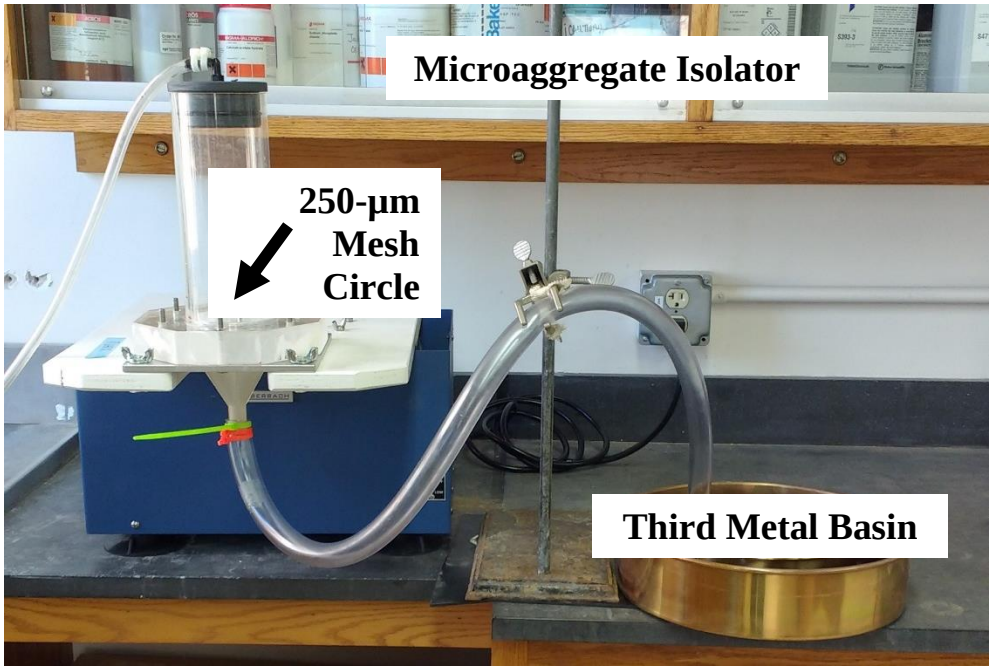
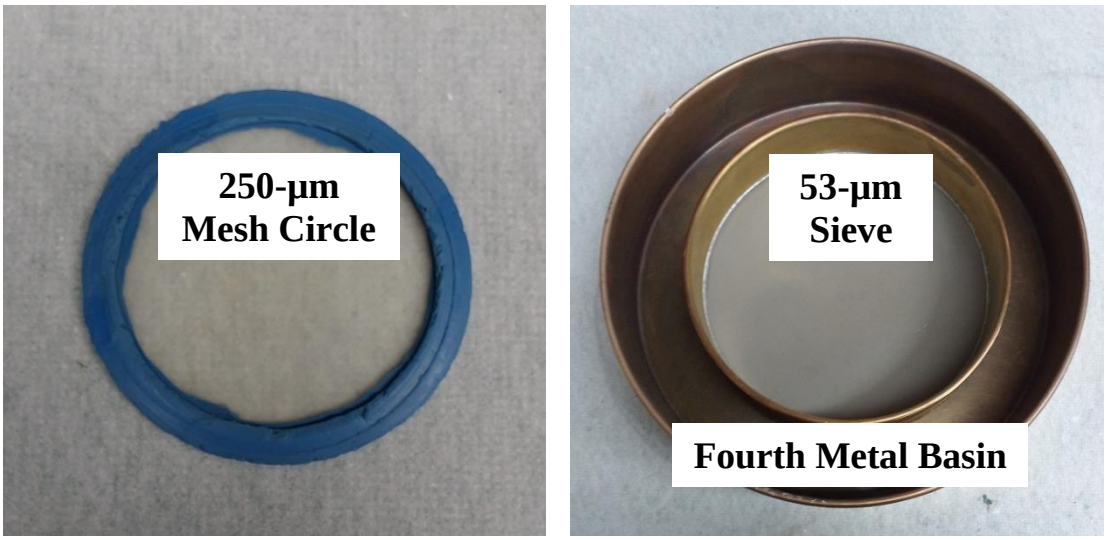


Figure A.3 250-µm mesh circle and final sieve and metal basin used in microaggregate isolation (Step B) of aggregate fractionation.



Appendix II: Statistical Code

Models for aggregate distribution, Chapter I

```
data soilprop;
infile 'C:/Users/Owner/Dropbox/Monica_Daane/Monica_Stats/Data Txt
Files/CERES soil wo BL.txt' firstobs = 2 expandtabs;
input loc$ year event$ plot sys$ crop$ rot$ block cov frac$ soil_wt
prop prop_ws c_pct actual_c_pct c_mg_g cpool_g biomass FB;

proc sort data=soilprop;
by frac;
run;

title 'aggregate proportions';
proc glimmix data=soilprop nobound plots=studentpanel;
by frac;
class year crop block;
model prop_ws=year crop year*crop cov; *using sp_trt as a covariate
does improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;
```

Models for aggregate C content, Chapter I

```
data soilprop;
infile 'C:/Users/Owner/Dropbox/Monica_Daane/Monica_Stats/Data Txt
Files/CERES soil wo BL.txt' firstobs = 2 expandtabs;
input loc$ year event$ plot sys$ crop$ rot$ block cov frac$ soil_wt
prop prop_ws c_pct actual_c_pct c_mg_g cpool_g biomass FB;

proc sort data=soilprop;
by frac;
run;

title 'aggregate proportions';
proc glimmix data=soilprop nobound plots=studentpanel;
by frac;
class year crop block;
model c_pct=year crop year*crop cov/dist=lognormal ddfm=kr; *using
sp_trt as a covariate does improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;
```

Correlations of Soil Aggregation (Macroaggregate Prop.) vs. C Inputs, Chapter I

```
data carbonagg;
input year  plot  crop$ AG    BG    Total macros      macroerr;
datalines;
2014  102   corn  5644  2415  8058  0.86937     0.007624
2014  313   corn  4660  1835  6495  0.85562     0.017984
2014  407   corn  5712  2455  8167  0.88343     0.008035
2014  106   soybean     2049  1084  3133  0.63115     0.036885
2014  307   soybean     2185  1155  3340  0.61655     0.01363
2014  411   soybean     2217  1172  3389  0.62043     0.012965
2014  104   wheat 2403  348   2751  0.69192     0.007483
2014  301   wheat 2546  560   3106  0.61668     0.018047
2014  402   wheat 2523  526   3048  0.63745     0.029738
2015  104   corn  6023  2559  8582  0.74265     0.01066
2015  301   corn  5976  2531  8507  0.67472     0.023569
2015  402   corn  5516  2260  7776  0.74303     0.013074
2015  102   soybean     2154  1139  3293  0.73375     0.013127
2015  313   soybean     1959  1036  2994  0.68645     0.015336
2015  407   soybean     2039  1078  3118  0.72288     0.012156
2015  106   wheat 2433  811   3244  0.6172      0.020913
2015  307   wheat 2460  863   3323  0.65923     0.021418
2015  411   wheat 2466  848   3314  0.64305     0.021112
;

proc corr;
var macros total; *macroaggregate corr. with total c input
run;

proc corr;
var macros AG; *macroaggregate corr. with aboveground c input
run;

proc corr;
var macros BG; *macroaggregate corr. with belowground c input
run;
```

Models for Total Microbial Biomass and F:B, Chapter II

```
data biofb;
infile 'C:/Users/Owner/Dropbox/Monica_Daane/Monica_Stats/Data Txt
Files/Microbial Data/Biomass FB.txt' firstobs = 2 expandtabs;
input Year Block Plot Crop$ cov Fraction$ Total Bac Fungi FB;

data a; set biofb; *removal of outliers, points with errors far beyond
2 SD of mean
if FB=2.2192 then delete;
if FB=2.3492 then delete;
if FB=1.7760 then delete;
if FB=1.6892 then delete;
if FB=0.7092 then delete;

if Total=0.9289 then delete;
```

```
if Total=0.5576 then delete;
if Total=0.4940 then delete;
if Total=0.3386 then delete;
if Total=0.2829 then delete;
if Total=0.2687 then delete;
run;

proc sort data=a;
by fraction;
run;

title 'microbial biomass';
proc glimmix data=a nobound plots=studentpanel;
by fraction;
class year crop block;
model total=year crop year*crop cov; *using sp_trt as a covariate does
improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;

title 'F:B';
proc glimmix data=a nobound plots=studentpanel;
by fraction;
class year crop block;
model FB=year crop year*crop cov; *using sp_trt as a covariate does
improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;
```

Models for Microbial Ecological Group Rel. Abundance, Chapter II

```
data relabund;
infile 'C:/Users/Owner/Dropbox/Monica_Daane/Monica_Stats/Data Txt
Files/Microbial Data/Guilds relabund.txt' firstobs = 2 expandtabs;
input Year Block Plot Crop$ Cov Fraction$ Gmpos Gmneg AMF Sapro Act;

proc sort data=relabund;
by fraction;
run;

data gmp; set relabund; *removal of outliers
if Gmpos=1.400 then delete;
run;

proc sort data=gmp;
by fraction;
run;


data gmn; set relabund; *removal of outliers
```

```
if Gmneg=2.001 then delete;
if Gmneg=4.364 then delete;
if Gmneg=4.167 then delete;
if Gmneg=4.177 then delete;
run;

proc sort data=gmn;
by fraction;
run;

data act; set relabund; *removal of outliers
if Act=0.922 then delete;
run;

proc sort data=act;
by fraction;
run;

data amf; set relabund; *removal of outliers
if AMF=1.407 then delete;
if AMF=1.400 then delete;
if AMF=14.619 then delete;
if AMF=8.653 then delete;
if AMF=10.458 then delete;
run;

proc sort data=amf;
by fraction;
run;

data sf; set relabund; *removal of outliers
if Sapro=9.446 then delete;
if Sapro=2.389 then delete;
if Sapro=8.484 then delete;
if Sapro=2.174 then delete;
if Sapro=7.180 then delete;
run;
proc sort data=sf;
by fraction;
run;

title 'Gm+';
proc glimmix data=gmp nobound plots=studentpanel;
by fraction;
class year crop block;
model gmpos=year crop year*crop cov; *using sp_trt as a covariate does
improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;


title 'Gm-';
```

```
proc glimmix data=gmn nobound plots=studentpanel;
by fraction;
class year crop block;
model gmneg=year crop year*crop cov; *using sp_trt as a covariate does
improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;

title 'Act';
proc glimmix data=act nobound plots=studentpanel;
by fraction;
class year crop block;
model act=year crop year*crop cov; *using sp_trt as a covariate does
improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;

title 'AMF';
proc glimmix data=amf nobound plots=studentpanel;
by fraction;
class year crop block;
model amf=year crop year*crop cov/dist=lognormal ddfm=kr; *using sp_trt
as a covariate does improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;

title 'SF';
proc glimmix data=sf nobound plots=studentpanel;
by fraction;
class year crop block;
model sapro=year crop year*crop cov/dist=lognormal ddfm=kr; *using
sp_trt as a covariate does improve residuals plots;
random block block*crop;
lsmeans year*crop/slice=year lines adjust=tukey;
lsmeans year*crop/slicediff=year lines adjust=tukey;
run;
```

Principal Component Analysis Performed in JMP, Chapter II

```
Principal Components(
      Y(
            :Name( "14:0 ISO" ),
            :Name( "15:0 ANTEISO" ),
            :Name( "15:0 ISO" ),
            :Name( "16:0 10 Methyl" ),
            :Name( "16:0 ISO" ),
            :Name( "16:1 w5c" ),
```

```
            :Name( "16:1 w7c" ),
            :Name( "17:0 10 Methyl" ),
            :Name( "17:0 ANTEISO" ),
            :Name( "17:0 CYCLO" ),
            :Name( "17:0 ISO" ),
            :Name( "18:0 10 Methyl" ),
            :Name( "18:1 w7c" ),
            :Name( "18:1 w9c" ),
            :Name( "18:2 w6,9c" ),
            :Name( "19:0 CYCLO" )
      ),
      Estimation Method( "Row-wise" ),
      "on Correlations",
      Arrow Lines( 1 ),
            Where( :Fraction == "mac" ),  **This analysis for macroaggregates
      specifically; Fraction == “mic” or “sc” for microaggregates or silt and
      clay, respectively**
      SendToReport(
            Dispatch(
                  {"Summary Plots"},
                  "PCA Summary Plots",
                  FrameBox,
                  {Frame Size( 49, 37 )}
            ),
            Dispatch(
                  {"Summary Plots"},
                  "PCA Summary Plots",
                  FrameBox( 2 ),
                  {Frame Size( 52, 37 )}
            )
```

Linear Mixed-Effect Models for Determination of Variability Contributed by Principal Component Analysis Factors performed in R, Chapter II

```
> setwd("C:/Users/Owner/Dropbox/Monica_Daane PCA")
> PCA1 <- read.table("PCs 1415.txt",header=TRUE,row.names=NULL)
>
> PCA1mac <-subset(PCA1, Fraction=="mac") *PC1, macroaggregates
> attach(PCA1mac)
> names(PCA1mac)
[1] "Year"     "Block"    "Plot"     "Crop"     "Subplot"  "Fraction"
[7] "Prin1"    "Prin2"    "Prin3"
>
> library(nlme)
>
> pc1.var.lme.mac<-lme(Prin1~1,PCA1mac,~1|Year/Block/Crop/Subplot)
>
> summary(pc1.var.lme.mac)
Linear mixed-effects model fit by REML
 Data: PCA1mac
       AIC      BIC    logLik
  548.3213 564.5785 -268.1607
Random effects:
```

```
 Formula: ~1 | Year
        (Intercept)
StdDev:    1.110597

 Formula: ~1 | Block %in% Year
         (Intercept)
StdDev: 5.325987e-06

 Formula: ~1 | Crop %in% Block %in% Year
        (Intercept)
StdDev:    1.752688

 Formula: ~1 | Subplot %in% Crop %in% Block %in% Year
         (Intercept) Residual
StdDev: 0.0002572494 2.352226

Fixed effects: Prin1 ~ 1
                  Value Std.Error DF     t-value p-value
(Intercept) -0.01602579 0.9148477 90 -0.01751744  0.9861

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-1.79587912 -0.66653459 -0.09785667  0.51711774  3.15373454

Number of Observations: 112
Number of Groups:
                                    Year
                                       2
                         Block %in% Year
                                       6
              Crop %in% Block %in% Year
                                      18
Subplot %in% Crop %in% Block %in% Year
                                     108
>
> 1.110597/-0.01602579
[1] -69.30061
>
> 5.325987e-06/-0.01602579
[1] -0.0003323385
>
> 1.752688/-0.01602579
[1] -109.3667
>
> 0.0002572494/-0.01602579
[1] -0.01605221
>
> 2.352226/-0.01602579
[1] -146.7775
>
> -69.30061+-0.0003323385+-109.3667+-0.01605221+-146.7775
[1] -325.4612
>
> -69.30061/-325.4612
[1] 0.2129305
```

```
>
> -0.0003323385/-325.4612
[1] 1.021131e-06
>
> -109.3667/-325.4612
[1] 0.3360361
>
> -0.01605221/-325.4612
[1] 4.932142e-05
>
> -146.7775/-325.4612
[1] 0.4509831
>
> PCA1mic <-subset(PCA1, Fraction=="mic") *PC1, microaggregates
> attach(PCA1mic)

> names(PCA1mic)
[1] "Year"     "Block"    "Plot"     "Crop"     "Subplot"  "Fraction"
[7] "Prin1"    "Prin2"    "Prin3"
>
> pc1.var.lme.mic<-lme(Prin1~1,PCA1mic,~1|Year/Block/Crop/Subplot)
>
> summary(pc1.var.lme.mic)
Linear mixed-effects model fit by REML
 Data: PCA1mic
       AIC      BIC    logLik
  523.5587 539.5956 -255.7793

Random effects:
 Formula: ~1 | Year
        (Intercept)
StdDev:   0.5404675

 Formula: ~1 | Block %in% Year
        (Intercept)
StdDev:   0.2234106

 Formula: ~1 | Crop %in% Block %in% Year
        (Intercept)
StdDev:   0.9960437

 Formula: ~1 | Subplot %in% Crop %in% Block %in% Year
        (Intercept)  Residual
StdDev:    2.306813 0.7714505

Fixed effects: Prin1 ~ 1
                    Value Std.Error DF      t-value p-value
(Intercept) 2.777789e-11 0.5140727 90 5.403495e-11       1

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-0.67911171 -0.15649059 -0.01526728  0.13150313  1.27149720
```

```
Number of Observations: 108
Number of Groups:
                                    Year
                                       2
                         Block %in% Year
                                       6
                Crop %in% Block %in% Year
                                      18
Subplot %in% Crop %in% Block %in% Year
                                     108
>
> 0.5404675/2.777789e-11
[1] 19456751395
>
> 0.2234106/2.777789e-11
[1] 8042749107
>
> 0.9960437/2.777789e-11
[1] 35857428336
>
> 2.306813/2.777789e-11
[1] 83044932498
>
> 0.7714505/2.777789e-11
[1] 27772105801
>
> 19456751395+8042749107+35857428336+83044932498+27772105801
[1] 1.74174e+11
>
> 19456751395/1.74174e+11
[1] 0.1117087
>
> 8042749107/1.74174e+11
[1] 0.04617652
>
> 35857428336/1.74174e+11
[1] 0.2058713
>
> 83044932498/1.74174e+11
[1] 0.4767929
>
> 27772105801/1.74174e+11
[1] 0.1594504
>
>
> PCA1sc <-subset(PCA1, Fraction=="sc") *PC1, silt and clay
> attach(PCA1sc)

> names(PCA1sc)
[1] "Year"     "Block"    "Plot"     "Crop"     "Subplot"  "Fraction"
[7] "Prin1"    "Prin2"    "Prin3"
>
> pc1.var.lme.sc<-lme(Prin1~1,PCA1sc,~1|Year/Block/Crop/Subplot)
>
```

```
> summary(pc1.var.lme.sc)

Linear mixed-effects model fit by REML
 Data: PCA1sc
       AIC      BIC    logLik
  482.5479 498.2386 -235.2739

Random effects:
 Formula: ~1 | Year
        (Intercept)
StdDev:    2.382048

 Formula: ~1 | Block %in% Year
         (Intercept)
StdDev: 0.0004218595

 Formula: ~1 | Crop %in% Block %in% Year
        (Intercept)
StdDev:    1.381152

 Formula: ~1 | Subplot %in% Crop %in% Block %in% Year
        (Intercept)  Residual
StdDev:    2.158795 0.1693727


Fixed effects: Prin1 ~ 1
                 Value Std.Error DF    t-value p-value
(Intercept) 0.08903537  1.729049 84 0.05149383  0.9591

Standardized Within-Group Residuals:
          Min            Q1           Med            Q3           Max
-0.2090626267 -0.0300834045  0.0006139695  0.0272240516  0.2595234294

Number of Observations: 102
Number of Groups:
                                    Year
                                       2
                         Block %in% Year
                                       6
             Crop %in% Block %in% Year
                                      18
Subplot %in% Crop %in% Block %in% Year
                                     102
>
> 2.382048/0.08903537
[1] 26.75395
>
> 0.0004218595/0.08903537
[1] 0.004738111
>
> 1.381152/0.08903537
[1] 15.5124
>
> 2.158795/0.08903537
[1] 24.24649
```

```
> 0.1693727/0.08903537
[1] 1.902308
>
> 26.75395+0.004738111+15.5124+24.24649+1.902308
[1] 68.41989
>
> 26.75395/68.41989
[1] 0.3910259
>
> 0.004738111/68.41989
[1] 6.925049e-05
>
> 15.5124/68.41989
[1] 0.2267235
>
> 24.24649/68.41989
[1] 0.3543778
>
> 1.902308/68.41989
[1] 0.02780344
>
> attach(PCA1mac)
>

> pc2.var.lme.mac<-lme(Prin2~1,PCA1mac,~1|Year/Block/Crop/Subplot)
>
> summary(pc2.var.lme.mac) *PC2, macroaggregates
Linear mixed-effects model fit by REML
 Data: PCA1mac
       AIC      BIC    logLik
  415.1136 431.3708 -201.5568

Random effects:
 Formula: ~1 | Year
        (Intercept)
StdDev:   0.4882906

 Formula: ~1 | Block %in% Year
         (Intercept)
StdDev: 6.040318e-05

 Formula: ~1 | Crop %in% Block %in% Year
        (Intercept)
StdDev:   0.7583802

 Formula: ~1 | Subplot %in% Crop %in% Block %in% Year
        (Intercept)  Residual
StdDev:    1.138344 0.7285904

Fixed effects: Prin2 ~ 1
                   Value Std.Error DF     t-value p-value
(Intercept) -0.006878904 0.4098482 90 -0.01678403  0.9866
```

```
Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-1.01046325 -0.37964047 -0.09696861  0.32589003  1.79108054
Number of Observations: 112
Number of Groups:
                                    Year
                                       2
                           Block %in% Year
                                       6
                  Crop %in% Block %in% Year
                                      18
Subplot %in% Crop %in% Block %in% Year
                                     108
>
> 0.4882906/-0.006878904
[1] -70.98378
>
> 6.040318e-05/-0.006878904
[1] -0.008780931
>
> 0.7583802/-0.006878904
[1] -110.2472
>
> 1.138344/-0.006878904
[1] -165.4833
>
> 0.7285904/-0.006878904
[1] -105.9166
>
> -70.98378+-0.008780931+-110.2472+-165.4833+-105.9166
[1] -452.6397
>
> -70.98378/-452.6397
[1] 0.1568218
>
> -0.008780931/-452.6397
[1] 1.939938e-05
>
> -110.2472/-452.6397
[1] 0.243565
>
> -165.4833/-452.6397
[1] 0.3655961
>
> -105.9166/-452.6397
[1] 0.2339976
>
> pc2.var.lme.mic<-lme(Prin2~1,PCA1mic,~1|Year/Block/Crop/Subplot)
>
> summary(pc2.var.lme.mic) *PC2, microaggregates
Linear mixed-effects model fit by REML
 Data: PCA1mic
       AIC      BIC    logLik
  327.3595 343.3965 -157.6798
```

```
Random effects:
 Formula: ~1 | Year
        (Intercept)
StdDev:    1.403704

 Formula: ~1 | Block %in% Year
        (Intercept)
StdDev: 0.000484313

 Formula: ~1 | Crop %in% Block %in% Year
        (Intercept)
StdDev:    1.053817

 Formula: ~1 | Subplot %in% Crop %in% Block %in% Year
        (Intercept)   Residual
StdDev:      0.8429 0.09211367

Fixed effects: Prin2 ~ 1
                   Value Std.Error DF       t-value p-value
(Intercept) -4.32993e-15  1.026424 90 -4.218463e-15       1

Standardized Within-Group Residuals:
         Min           Q1          Med           Q3          Max
-0.235957748 -0.064895781  0.005482075  0.062201503  0.259615893


Number of Observations: 108
Number of Groups:
                                    Year
                                       2
                         Block %in% Year
                                       6
              Crop %in% Block %in% Year
                                      18
Subplot %in% Crop %in% Block %in% Year
                                     108
>
> 1.403704/-4.32993e-15
[1] -3.241863e+14
>
> 0.000484313/-4.32993e-15
[1] -111852385604
>

> 1.053817/-4.32993e-15
[1] -2.433797e+14
>
> 0.8429/-4.32993e-15
[1] -1.946683e+14
>
> 0.09211367/-4.32993e-15
[1] -2.127371e+13
>
> -3.241863e+14+-111852385604+-2.433797e+14+-1.946683e+14+-2.127371e+13
[1] -7.836199e+14
```

```
> -3.241863e+14/-7.836199e+14
[1] 0.4137035
>
> -111852385604/-7.836199e+14
[1] 0.0001427381
>
> -2.433797e+14/-7.836199e+14
[1] 0.3105839
>
> -1.946683e+14/-7.836199e+14
[1] 0.2484218
>
> -2.127371e+13/-7.836199e+14
[1] 0.027148
>
> pc2.var.lme.sc<-lme(Prin2~1,PCA1sc,~1|Year/Block/Crop/Subplot)
>
> summary(pc2.var.lme.sc) *PC2, silt and clay
Linear mixed-effects model fit by REML
 Data: PCA1sc
       AIC      BIC    logLik
  315.6114 331.3021 -151.8057

Random effects:
 Formula: ~1 | Year
        (Intercept)
StdDev:   0.7038412

 Formula: ~1 | Block %in% Year
        (Intercept)
StdDev: 9.02088e-05

 Formula: ~1 | Crop %in% Block %in% Year
        (Intercept)
StdDev:   0.6912488

 Formula: ~1 | Subplot %in% Crop %in% Block %in% Year
        (Intercept)   Residual
StdDev:   0.9322261 0.06927908

Fixed effects: Prin2 ~ 1
               Value Std.Error DF    t-value p-value
(Intercept) 0.045566 0.5319078 84 0.08566523  0.9319


Standardized Within-Group Residuals:
         Min           Q1          Med           Q3          Max
-0.299610966 -0.024371343  0.001390094  0.031922460  0.201064704
```

```
Number of Observations: 102
Number of Groups:
                                    Year
                                       2
                         Block %in% Year
                                       6
                Crop %in% Block %in% Year
                                      18
Subplot %in% Crop %in% Block %in% Year
                                     102
> 0.7038412/0.045566
[1] 15.44663
>
> 9.02088e-05/0.045566
[1] 0.001979739
>
> 0.6912488/0.045566
[1] 15.17028
>
> 0.9322261/0.045566
[1] 20.45881
>
> 0.06927908/0.045566
[1] 1.520412
>
> 15.44663+0.001979739+15.17028+20.45881+1.520412
[1] 52.59811
>

> 15.44663/52.59811
[1] 0.2936727
>
> 0.001979739/52.59811
[1] 3.763898e-05
>
> 15.17028/52.59811
[1] 0.2884187
>
> 20.45881/52.59811
[1] 0.3889647
>
> 1.520412/52.59811
[1] 0.02890621
```

Models for Determination of Significant Differences in Principal Component Values by Crop Phase, Chapter II

```
data princomp;
infile 'C:/Users/Owner/Dropbox/Monica_Daane/Monica_Stats/Data Txt
Files/Microbial Data/PCs.txt' firstobs = 2 expandtabs;
input Year Block Plot Crop$ Subplot Fraction$ PC1 PC2 PC3 pc1adj
pc2adj;
```

```
proc sort data=princomp;
by Fraction Year;
run;

data c; set princomp; *removal of outliers in silt clay fraction
where Fraction='sc';
if pc2adj > 2.2;
run;

proc sort data=c;
by Year;
run;

title 'Prin comp 1';
proc glimmix data=princomp nobound plots=studentpanel;
by Fraction Year;
class Crop Block;
model pc1adj= Crop Subplot/dist=lognormal ddfm=kr;
random Block Block*Crop;
lsmeans Crop/diff adjust=tukey lines;
run;

title 'Prin comp 2';
proc glimmix data=princomp nobound plots=studentpanel;
by Fraction Year;
class Crop Block;
model pc2adj= Crop Subplot/dist=lognormal ddfm=kr;
random Block Block*Crop;
lsmeans Crop/diff adjust=tukey lines;
run;
title 'Prin comp 2 sc only';
proc glimmix data=c nobound plots=studentpanel;
by Year;
class Crop Block;
model pc2adj= Crop Subplot/dist=lognormal ddfm=kr;
random Block Block*Crop;
lsmeans Crop/diff adjust=tukey lines;
run;
```