ROOT PRODUCTION AND SOIL CARBON ACCUMULATION IN ANNUAL, PERENNIAL, AND DIVERSE CROPPING SYSTEMS

By

Christine Dazil Sprunger

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ABSTRACT

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Soil carbon (C) accumulation in agricultural landscapes can improve soil health and concurrently mitigate climate change. My dissertation addresses three major knowledge gaps with respect to root production and soil C accumulation within agricultural landscapes: Nitrogen fertilizer additions, life history (annual versus perennial), and biodiversity. In addition, I investigate how farmers perceive soil C on their fields and determine which soil C indicators best reflect their perceptions of soil health.

Planting perennial grain crops in place of annual row crops could lead to C sequestration due to their extensive root systems. In chapters 2 and 3, I test the optimal partitioning theory and examine soil C cycling of annual winter wheat (*Triticum aestivum*) and perennial intermediate wheatgrass (*Thinopyrum intermidum;* IWG*)* under three nitrogen levels (Low N (Organic N), Mid N, High N). I found that IWG had significantly greater root biomass at surface depths compared to wheat ($P<0.05$), but there were no differences at subsurface depths between the two crops. In 2011 and 2012, total root biomass remained stable across the three N levels for both crops but in 2013, IWG root biomass in the High N level was significantly greater than in the Low N (Organic N) and Mid N levels ($p<0.05$). Despite significantly greater root C in IWG, there were no differences in labile or recalcitrant C pools compared to wheat. Overall, these results fail to support the optimal portioning theory and findings suggest that a longer period of time is needed in order for soil C to accumulate under perennial grain crops.

The ability to sequester C could be a major benefit of perennial cellulosic biofuels. In chapters 4 and 5, I examine fine root production and soil C dynamics via a long-term incubation in candidate biofuel cropping systems that differ in life histories (annual vs. perennial) and diversity (monoculture vs. polyculture) in contrasting soils. I found that the native grasses and restored prairie systems had greater root production compared to the monoculture perennials ($p<0.05$). At the low fertility site, I found substantial differences in active C pools between annual and perennial polyculture crops. Active C pools under polycultures were over 2.5 times greater than under continuous corn. At the high fertility site, most system differences were insignificant except the restored prairie and rotational corn had 3.4 times more active C than other systems. I conclude that diverse perennial biofuel crops grown on marginal lands are more effective at C accumulation compared to diverse perennials grown on high fertility soils.

In chapter 6, I compare the total soil organic matter test to the C mineralization (active C) test to determine which soil C indicator reflected differences in management across 52 farm fields in Michigan and whether test results reflect farmer perceptions of soil C. Results from the active C test strongly supported investigator field observations and farmer perceptions of soil C. My findings demonstrate that the active C test should be widely offered at university and commercial laboratories.

Overall, these results show that roots of established perennial grain crops increase with greater N additions, which can lead to large C stores and N retention in roots. However, in two separate experiments, I found no evidence for enhanced soil C accumulation over the first 4-5 years under monoculture perennial cropping systems relative to annual row-crops. This suggests that crop diversity in perennial based cropping systems should be promoted to replenish soil C for increased soil health and climate change mitigation.

Dedicated to my Parents, who have always believed in me.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: DISSERTATION INTRODUCTION

OVERVIEW

Society is currently facing several unprecedented challenges including global climate change, historic losses of arable land, fuel insecurity, and malnutrition with over 1 billion food insecure people worldwide (FAO, 2009; Lal, 2011). Current atmospheric CO_2 concentrations are at 398 ppmv and are projected to increase at a rate of 2.2 ppmv/yr (IPCC, 2007). Projections for food insecurity are also dire, as the world's population will reach 9.1 billion by 2050 (FAO, 2009) and demands for food will prompt even more land conversion and intensive agriculture. Food insecurity and global climate change are intertwined as both of these challenges can be alleviated or further exacerbated depending on soil carbon (C) management (Lal, 2010).

At the farm scale, soil C provides ecosystem services by increasing soil health and crop productivity. For example, an increase in soil C can improve water holding capacity, regulate nutrient cycling and retention, enhance soil physical structure, provide a better medium for plant roots to obtain water and nutrients by reducing porosity, and increase soil biodiversity (West and Post, 2002; Johnston et al., 2009).

The importance of soil C can also be realized at the global scale as soils contain between 1500-2000 Pg of C (Janzen, 2004). The exchange of CO_2 between terrestrial landscapes and the atmosphere has a major role in regulating the global C cycle. CO_2 is assimilated into the terrestrial biome through photosynthesis; however half of this CO_2 is soon released back to the atmosphere through plant respiration (Schlesinger, 1997). Globally, soils hold twice the amount of C that is found in the atmosphere and thus serve as an important C pool (Swift, 2001).

However, due to land conversion and intensive agricultural practices soil C has been reduced by up to 75% in agricultural landscapes (Lal, 2010). The consequences of soil C loss

include reductions in soil health and crop productivity, as well as enhanced CO_2 emissions. Currently, CO_2 emissions from land use change account for approximately 17% of total GHG emissions caused from anthropogenic activities (IPCC, 2007) and historically they account for approximately 124 Pg C of CO_2 emissions to the atmosphere between the years 1850 and 1990 (Houghton, 1998).

The factors needed to replenish the soil C pool include increasing soil organic matter inputs while slowing decomposition of C inputs, placing soil C deeper in the ground where there is reduced microbial activity, and enhancing the physical protection of C through aggregation (Post and Kwon, 2000). However, increasing soil C is challenging because the total C pool is large and dynamic and consists of different pools that vary in turnover times (Paul, 2001; Wander, 2004). The active C pool consists of freshly decomposing material and has a mean residence time of up to a year, while the slow C pool consists of material that is more lignified and typically has a mean residence time of a few decades. The resistant pool is the largest and oldest pool of C and mainly consists of inorganic and non-hydrolyzable organic C. Since the resistant pool reflects the largest and most recalcitrant pool of total C, it often takes decades to detect differences in soil C following a change in management.

Restoring C pools in agricultural systems is attractive because increasing soil C can lead to healthier soils and increase crop production while simultaneously mitigating climate change through C sequestration. Furthermore, there are several management practices that have proven to be effective at sequestering C over time. For instance, utilizing no-till management in place of conventional tillage can result in sequestration rates of 57 g C m^{-2} yr^{-1} and increasing rotational complexity has been found to sequester 20 g C m^{-2} yr^{-1} (West et al., 2002). Sainju et al. (2008) found that poultry additions lead to C sequestration rates of 510 kg C ha^{-1} yr^{-1}. Perhaps the

management strategy that has proven to be most effective for C sequestration is converting agricultural systems back to perennial vegetation (Post and Kwon, 2000; Syswerda et al., 2011). Converting annual row crop systems to perennial vegetation or successional systems has resulted in sequestration rates of up to 60 g C m^{-2} yr^{-1} (Council for Agricultural Science and Technology, 2004).

Perennial systems are effective at sequestering soil C due to their extensive root systems, year-round ground cover, and lack of disturbance after the initial cultivation (Post and Kwon, 2000; Glover et al., 2010). Perennial systems tend to have at least three times more root biomass than annual systems (Dupont et al., 2014). Since root production and decay represent the primary source of C in most terrestrial ecosystems, perennial systems with extensive roots can be major contributors to soil C sequestration. Furthermore, roots tend to persist in soil longer than aboveground material and can thus play a key role in C stabilization (Kong and Six, 2010; Rasse et al., 2005). Thus, the development of perennial cropping systems for food or fuel is attractive.

Two relatively new options for incorporating perennial crops into agricultural landscapes include the development of perennial grain crops and perennial cellulosic biofuels. Breeders are working to develop perennial grain crops that achieve yields comparable to annual row crops with extensive roots that could provide ecosystem services. The concept of perennial cellulosic biofuels has gained an increasing amount of traction since the U.S. Congress mandated that 136 billion liters of renewable fuel be produced annually by the year 2020 (Sissine, 2007). Currently, the main source of bioethanol production is corn, which has capped at 56.8 billion liters (Sissine, 2007), indicating that other biofuel sources are needed to meet Energy Independence and Security Act requirements. Furthermore, perennial cellulosic biofuels are more attractive than corn production due to their C sequestration potential (Lemus and Lal, 2005).

DISSERTATION OBJECTIVES

My overall objective is to determine root production and C accumulation in annual and perennial crops used for food or fuel that receive different amounts of fertilizer and vary in biodiversity. The focal questions that I address include: How do organic and inorganic sources of N impact above and belowground biomass allocation and C storage? Do perennial crops for both food and biofuel cropping systems enhance labile and recalcitrant C? How does biodiversity influence fine root production and C accumulation in active, slow, and resistant pools? Which soil C tests detect changes in management across farmer fields and align with farmer perceptions of soil C? To address these questions, I utilize methods from soil science, biogeochemistry, agroecology, and qualitative social-science research.

CHAPTER ORGANIZATION

To date, much of the research regarding perennial grain crops has been devoted to breeding efforts and aboveground productivity (Jaikumar et al., 2012; Murphy et al., 2010), while no study has investigated belowground production in situ. Moreover, little is known about the effect of fertilizer types and rates on biomass allocation and vertical root distribution in annual or perennial cropping systems. Although empirical field data regarding the effects of fertilizer on biomass allocation and root production are scarce, understanding plant resource allocation in nutrient rich and nutrient poor systems is a concept that has received widespread attention in ecology. For example, the optimal partitioning theory posits that systems where essential nutrients are lacking will have increased belowground production and in cases where excess fertilizer is added, root biomass will decrease (Bloom, 1985). If increased fertilizer leads to reductions in root growth, important ecosystem services provided by roots could ultimately be lost. In chapter 2, I test the optimal portioning theory by comparing plant biomass allocation and

coarse and fine root production of annual winter wheat *(Triticum aestivum* L. var. *Caledonia)* and perennial intermediate wheatgrass [(*Thinopyrum intermedium* (Host) Barworkth and D.R. Dewey); IWG] across a Nitrogen (N) fertilizer gradient. I also examine vertical root distribution to determine if perennial root biomass increases at depth in systems receiving lower rates of N compared to annuals due to more persistent roots. Finally, I quantify whole-plant nitrogen use efficiency across the different N levels in wheat and IWG.

Initial results of aboveground productivity in IWG reveal that yields are low and decline further in their third or fourth year (Culman et al., unpublished). Since C sequestration is one of the main motivations for the development of perennial grains, it is important to understand how much time is required before soil C starts to accumulate in these systems. The decline in yields after three years would require farmers to either replant or switch to another crop, in which case C sequestration in these systems might never be realized. In chapter 3, I compare coarse and fine root C mass of wheat and IWG down to a 1 m depth as well as labile and recalcitrant C pools to determine if IWG accumulates more soil C relative to wheat four years after establishment.

In contrast to chapters 2 and 3 where I compare belowground C dynamics of a monoculture annual and perennial grain system, in chapters 4 and 5, I examine belowground production and C accumulation in annual and perennial biofuel cropping systems differing in diversity. The effects of crop diversity on aboveground productivity have been extensively studied and are well known; typically crop diversity leads to increased aboveground productivity, especially in low fertility systems (Tilman, 1996; Smith et al., 2008). The effects of crop diversity on belowground production are less well known. Despite this knowledge gap, several hypotheses posit that crop diversity will lead to increased root production and C accumulation. For example, Hooper and Vitousek (1997) suggest that root production will be

greater in more diverse cropping systems due to plant complementarity effects and differences in phenology and nutrient demand. de Kroon et al. (2012) hypothesize that pathogens constrain root growth in monocultures compared to mixed species communities and that due to competition for nutrients, root production in mixed species systems will be more extensive. Given the wide variety of candidate biofuel cropping systems, understanding how species composition and functional diversity influences belowground C dynamics is crucial for determining short and long term C sequestration potentials in these systems.

Scientists and policy makers strongly encourage farmers to adopt sustainable management practices that could result in soil health improvements and C sequestration. However, farmers largely base their management decisions on soil test results. To date, total Soil organic matter (SOM) is the most common soil C indicator used by farmers but often times total SOM is not sensitive to short-term management changes (Culman, 2013). C mineralization (active C), a test that is more sensitive to changes in management and reflects the labile soil C pool, is not widely offered at university and commercial laboratories. In chapter 6, I combined soil science field-based research with qualitative social-science methodology to determine if the active C test is able to detect differences across fields varying in soil health and performance and how well measured active C reflects farmer perceptions of soil C compared to the total SOM measurement.

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CHAPTER 2: ROOT ALLOCATION RESPONSES TO NITROGEN FERTILIZER IN AN ANNUAL WHEAT VERSUS PERENNIAL WHEATGRASS CROPPING SYSTEM

ABSTRACT

Perennial cropping systems typically exhibit extensive root systems that have been shown to contribute to important ecosystem services. Optimal partitioning theory predicts that plants that lack access to essential soil nutrients increase belowground productivity for root foraging potential and plants that receive excessive nutrients reduce belowground biomass and productivity and instead allocate resources aboveground. To test this theory, I quantified biomass distribution, crop biomass allocation, and whole crop nitrogen use efficiency (NUE) in annual winter wheat *(Triticum aestivum* L. var. *Caledonia)* and perennial intermediate wheatgrass (IWG), *Thinopyrum intermedium* (Host) Barworkth and D.R. Dewey across three nitrogen levels. The N levels were Low N (Organic N) (90 kg N ha^{-1} of poultry manure), Mid N (90 kg N ha^{-1} of urea), and High N (135 kg N ha^{-1} of urea). In the first two years, N level had no effect on coarse ($p>0.05$, $n=4$) or fine root biomass ($p>0.05$ $n=4$) in either crop. In year three, when IWG was fully established, both coarse and fine root biomass were significantly greater under the High N addition ($p<0.05$, $n=4$) in the surface 0-10 cm depth. There were no differences in root biomass at lower depths across N levels ($P>0.05$, $n=4$). IWG had significantly greater root biomass compared to wheat to 40 cm ($p<0.05$, $n=4$) but no differences were found between the two crops at deeper depths. Root:shoot ratios remained stable across the three N levels in both wheat and IWG systems ($P>0.05$, $n=4$). Regardless of N level, however, IWG always had greater whole crop NUE compared to wheat ($P<0.05$,$n=4$). NUE did not significantly differ across N level for wheat, while IWG was most efficient in the Mid N system ($p<0.05$, $n=4$). I thus found

no evidence for root foraging in the systems receiving less N and overall results fail to support optimal portioning theory: coarse and fine root production either remained the same or increased with higher levels of N and was proportional to aboveground production.

INTRODUCTION

Perennial grains have been promoted to meet food security demands while providing important ecosystem services in agriculture (Glover, 2010). In contrast to annual crops, perennials have extensive root systems and year-round ground cover, which can be important for the delivery of numerous ecosystem services (Snapp et al., 2015; Syswerda and Robterson, 2014; Glover et al., 2010). For example, perennial cropping systems have been shown to reduce nitrate leaching by up to 90% compared to annual crops (Syswerda et al., 2012; Culman et al., 2013). Furthermore, perennial systems are more efficient at building soil organic matter and reducing erosion (McLauchlan et al., 2006; Syswerda et al., 2011).

In order for perennial grain systems to achieve full yield potential, farmers will need to apply N fertilizer, which will likely influence belowground biomass allocation. Optimal partitioning theory (OPT: Bloom et al., 1985) posits that plants will respond to nutrient limited environments by increasing root productivity while allocating less energy to aboveground crop components. In cases where access to nutrients is adequate or excessive, OPT predicts a reduction in root production and an increase aboveground (grain and shoot) production. Bloom et al. (1985) predict that cropping systems receiving heavy fertilizer additions will ultimately reduce their root:shoot ratios. If predictions from OPT are extended to perennial grain crops, fertilization could result in reduced ecosystem services (e.g., belowground C inputs and nutrient capture) sought with perennial crops.

Belowground crop responses to N fertilizers in both annual and perennial crops are poorly understood and contradictory. For example, Jarchow et al. (2012) found support for OPT and reported lower root production in systems receiving greater N additions in both annual and perennial mixed grass systems. In contrast, Hegenstaller et al. (2009) reported that third and fourth year switchgrass (*Panicum virgatum L.*) and big bluestem (*Andropogon gerardii* Vitman) stands had greater root biomass with increased fertilizer additions, while eastern gamagrass [*Tripsacum dactyloides* (L.) L.] consistently had reduced root biomass with increased fertilizer. Others have found no root biomass response to increased inorganic fertilizer additions in corn and switchgrass systems (Russell et al., 2009; Jung and Lal, 2011).

Roots are dynamic and plastic by nature and are affected by nutrient resources and water availability throughout the soil profile. Since resource limitation becomes more apparent at depth, OPT may be more relevant to perennials because they have longer growing seasons, and their roots spend a greater proportion of the year at depth compared to annual systems. For example, in drought conditions, fine roots extend to greater depths in order to obtain water and nutrients (Poorter and Nagel, 2000; Canadell et al., 2006). Thus, root responses to nutrient resource limitation might primarily occur at subsurface depths. However, previous work examining root response to N additions has typically only measured root biomass in surface horizons (Offocer, et al., 2009; Jung and Lal, 2011) and in cases where subsurface horizons have been sampled, authors rarely report root biomass by depth (Hegenstaller et al., 2009; Russell et al., 2009). Understanding how different rates and sources of N fertilization influence belowground productivity by depth and overall crop N uptake in annual versus perennial cropping systems could have important implications for agronomic productivity and whole crop nitrogen use-efficiency (Dawson et al., 2008).

Here I compare crop biomass allocation, coarse and fine root vertical distribution, and nitrogen use efficiency (NUE) in annual winter wheat *(Triticum aestivum* var. *Caledonia)* and a novel perennial grain, perennial intermediate wheatgrass (IWG), *Thinopyrum intermedium* (Host) Barworkth and D.R. Dewey across a nitrogen fertilizer gradient. Consistent with OPT theory, I hypothesized that i) Root biomass and root:shoot ratios of both annual wheat (wheat) and perennial IWG (IWG) will decrease under increased fertilizer additions; and ii) IWG will have a greater response to lower N at subsurface depths than will wheat, because of a more persistent root system. Furthermore, I hypothesize that both wheat and IWG whole crop NUE will be reduced in systems receiving greater amounts of N fertilizer. However, since perennials have more root biomass, IWG will have greater NUE compared to wheat.

METHODS

Site description

The experiment was conducted at the W.K. Kellogg Biological Station (KBS) Long-term Ecological Research site, located in southwest Michigan, USA (42º 24' N, 85º 24' W, elevation 288 m). The mean annual precipitation and temperature are 1005 mm and 10.1ºC. KBS soils are in the Kalamazoo soil series (fine loamy) and Oshtemo (coarse loamy), mixed, mesic Typic Hapludalfs). These soils typically have an A horizon of 30 cm, a deep Bw/Bt horizon that reaches to 80+ cm, and a BC horizon to 140 cm. Prior to establishment in 2009, the field was under a corn (*Zea mays* L.*)*-soybean [*Glycine max* (L.) Meer.]-wheat (*Triticum aestivum)* rotation.

Experimental design

The study was established in 2009 as a split plot (3.1 m by 4.6 m) randomized complete block design experiment with four replicate blocks. The main factor is N treatments and the sub-factor is crop type. The N levels are an Low N (Organic N) treatment, which received 90 kg N ha^{-1} of poultry manure; Mid N, which received 90 kg N ha^{-1} of urea; and High N, which received 135 kg N ha^{-1} of urea. N release from manure is typically slower than N release from urea and other inorganic fertilizers (Rees and Castle, 2002) such that N availability is ordered as Low N (Organic N) < Mid N< High N).

The crop types assessed in this experiment were i) annual winter wheat var. Caledonia (soft wheat) and ii) *Kernza*^{TM} (IWG), which was developed through bulk breeding and mass selection at the Land Institute located in Salina, KS (DeHaan et al, 2004; Cox et al., 2010).

Prior to planting, both plots were chisel plowed in September 2009. Every October, 2.24 Mg ha^{-1} of pelletized poultry manure and sawdust at 4-3-2 N-P-K (Herbruck's Poultry Ranch, Saranac, MI), was applied to the Low N (Organic N) system. The application rate delivered 90 kg N ha^{-1} total N. The Mid N level is the recommended rate for conventionally grown wheat in the state of Michigan, while the High N (135 kg N ha^{-1}) level received 50% more N than the Mid N level. Both the Mid N and High N systems received pelleted urea at three different times throughout the growing season. In the conventional systems, a starter of 33.6 kg N ha^{-1} and 53.8 kg K ha^{-1} as K_2O for both Mid N and High N systems were applied immediately before planting. The following spring, plots were top-dressed with urea at 28 and 50.4 kg N ha^{-1} for Mid N and High N, respectively, typically at the beginning of April. In-depth details regarding timing of planting and chemical application can be found in Culman et al. (2013).

Aboveground biomass sampling

Aboveground biomass was measured at grain maturity for both crops. In general, wheat was harvested in early to mid July and IWG was harvested at the end of July or early August. Aboveground biomass and yields were determined by randomly placing two 0.25-m^2 quadrats in every plot and clipping the crop biomass to 10 cm above the soil. Samples were then threshed to separate grain from straw and dried at 60ºC for 48 hours before weighing.

Belowground biomass and soil sampling

Belowground biomass and soil samples were collected near peak biomass and anthesis (mid June 2012) for both wheat and IWG. In 2011, belowground biomass was measured only in the Low N (Organic N) and High N levels. A hydraulic direct-push soil sampler (Geoprobe, Salina, KS) was used to take three 1-m cores per plot. The cores were 6 cm in diameter and subsequently divided into five depths (0-10 cm, 10-20 cm, 20-40 cm, 40-70 cm, and 70-100 cm). The three cores were composited by depth interval and a subsample of 400 g from each depth was taken for root analyses and the remainder used for soil analyses. Soil moisture was determined gravimetrically.

Roots were separated into two size classes coarse (>6 mm) and fine (1- 6 mm). Coarse roots were separated from soil by dry sieving through 6 mm sieves. Fine roots were obtained from soil sieved through 1 mm sieves by wet sieving. No attempt was made to determine live versus dead roots. To clean roots prior to weighing and drying, I soaked roots in deionized water and hand washed. Both coarse and fine roots were dried at 60ºC for 48 hours prior to weighing.

Crop carbon (C) and nitrogen (N) analysis

Dried coarse and fine roots were frozen in liquid N and then immediately ground to a fine powder using a mortar and pestle. Dried grain and stem crop components were ground separately to 1 mm with a Wiley mill. Both above and belowground crop parts were analyzed for C and N in a CHNS analyzer (Costech Analyzer ECS 4010, Costech Analytical Technologies, Valencia, CA). In 2011, crop material was not analyzed for C and N.

Nitrogen use efficiency

I used the mass balance approach to calculate NUE on the basis of fertilizer applied. Total Plant N (straw kg N ha^{-1} + grain kg N ha^{-1} + Coarse and Fine Root kg N ha^{-1})/ Total N applied (kg N ha^{-1}); Aboveground N (straw kg N ha^{-1} + grain kg N ha^{-1})/ Total N applied (kg N ha^{-1}); and Root NUE (Coarse and Fine Root kg N ha^{-1})/Total N applied (kg N ha^{-1}). Ratios over 1.0 are an indication that the crop took up more N then was applied in that given growing season.

Statistics

All crop and soil responses were analyzed using Proc Mixed of SAS (version 9.3; SAS Institute, Cary, NC, USA). Crop, N level, and depth were treated as fixed effects and block as a random effect. Significant differences were determined at $\alpha = 0.001$, 0.01, and 0.1. Double repeated measures were used to account for both depth and year in the model. Means were compared with an adjusted Tukey's pairwise means comparison.

RESULTS

Weather and soil moisture

Cumulative precipitation and growing degree-days between the months of March and October in 2011 and 2013 varied substantially from 2012. KBS received above average precipitation during the 2011 and 2013 growing seasons, receiving 858 mm and 752 mm respectively. Growing degree days (GDD) were similar in 2011 and 2013 (2497 and 2435, respectively), and were comparable to the 30-year average (2431). In 2012, the Midwest experienced severe drought conditions from June to August. There were 276 and 337 more GDD in 2012 compared to 2011 and 2013; and 267 more than the 30-year average. In 2012, KBS received a cumulative 557 mm of precipitation between March and October, which was substantially lower than the average (721 mm).

There were no significant differences in gravimetric soil moisture across the N gradient, and thus reported values are averaged across the Low N (Organic N), Mid N, and High N systems. Gravimetric soil moisture was heavily influenced by crop, year, and depth ($p=0.01$, 0.0001, and 0.03, respectively). Although wheat consistently had greater gravimetric soil moisture than IWG, pairwise comparisons reveal that significant differences were mainly found at 40-70 cm and 70-100 cm (Table 2.1). In 2013 surface soil moisture (0-10 cm, 10-20 cm, 20-40 cm) was 18% greater than in 2011 and 2012 for both crops (Table 2.1).

Aboveground biomass

Aboveground biomass greatly differed between the two crops (Table 2.2, $p=0.03$). In 2011 and 2013, IWG had consistently greater aboveground biomass compared to wheat (Table 2.2). In the 2012 drought year, both crops had lower productivity; averaging across N levels IWG biomass decreased by 69% and wheat decreased by 53% between 2011 and 2012. In 2013, IWG

aboveground biomass increased with greater levels of applied N; however, the N level effect was only marginally significant ($p=0.08$).

Belowground biomass

IWG consistently had greater fine and coarse root biomass compared to wheat (Table 2.2, $p=0.0001$). Depending on the N level and year, IWG had between 3 and 12 times greater coarse and fine root biomass than wheat. There was no overall N level effect on coarse root biomass ($p=0.3$). However, the significant crop by N level by year interaction ($p=0.01$) shows that IWG was more affected by N level than wheat, especially in 2013. For instance, pairwise comparisons revealed that IWG coarse root biomass under High N was significantly greater than Mid N and Low N (Organic N) coarse root biomass (Table 2.2, $p=0.01$, and $p=0.002$).

There was a significant overall N level effect on fine root biomass ($p<0.05$). For IWG fine root biomass under Mid and High N were significantly greater than in the Low N (Organic N) system ($p<0.5$). The crop by N level effect was significant ($p=0.04$) because increasing levels of N influenced only IWG.

Crop allocation and root:shoot ratios

Differences in crop biomass allocation were evident for wheat and IWG (Figure 2.1). In non-drought years, IWG allocated between 23 and 50% of its total biomass to roots as compared to wheat, which allocated approximately 10% to roots. In 2011 and 2013, IWG root:shoot ratios were two-times greater than wheat root:shoot ratio (Figure 2.1, $p<0.0001$). The significantly higher root:shoot ratios evident in 2012 in comparison to 2011 and 2013 were caused by large reductions in aboveground biomass rather than gains in belowground biomass. In 2012 IWG root

biomass was equal to 2011 and slightly lower than 2013 (Table 2.2). There were no significant N level effects on root:shoot ratios (Figure 2.1, $p=0.8$).

Coarse and fine root biomass by depth

IWG coarse root biomass was between 3.4 and 8 times greater than wheat coarse root biomass at surface depth intervals of 0-10 cm, and 10-20 cm (Figure 2.2, $p<0.0001$). There were a few marginally significant differences at mid (20-40 cm) and subsurface (40-70 and 70-100 cm) depth intervals ($p=0.05$), but in general there were few differences between wheat and IWG coarse root biomass at lower depths (Figure 2.2). Wheat vertical coarse root distribution was fairly consistent across the three years with 93% of the roots found in the top 40 cm. IWG had similar vertical coarse root distributions with 94% of roots typically found in the top 40 cm, however, IWG root biomass increased significantly in the surface depths over time (Figure 2.2).

Despite the fact that there were no overall N level effects on coarse root biomass, there was a four-way interaction between year, crop, N level, and depth ($p=0.01$). Notable differences in IWG coarse root biomass across the different N levels at the surface depths likely caused this significant interaction. For example, pairwise comparisons found that for 2011 and 2013 High N coarse root biomass at 0-10 cm was significantly greater than Low N (Organic N) coarse root biomass ($P=0.0001$ and $P=0.0001$, respectively). In 2012, the Low N (Organic N) system had greater coarse root biomass than Mid N ($P<0.05$), but was not significantly different from High N. There were no differences between N levels at subsurface depths. Greater amounts of variability occurred at surface depths compared to subsurface depths, especially in 2012.

Fine root biomass distributions were very similar to coarse root biomass distributions with the majority of roots concentrated in the top 20 cm. However, IWG allocated a greater amount of biomass to fine roots compared to coarse roots below 40 cm (Figure 2.3). There were strong

differences in fine root biomass between crops ($p<0.0001$), across N levels ($p=0.002$), over time (0.0003), and by depth ($P<0.0001$). There was also a significant three-way interaction among depth, year, and crop ($p=0.03$), which reflected the variation in fine root production over time for IWG at surface depths compared to greater stability in wheat. Furthermore, significant differences in fine root production between IWG and wheat were typically only in the top 0-40 cm. At the surface, IWG fine root biomass was typically between 1.5 and 4 times greater than wheat. IWG fine root biomass tended to increase with increasing levels of fertilizer, especially at 0-10 cm, while wheat did not. IWG fine root biomass increased over time, with the greatest values occurring in 2013 under Mid N and High N systems.

Crop N and nitrogen use efficiency

The total N contained in IWG coarse and fine roots consistently was greater compared to wheat (Table 2.3, $P<0.0001$). Aboveground biomass N content, however, was statistically similar between the two crops. Aboveground N content significantly differed across the N gradient, with greater N content typically found in the High N system ($p=0.01$) for both crops. There were significant pairwise comparisons across N levels for aboveground N in 2013 but not in 2012.

The total N contained in coarse roots generally increased with increasing N fertilizer additions (Table 2.3). The significant crop by N level interaction ($p=0.004$) along with pairwise comparisons indicate that N levels had a much stronger influence on IWG compared to wheat, especially in 2013. There was an overall N level effect on fine root N content ($P=0.02$). For IWG in 2012 and 2013, Mid N and High N had significantly greater fine root N compared to the Low N (Organic N) system (Table 2.3). Although there was an overall crop by N level interaction ($p=0.2$), wheat fine root N did not appear to be as strongly influenced by increasing N levels

compared to IWG. Total N strongly differed by crop ($p<0.0001$) and N level (0.0002), and was substantially greater in IWG and always larger in the High N level.

There was a significant year effect for all crop parts, with N content typically greater in 2013. Coarse and Fine root N content were also examined by depth (data not shown) and exhibited very similar trends to coarse and fine root biomass by depth (Figure 2.2 and 2.3).

The NUE for above and belowground biomass components were also calculated separately (Table 2.4). In terms of total crop NUE, IWG was more efficient at using N compared to wheat ($p<0.0001$). Across both years and N level, IWG NUE ratios ranged from 0.8 to 1.5 and wheat NUE ranged from 0.56 to 0.86 (Table 2.4).

IWG within the Mid N system exhibited greater NUE compared to Low N (Organic N) and High N systems ($p=0.03$). The significant interaction between crop and N level ($P=0.03$) is an indicator that N level had little effect on wheat NUE. Significant gains in NUE from 2012 to 2013 were visible in all three N levels for wheat and were most noticeable in the Mid N system for IWG. NUE increased by up to 53% in wheat from 2012 to 2013 by up to 43% in IWG (Table 2.4). There was no crop effect on aboveground NUE, as wheat and IWG were statistically similar to one another ($p=0.9$). However there was an overall N level effect, where wheat aboveground NUE was greater in Low N (Organic N) systems and IWG aboveground NUE was greater under Mid N. Root NUE was substantially greater in IWG compared to wheat ($p<0.0001$).

DISCUSSION

OPT predicts that root biomass will decrease proportionately in systems receiving increased fertilizer or otherwise supplied with limiting nutrients at levels greater than crop need

(Bloom et al., 1985). In cases where nutrients are limiting, OPT predicts that plants will proportionally increase allocation to root growth. To accept OPT I would expect to find 1) a reduction in root biomass in systems receiving greater amounts of fertilizer and 2) increased root resource foraging under nutrient limited systems. I found neither expectation for either wheat or IWG, instead finding that annual root biomass remained stable across the N fertilizer gradient in all three years and root biomass of established IWG increased rather than decreased with greater amounts of N additions in 2013. Furthermore, there was no evidence for increased root foraging at depth in reduced N systems under either crop. OPT thus failed to adequately predict N level effects on root biomass and crop biomass allocation in situ. Nitrogen use efficiency on the other hand, was greater in IWG than in wheat, which is consistent with predictions that perennial crops will use N more efficiently than annual systems (Jordan et al. 2007; Dawson et al., 2008; Glover et al., 2010; Hirose, 2011).

Root responses to added N

The lack of a root response to increased N additions in IWG could be due to environmental and developmental factors. In 2011, IWG stands were two years old and still establishing, which could prevent observed responses to increased N fertilizer (Jung and Lal, 2011). While I would then expect to see a root response to N level during the 3rd year, 2012 was a drought year, which apparently negated any response to N. However, I also failed to find a root response to N in 2013, when IWG was mature and growing conditions were favorable. In fact, in 2013 IWG root biomass significantly increased with higher levels of N, while root:shoot ratios remained stable, which is inconsistent with OPT. I also failed to find evidence for OPT in the wheat system, as root biomass remained stable across N levels. This could suggest that N was not the primary

limiting factor (Meinke et al., 1997). Instead the wheat system could have been limited by moisture, disease or another nutrient. In 2011, the Mid N, yields (63.7 ± 6.3 bu) were slightly lower than the wheat yield average at the regional level for southwest MI (69.5 bu, NASS, USDA). In subsequent years, the wheat yield dropped in 2012 (41.3± 8.3 bu) and 2013 (52.8 ± 6.9 bu). The lower yields in 2012 are the result of a drought year, however even in favorable growing conditions evident in 2011 and 2013, the wheat yield tended to be lower than county averages, which could indicate that this system was limited by factors other than nitrogen.

Another explanation for this lack of response to N could be the methodological approaches used in this study. For example, I sampled once during the growing season, perhaps at a time when nutrient resources were not limiting. However, I sampled near peak aboveground biomass, when crop nutrient demand is still high. The more likely difference between this study and research in support of OPT is that this study was conducted in situ, rather than in greenhouse pots or mesocosms (Davidson et al., 1969; Christie and Moorby, 1975; Brewster et al., 1976). Growing conditions in greenhouses can be substantially different than field growing conditions and thus could influence root dynamics differently. That said, at least one in situ study has found support for OPT. For example, Jarchow et al. (2012) reported greater root biomass of C_4 grasses in unfertilized systems compared to unfertilized system. Interpretation is clouded by extreme nutrient limitations in the unfertilized system, which contrasts from this study, where each treatment received at least some N fertilizer.

Heggenstaller et al. (2009) also report results for in situ study of switchgrass and big bluestem that are consistent with reported results. Increases in root diameter due to greater nutrient uptake in nutrient rich environments could explain greater root biomass in systems receiving higher N additions (Ryser and Lambers, 1995).

OPT further predicts that when nutrients or water are limiting, crop allocation should shift to the production of fine roots that can capture resources available at greater depths (Bloom et al., 1985). I found no evidence for enhanced fine root production in either wheat or IWG within the Low N (Organic N) level at any depth to 1 m. Likewise, Jarchow et al., (2012), who found greater root biomass in an unfertilized C_4 grass system at the surface, also found no evidence of increased root production at depth, (although they did not distinguish between coarse and fine root biomass).

These findings corroborate other calls for reconsideration of OPT (Coleman and McConnaughey, 1995; Reich, 2002; Janecek et al., 2014). OPT, may in fact be less useful for describing belowground resource allocation or simple developmental patterns compared to the optimal foraging theory, where plants are expected to invest roots in highly enriched areas versus more depauperate patches (Charnov, 1976; Loecke and Robertson, 2009; McNickle and Cahil, Jr., 2009). This seems consistent with results reported in this study, whereby roots increased under High N and were mainly concentrated in the top 0-10 cm, rather than foraging deeper in the soil profile to obtain other available nutrients. Others have suggested that biomass partitioning is a function of ontogenetic drift, wherein biomass allocation is determined by growth and development rather than shifts in reallocation due to limiting resources, as suggested by OPT (Coleman and McConnaughay, 1995; Reich, 2002; Mcarthy and Enquist, 2007). The growth patterns of IWG in this study are consistent with this theory, as root biomass increased overtime, especially in the High N system. Perhaps in this system, the increased root biomass within the established IWG under High N is simply due to changes in development, allowing IWG to gain access to greater nutrient capture.

Nitrogen use efficiency in annual vs. perennial systems

Above and belowground biomass responses to N additions can have profound impacts on internal and external crop N cycling. For this reason, I was also interested in determining whole-crop N use efficiency (NUE). While there are many ways to define and calculate NUE (Dawson et al., 2008), in this study I consider whole-crop NUE to be total crop N (above +belowground biomass N) per N added, both in units of kg N/ha (Robertson and Vitousek, 2009). This mass balance approach allows us to determine the efficiency with which wheat and IWG assimilate added N. My hypothesis that IWG would have greater whole-crop NUE compared to wheat was supported, regardless of N level.

Since aboveground NUE was not significantly different between wheat and IWG, it is likely that the extensive roots of IWG as well their large capacity for N storage are the main drivers for their high NUE values, which gives them an efficiency advantage over wheat. For example, in 2013, IWG root NUE increased by 40% in the Low N (Organic N) and Mid N and by 87% in the High N treatments. Traditionally, root N content has not been included in NUE calculations (Weih, 2011). In three cases the IWG whole-plant NUE was greater than one, indicating that the crop took up more N than was applied, which demonstrates their ability to assimilate large amounts of N.

NUE significantly differed across N level in IWG but not in wheat. IWG NUE was greatest in the Mid N level compared to the Low N (Organic N) and High N levels. This does not support my hypothesis that NUE decreases with increasing levels of N. One explanation for greater NUE in the Mid N level could be that biomass production and N uptake kept up with N supply, compared to in the Low N (Organic N) system, which always had lower above and belowground biomass.

Implications for enhanced ecosystem services by perennial grain crops

These findings demonstrate that perennial grain cropping systems can significantly enhance ecosystem services in agriculture by increasing root biomass. Under a range of N additions, IWG produced up to 8 times more total root biomass than wheat in the top 40 cm of soil. No differences were found between the two crops deeper in the profile, refuting the hypothesis that perennial grain crops are likely have greater root biomass at depth compared to wheat (Cox et al., 2006; Glover et al., 2010; Kell, 2011).

Greater total root biomass in the perennial crop will likely lead to increases in soil organic matter, based on findings by others, who have found increased C sequestration under perennial systems (Robertson, 2000; West and Post, 2000; Syswerda, 2010). As perennial crops age, a greater standing stock of belowground biomass is established (Craine et al., 2003). This allows more C to accumulate in root biomass and soil due to root turnover, which provides between 30 and 80% of organic C inputs to soil (Kalyn and Van Rees, 2006). In this study, IWG root biomass increased by 51% from 2011 to 2013. While I did not measure total soil C, early results from this experiment show greater labile C under IWG compared to wheat in surface soil horizons (Culman et al., 2013).

Increased root biomass has also been shown to enhance N cycling and accumulation (Fornara and Tilman, 2008). For example, increased root biomass in perennial systems can lead to N immobilization, and the quick release of fine root N during turnover can lead to N retention and accrual (Fornara et al., 2009). These results demonstrate that increased root biomass enabled IWG to take up large amounts of N and contributed to overall high NUE. As a result, minimal N losses likely occur in these systems; for example, relative to wheat, IWG at this site reduced nitrate leaching by up to 99% in 2011 (Culman et al., 2013).

CONCLUSIONS

In this study, established IWG stands increased root biomass with increasing levels of N fertilizer, while wheat root biomass remained stable despite varying levels of N. I found no evidence for increased root foraging at depth in reduced N systems under either crop. These results suggest that the optimal foraging theory is a more adequate explanation for biomass allocation than OPT in these systems. Roots of IWG enhance N uptake and nitrogen use efficiency and appear to have contributed to the reduction of nitrate leaching. Given the C and N accrual and the retention of N by their extensive root systems, perennial grain crops could contribute significantly to the environmental sustainability of agricultural systems. However their role in the longer-term sequestration of non-living organic carbon in soils remains uncertain.

APPENDIX

Table 2.1 Gravimetric soil moisture at five depths throughout the soil profile in wheat and IWG in 2011, 2012, and 2013, averaged across N levels (means ± se). Different superscript letters within years denote significant differences between crops for each depth and year combination at ($p<0.05$).

	2011		2012		2013	
	Wheat	IWG	Wheat	IWG	Wheat	IWG
	g kg^{-1}					
Depth cm						
0-10	9.1 (0.6)[a]	8.0 (0.5)[a]	9.4 (0.6)[a]	8.4 (0.5)[a]	11.4 (0.5)[a]	10.8 (1.2)[a]
10-20	8.0 (0.5)[a]	7.3 (0.4)[a]	9.6 (0.5)[a]	8.0 (0.4)[b]	11.3 (0.5)[a]	10.2 (0.4)[a]
20-40	8.8 (0.6) [a]	8.5 (0.7)[a]	8.0 (0.4)[a]	7.3 (0.6)[a]	11.8 (0.6)[a]	10.6 (0.6)[a]
40-70	10.4 (0.5)[a]	8.5 (0.4)[b]	8.9 (0.5)[a]	7.7 (0.5)[b]	11.7 (0.6)[a]	9.5 (0.6)[b]
70-100	8.4 (0.6)[a]	7.6 (0.5)[a]	7.9 (0.6) [a]	5.6 (0.5)[b]	9.9 (0.9)[a]	7.8 (0.5)[b]

Table 2.2 Total biomass in wheat and IWG across three N levels in 2011, 2012, and 2013 (means ± se). Comparisons of cropping system means within a given year followed by same superscript letters are not significant. Different letters within a column of a given year denotes significant differences across N level.

	Coarse Roots		Fine Roots		Aboveground		Total Crop Biomass	
	Wheat	IWG	Wheat	IWG	Wheat	IWG	Wheat	IWG
				Mg ha^{-1}				
2011								
Low N (Organic N)	1.1 (0.6)^{b}	3.4 (0.5)^{a}	0.31(0.02)^{a}	0.99 (0.1)^{b}	12.63 (1.8)^{c}	14.9 (1.1)^{b}	14.0 (2.2)^{b}	17.34 (2.4)^{a}
Mid N	NA	NA	NA	NA	12.53 (1.2)^{c}	19.91 (1.2)^{a}	NA	NA
High N	0.4 (0.1)^{b}	5.0 (0.7)^{a}	0.34 (0.05)^{a}	0.99 (0.3)^{b}	14.67^{b}	15.94^{b}	15.4 (0.8)^{b}	21.89 (1.1)^{a}
2012								
Low N (Organic N)	0.7 (0.2)^{b}	5.93 (0.5)^{a}	0.26 (0.06)^{b}	0.43 (0.07)^{a}	5.99 (0.58)^{a}	4.3 (0.6)^{b}	7.02 (0.7)^{b}	10.65 (0.8)^{a}
Mid N	0.9 (0.3)^{b}	5.75 (0.5)^{a}	0.24 (0.03)^{b}	0.82 (0.2)^{a}	5.16 (0.9)^{a}	5.78 (0.9)^{a}	6.25(0.8)^{b}	12.36 (1.3)^{a}
High N	1.6 (0.4)^{b}	5.8 (1.4)^{a}	0.27 (0.03)^{b}	0.78 (0.2)^{a}	7.4 (0.8)^{a}	5.33 (0.5)^{a}	9.3 (0.3)^{b}	11.9 (1.2)^{a}
2013								
Low N (Organic N)	1.1 (0.3)^{c}	5.3 (0.7)^{b}	0.21 (0.02)^{c}	0.76 (0.07)^{b}	8.4 (0.81)^{b}	10.6 (0.7)^{a}	9.77 (0.9)^{c}	16.6 (0.6)^{b}
Mid N	0.8 (0.2)^{c}	6.1 (1.0)^{b}	0.33 (0.06)^{c}	1.8 (0.33)^{a}	9.68 (0.2)^{b}	11.9 (0.8)^{a}	10.8 (0.2)^{c}	19.86 (1.5)^{a}
High N	0.8 (0.2)^{c}	8.45 (0.6)^{a}	0.43 (0.2)^{c}	1.9 (0.4)^{a}	8.99 (0.6)^{b}	12.22^{a}	10.2 (0.5)^{c}	22.54 (1.4)^{a}

Table 2.3 Total N Content in wheat and IWG across three nitrogen levels in 2012 and 2013 (means ± se). Comparisons of means within rows (among cropping system) followed by same lowercase letters are not significant. Different letters within a column of a given year denotes significant differences across N level.

	Coarse Roots		Fine Roots		Aboveground		Total Crop Biomass	
	Wheat	IWG	Wheat	IWG	Wheat	IWG	Wheat	IWG
	kg N ha^{-1}							
2012								
Low N (Organic N)	5.84 (1.6)^{b}	41.4 (6.1)^{a}	1.5 (0.3)^{c}	3.2 (0.3)^{b}	53.4 (7.1)^{a}	35.9 (3.5)^{a}	60.9 (8.1)^{c}	80.5 (9.4)^{b}
Mid N	6.86 (2.1)^{b}	40.7(2.7)^{a}	2.2 (0.3)^{c}	5.24 (0.7)^{a}	53.2 (14.2)^{a}	49.2 (6.2)^{a}	62.3 (10.1)^{c}	95.1 (8.3)^{b}
High N	13.2 (4.1)^{b}	55.3(8.5)^{a}	2.5(0.2)^{c}	5.5 (1.6)^{a}	67.6 (10.3)^{a}	49.8 (3.0)^{a}	83.3 (4.8)^{b}	110.6 (5.0)^{a}
2013								
Low N (Organic N)	6.9 (1.9)^{d}	20.0 (2.9)^{c}	2.0 (0.2)^{c}	5.0 (0.5)^{b}	58.2 (5.0)^{a}	63.8 (5.4)^{ac}	67 (5.8)^{d}	88.8 (1.9)^{c}
Mid N	6.1(1.6)^{d}	34 (6.0)^{c}	3.3 (0.5)^{c}	12.0 (2.4)^{a}	68.1 (6.1)^{c}	88.5 (10.7)^{b}	77.5 (5.5)^{cd}	134.5 (8.7)^{a}
High N	6.3 (1.6)^{d}	52.1 (3.5)^{a}	3.9 (1.6)^{c}	13.5 (3.7)^{a}	102.2 (15.8)^{a}	76.2 (4.3)^{bc}	112 (15.2)^{b}	141.8 (9.9)^{a}

Table 2.4 Nitrogen Use Efficiency in Harvested N, Root N, and Total Plant N. NUE was calculated as biomass N/total N applied. NUE ratios greater than 1.0 indicate that the crop took up more N than what was applied during the growing season. Comparisons of means within rows (among cropping system) followed by same lowercase letters are not significant. Different letters within a column of a given year
denotes significant differences across N level.

	Aboveground NUE		Root NUE		Whole Plant NUE	
	Wheat	IWG	Wheat	IWG	Wheat	IWG
2012						
Low N (Organic N)	0.52 (0.05)[a]	0.4 (0.04)[a]	0.07 (0.01)[b]	0.5 (0.07)[a]	0.59 (0.04)[c]	0.89 (0.1)[b]
Mid N	0.46 (0.1)[a]	0.55 (0.07)[a]	0.1 (0.02)[b]	0.51 (0.02)[a]	0.56 (0.12)[c]	1.05 (0.09)[a]
High N	0.45 (0.07)[a]	0.37 (0.03)[a]	0.12 (0.04)[b]	0.45 (0.06)[a]	0.57 (0.03)[c]	0.83 (0.1) [b]
2013						
Low N (Organic N)	0.65 (0.06)[a]	0.71 (0.06)[a]	0.09 (0.02)[c]	0.27 (0.05)[b]	0.75 (0.06)[c]	0.98 (0.02)[b]
Mid N	0.76 (0.07)[a]	0.75 (0.06)[a]	0.1 (0.02)[c]	0.51 (0.09)[a]	0.86 (0.06)[c]	1.5 (0.09)[a]
High N	0.75 (0.1)[a]	0.56 (0.03)[a]	0.08 (0.01)[c]	0.49 (0.04)[a]	0.83 (0.1)[c]	1.05 (0.07)[b]

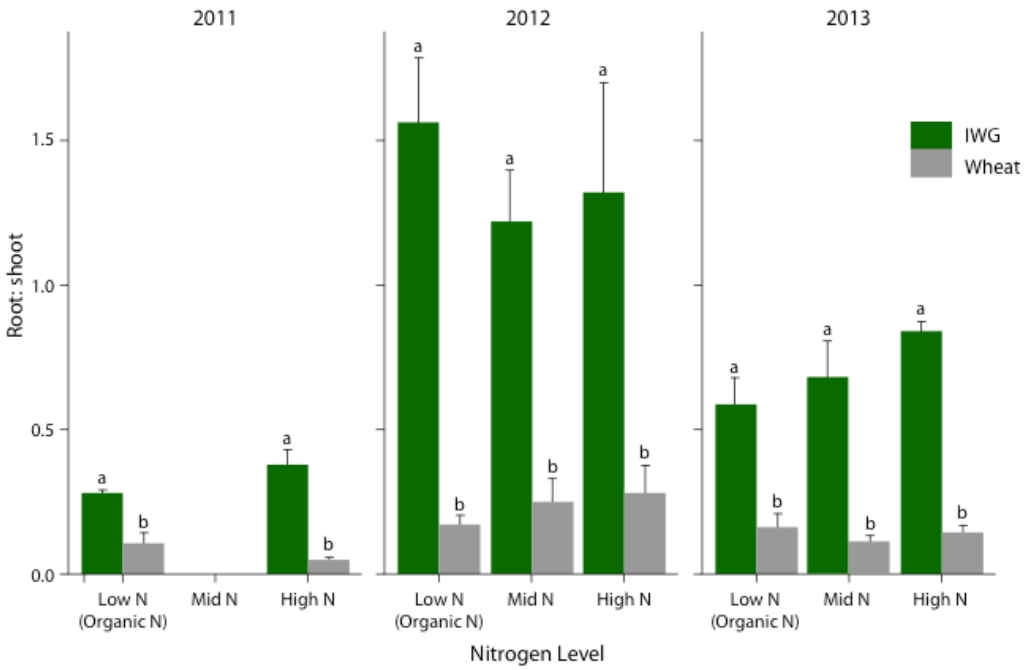

Figure 2.1 Root:shoot ratios of wheat and IWG in 2011, 2012, and 2013 for over three N levels (Low N (Organic N), Mid N, and High N). The sum of total coarse and total fine roots were used to calculate total root biomass. Total straw and grain were summed to determine total shoot biomass. Error bars represent the standard error of the mean and different letters denote significance at <0.05.

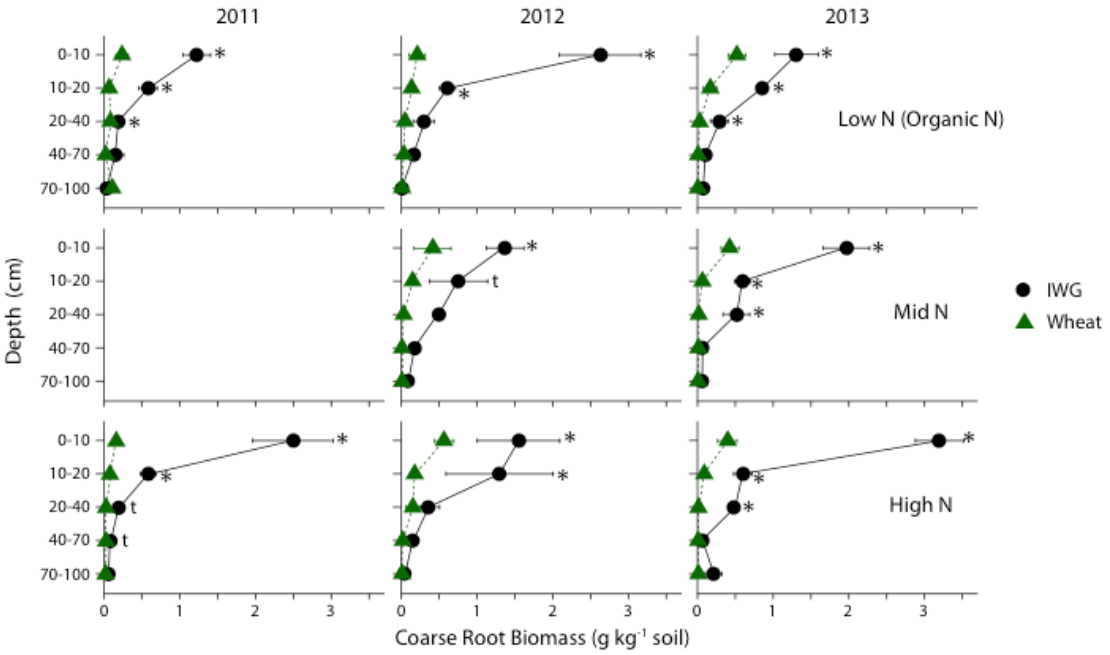

Figure 2.2 Coarse root biomass values in annual winter wheat (triangle) and IWG (circle) for three management practices (Low N(Organic N), Mid N, and High N) over three years (2011, 2012, 2013) at five different depths throughout the soil profile. Error bars represent the standard error of the mean and asterisks denotes significance at <0.05, t denotes significance at <0.1.

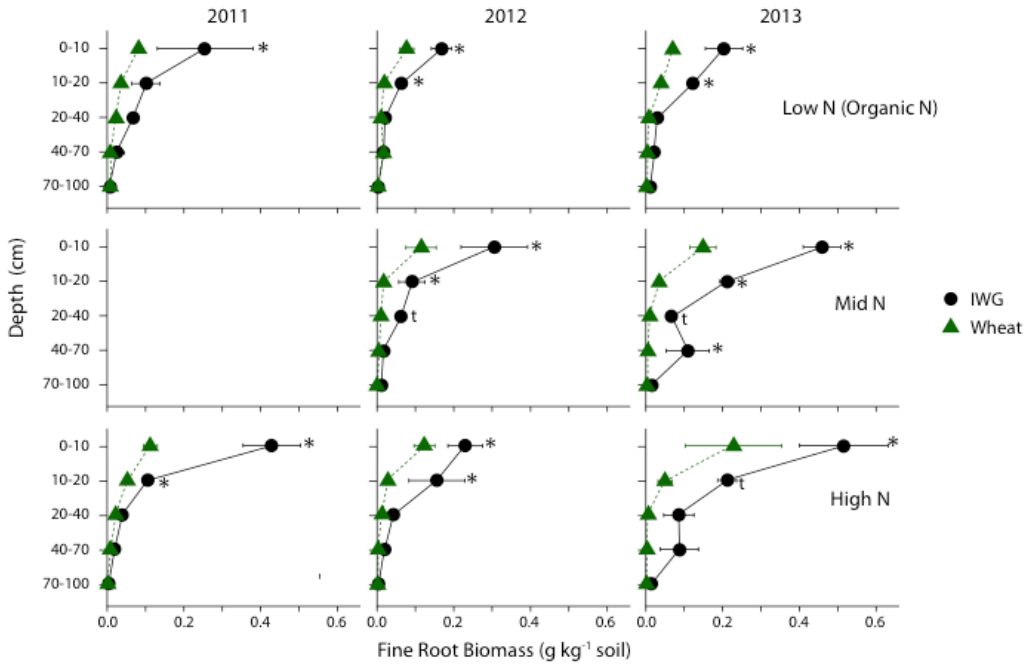

Figure 2.3 Fine root biomass values in annual winter wheat (triangle) and IWG (circle) for three management practices (Low N (Organic N), Mid N and High N) over three years (2011, 2012, 2013) at five different depths throughout the soil profile. Error bars represent the standard error of the mean and asterisks denotes significance at <0.05, t denotes significance at <0.1.

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CHAPTER 3: LITTLE EVIDENCE FOR EARLY SOIL CARBON CHANGE UNDER A PERENNIAL GRAIN CROP

ABSTRACT

Due to larger and more extensive root systems, perennial grain crops are expected to sequester carbon (C) and improve soil health. To examine the rate of soil C accumulation in a recently established perennial grain crop I compared C dynamics in perennial intermediate wheatgrass (IWG) against annual winter wheat (wheat). I tested whether or not different management practices influenced C dynamics under three available nitrogen levels, Low N (Organic N) system (90 kg N ha^{-1} poultry manure), Mid N (90 kg N ha^{-1} urea), and High N (135 kg N ha^{-1} urea). I measured aboveground C (grain + straw), and coarse and fine root C to a depth of one meter, and Particulate Organic Matter (POM), fractionated by size, was used to indicate labile and recalcitrant soil C pools. At harvest, IWG had 1.9 times more straw C and up to 15 times more root C compared to wheat. There were no significant differences in the large (6 mm-250 μm) or medium (250-53 μm) POM-C between wheat and IWG ($p>0.05$) in surface horizons (0-10 cm). Large POM-C under IWG ranged from 3.6 ±0.3 to 4.0 ±0.7 g C kg $soil^{-1}$ across the different levels of N, similar to wheat, where large POM-C ranged from 3.6 ±1.4 g C kg $soil^{-1}$ to 4.7 ±0.7 g C kg $soil^{-1}$ across N levels. Averaged across N level, medium POM-C was 11.3 ±0.7) g C kg $soil^{-1}$ and 11.1 ±0.8 g C kg $soil^{-1}$ for wheat and IWG, respectively. Despite larger pools of above and belowground C in IWG to 70 cm depth, I found no difference in labile or recalcitrant soil C pools between the two crops. Post-hoc power analysis revealed that in order to detect differences in the labile C pool at 0-10 cm with an acceptable power (~80%), 52 replicates or a

15% difference in C between wheat and IWG were needed. I thus found no evidence for more soil C accumulation under IWG other than greater standing stocks of root C.

INTRODUCTION

Intensive agricultural practices have depleted soil carbon (C) pools by up to 75% and contributed ~124 Pg C to the atmosphere over the past 140 years (Lal, 2011; Houghton and Hackler, 2001). Several management practices can replenish the soil C pool (West and Post, 2002; Jarecki and Lal, 2010); one of the most effective is to convert annual row crops to perennial vegetation (Post and Kwon, 2000; Syswerda et al., 2010; McLauchlan et al., 2006). For example, Post and Kwon (2000) reported average C accumulation rates following conversion to grasslands of 33.2 g C m^{-2} y^{-1} and Gebhart et al. (1994) found rates as high as 110 g C m^{-2} y^{-1} 12 years post conversion. Similar estimates have been reported for row crop conversion to forests although rates vary between tropical and temperate stands (Post et al., 2000). Evidence of C accrual in abandoned agricultural plots have also been reported with annual increases of 19.7 g C m^{-2} y^{-1} in surface soils (Knops and Tilman, 2000)

Post and Kwon (2000) explain that one of the most important drivers of C accumulation following conversion to perennials is an increase in soil organic matter inputs. Perennial systems often have between 3 and 10 times more belowground biomass compared to annual row crops (Culman et al., 2010; Zan et al., 2001; Dupont et al., 2014). Furthermore, C accrual will occur faster than respiration by heterotrophs in perennial vegetation because perennials are usually not tilled and are typically planted for longer intervals compared to annual crops (Huggins et al., 1998). Other important factors that lead to C accumulation under perennials are the inputs of soil organic matter deeper in the soil profile and enhancing the physical protection of soil C through

aggregation (Six et al., 1998; Grandy and Robertson, 2006; Syswerda et al., 2010; Tiemann and Grandy, 2015).

Less is known about the length of time required before increases in soil C are detectable or before soil C stabilizes post conversion (McLauchlan et al., 2006). In some cases, C accrual is detectable within the few years post conversion (Rehbein, 2015; McLauchlan et al., 2006) while in other cases detectable C accumulation can take over ten years (Syswerda et al., 2010). Much of this temporal variation reflects the rate of initial C accrual, which is largely dependent on the original C levels and how close a system is to reaching C equilibrium (Six et al., 2002). Further, soil C is comprised of different pools that turn over at different rates. A labile C pool with short turnover times can lead to only short-term C sequestration, whereas more recalcitrant C pools have longer residence times and consequently contribute to long-term C sequestration (Wander, 2004). The proportion of C in the labile pools compared to recalcitrant pools is rarely determined and thus C stabilization potential post conversion is poorly understood.

There is widespread interest to increase soil C in agricultural systems for both farm-scale and global benefits and one option could be to cultivate perennial grain crops in place of annual grain crops (Asbjornsen et al., 2013). Perennial wheat and perennial intermediate wheatgrass (IWG) are being developed to achieve the high yields of annual wheat and simultaneously have extensive root systems that could potentially increase soil C (Glover, 2010; Kell, 2011). For example, perennial grains developed by Dehaan et al. (2004) have significantly more coarse roots compared to wheat to 40 cm depth as well as more fine roots to 70 cm (Sprunger, Chapter 2). However, initial yields from perennial grain crops seem to peak after two or three years (Wagonner, 1990; Culman et al., unpublished), at which point a farmer would need to replant IWG or rotate to another crop. Understanding whether initial gains in soil C can occur within this

period is important for determining the value of perennial grains as a plausible strategy for C sequestration.

My objective here is to examine root C and labile and recalcitrant pools of soil C under an experimental perennial grain crop (IWG) four years post conversion compared to annual winter wheat (wheat). I hypothesized that 1) IWG will accumulate more C in labile and recalcitrant pools compared to wheat because of greater C inputs from both above and belowground sources; and 2) C pools will be greatest in systems receiving greater rates of N fertilization.

METHODS

Site description

This study was conducted at the W.K. Kellogg Biological Station (KBS), located in Southwest Michigan, USA (42° 24' N, 85° 24' W, elevation 288 m). The mean annual precipitation and temperature are 1005 mm and 10.1°C. KBS soils are in the Kalamazoo soil series (fine loamy) and Oshtemo (coarse loamy), mixed, mesic Typic Hapludalfs. These soils typically have an A horizon to a depth of 30 cm, a deep Bw/Bt horizon that reaches 80+ cm, and a BC horizon that extends to 140 cm. Prior to the establishment of this experiment, this field was under a corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Meer.]-wheat (*Triticum aestivum)* rotation.

Experimental design

The experiment was established in 2009 as split plot randomized complete block design with four replicated blocks. The main factor was N level and the sub-factor was crop type equaling 24 plots (3 N levels by 2 crops by 4 blocks). Each plot was 3.05 by 4.57 m, with 2.43 m buffers in between the plots and 0.9 m buffers on the perimeter. The three N levels included 1)

Low N (Organic N), which received 90 kg N ha^{-1} of poultry manure; 2) Mid N, which received 90 kg N ha^{-1} of urea; and 3) High N, which received 135 kg N ha^{-1} of urea. Details on fertilization application and timing can be found in (Sprunger, Chapter 2).

The two crops were i) annual winter wheat *(Triticum aestivum* L. var. *Caledonia)* and ii) IWG (*Thinopyrum intermedium* (Host) Barworkth and D.R. Dewey*)*. IWG was developed through bulk breeding and mass selection at the Land Institute in Salina, KS (DeHaan et al, 2004; Cox et al., 2010). The Land Institute has trademarked this experimental grain as *Kernza*^{TM}.

Prior to establishment this site was chisel plowed to 20 cm and in subsequent years the wheat plots were rototilled to 15 cm depth. Prior to establishment in 2009, the field was under a corn (*Zea mays* L.*)*-soybean [*Glycine max* (L.) Meer.]-wheat (*Triticum aestivum)* rotation.

Aboveground biomass sampling

For this study, aboveground biomass was measured at maturity for both crops, where wheat was harvested on July 15, 2013, and the IWG was harvested on August 26, 2013. Aboveground biomass was determined by randomly placing two 0.25-m^2 quadrats in every plot and clipping the crop biomass to 10 cm above the soil. The aboveground biomass was separated into seed heads and straw. Then dried at 60ºC for 48 hours and weighed. Seeds were separated from their hulls using a tabletop thresher.

Belowground biomass and soil sampling

Belowground biomass and soil samples were collected on June 7th and 8th, 2013, which was near peak aboveground biomass for both wheat and IWG. I used a hydraulic direct-push soil sampler (Geoprobe, Salina, KS) to extract three soil cores to 1 m depth, 6 cm in diameter from each plot. The three cores were subsequently divided into five depths (0-10 cm, 10-20 cm, 20-40

cm, 40-70 cm, and 70-100 cm) and composited by depth interval. From each depth interval, a sub-sample of 400 g was taken for root analysis. Roots were separated into two size classes, coarse (>6 mm) and fine (<1 mm). I obtained coarse roots by dry gently sieving soil samples through 6 mm sieves. I obtained fine roots by wet sieving the remaining soil through a 1 mm sieve. I made no attempt to separate live and dead roots. To ensure that roots were soil-free, I hand-washed roots by soaking them in deionized water. Both coarse and fine roots were dried at 60^{o}C for 48 hours and then weighed.

Crop C and N analysis

Dried grain and stems were ground separately to a fine powder. Dried roots were frozen in liquid N and then immediately ground to a fine powder using a mortar and pestle. I analyzed both above and belowground crop parts for C and N in a CHNS analyzer (Costech Analyzer ECS 4010, Costech Analytical Technologies, Valencia, CA). Crop C content was determined by multiplying crop biomass by C concentration.

Labile and recalcitrant C pools

I utilized physical size fractionation to determine particulate organic matter (POM), which has been shown to reflect both labile and more recalcitrant C pools (Cambardella and Elliot, 1992; Culman et al., 2012). First, I gently sieved 100 g of soil through 6 mm as not to disturb soil aggregates (Ontl, 2013). Next, 10 g of air-dried soils and 30 mL of 0.05 sodium hexametaphospate were combined in 50 mL centrifuge tubes and placed on a shaker for 8 hours at 120 oscillations min^{-1}. Using a water bottle filled with deionized water, I passed the solution of soil and sodium hexametaphospate through a 212 μm sieve (large POM), which was placed over a 0.053 μm mesh sieve (medium POM). The large POM fraction is associated with coarser

material and reflects the labile C pool, while the medium POM is comprised of silt and clay particles and is associated with more recalcitrant pools of C. The materials that were retained on both sieves were oven dried at 55° C until samples reached a constant weight. Dried samples were then ground using a mortar and pestle and analyzed for C and N as above. POM-C on an areal basis was determined by multiplying POM-C concentration, dry weight of POM-fraction, and length of depth interval.

Statistics

All above and belowground biomass as well as labile soil C data were analyzed separately using Proc Mixed of SAS (version 9.3; SAS Institute, Cary, NC, USA). Plant species, N level, and depth were treated as fixed effects and block as a random effect. Significant differences were determined at $\alpha = 0.05$. For labile C and roots, depth was analyzed as a repeated measure. Means were compared with an adjusted Tukey's pairwise means comparison.

I used a post-hoc statistical power analysis to determine if a type II error occurred during the POM-C statistical analysis. Power analyses have been widely used in soil science to determine if the lack of significance is more likely due to insufficient sampling (number of replications) or an absence of biogeochemical differences between treatments (Kravchenko and Robertson, 2011; Ladoni et al., 2015). Detailed explanations of power analyses that have been used for soil C studies can be found in Garten and Wullschlegar (1999), Poussart and Olsson, (2004), and Kravchenko and Robertson (2011). In brief, I conducted a post-hoc power analysis that included 1) hypothesizing a size difference in the Large POM-C between wheat and IWG; 2) estimating the variability; 3) specifying a significance level of $\alpha=0.05$; 4) specifying the probability of detecting statistical differences (power); and 5) calculating a proposed number of replications.

The power analysis was conducted using the PROC MIXED procedure in SAS (version 9.3; SAS Institute, Cary, NC, USA).

RESULTS

Aboveground C

Aboveground C significantly differed by crop type (Table 3.1). Grain C for wheat ranged from 1.28 ± 0.14, s.e.m to 1.39 ± 0.15 Mg C ha^{-1} across N levels and was up to 25 times greater than for IWG, where grain C ranged from 0.07 ± 0.002 to 0.54 ± 0.1 Mg C ha^{-1}. There was no overall N level effect as both crops had statistically similar grain C across N levels ($F=5$, $p<0.6$). Straw C was significantly greater in IWG compared to wheat ($F=1.5$, $p<0.2$). Averaging across N levels, IWG had 1.9 times greater straw C compared to wheat. Both wheat and IGW aboveground C was similar across the three N levels.

Root C and depth distribution

IWG coarse root C was up to 15 times greater than that of wheat, where IWG coarse root C ranged from 1.70 ±0.30 to 2.42 ±0.13 Mg C ha^{-1} and wheat C mass ranged from 0.29 ±0.07 to 0.11 ±0.05 Mg C ha^{-1}. Despite no overall N level effect ($F=1.4$, $p=0.3$), pairwise comparisons revealed that IWG coarse root C under high N was significantly greater than coarse root C under Mid N and Low N (Organic N) (Table 3.2, $p<0.03$). Wheat coarse root C was statistically similar across N levels. The majority of root C was concentrated at the surface for both crops. Averaging across N levels, 60 % of IWG total root C was in the top 10 cm and 81% was in the top 20 cm. Wheat root C was even more concentrated at the surface, where, on average 79% of root C was in the top 0-10 cm and 96% of root C was in the top 20 cm. IWG had significantly greater root C compared to wheat to 40 cm depths across all N levels (Figure 3.2).

Differences between wheat and IWG were also apparent for fine root C (Table 3.2), which was four times greater in IWG compared to wheat (F=34.6, p=0.0002). Total fine root C for IWG was 0.24±0.02, 0.47 ±0.09, and 0.47±0.10 Mg C ha^{-1} for Low N (Organic N), Mid N and High N respectively. In contrast, total fine root C was 0.063 ±0.001, 0.01 ±0.01, and 0.11 ±0.05 Mg C ha^{-1} for Low N (Organic N), Mid N and High N respectively. There was a marginal overall N level effect on fine root C (F=3.0, p=0.1). In addition, pairwise comparisons showed that IWG under High N and Mid N had significantly greater root C content compared to IWG under Low N (Organic N) (Table 3.2, 0.03, 0.02, respectively).

IWG fine root C was more evenly distributed throughout the soil profile compared to coarse root C, but still a large portion was in the top 20 cm. For example, averaging across N levels, 48% of root C was in the top 10 cm and 72% was in the top 20 cm. Fine root distributions in the wheat systems mirrored the coarse root biomass distributions with 67% found in the top 10 cm and 92% found in the top 90 cm. IWG had significantly more fine root C compared to wheat to 70 cm depth in Mid N and High N levels (Figure 3.2). Differences between the two crops were only visible in the top 20 cm under Low N (Organic N).

C and N concentrations and C:N ratios

There was a significant crop effect for root C concentrations (F=98.9, p<0.0001), but differences between wheat and IWG mainly occurred in top 10 cm (Table 3.3), which explains the significant crop by N level by depth interaction (F=2.34, p=0.03). At the surface depth interval, IWG root C concentrations ranged from 28.9 to 33.1% and were greater than in wheat, which ranged from 16.8% to 22.3%. Coarse root C did not significantly differ across N levels (F=3.1, F=0.07). Despite significant overall crop and N level effects on fine root C concentrations (F=0.05, p =0.01 and F=3.76, p =0.05), distinct trends between the two crops for

fine root C concentrations were not as apparent compared to those in coarse roots. In general, greater C concentrations were found under the Low N (Organic N) level compared to the Mid N and High N levels (Table 3.3).

Coarse root N concentrations were almost always greater in the wheat systems compared to IWG (Table. 3.4, $F=77$, $p<0.0001$) and decreased significantly by depth ($F=26$, $p<0.0001$). There was also a strong N level effect, where coarse root N concentrations were typically greatest in the High N level ($F=36$, $p<0.0001$). There was a significant N level by crop by depth interaction ($F=2.7$, $p=0.01$), most likely caused by lack of differences across N level and between crops at depths below 40 cm. Fine root N concentrations differed by crop ($F=75.7$, $p<0.0001$) but not by N level ($F=0.6$, $p<0.6$). Wheat had greater N concentrations compared to IWG at almost every depth (Table 3.3). On average, fine root N concentrations were 36% greater than coarse root N concentrations for both crops.

The C:N ratio for coarse roots was significantly greater in IWG systems compared to wheat at almost every depth (Figure 3.3, $F=269$, $p<0.0001$). There was also a strong overall N level effect ($F=74.8$, $p<0.0001$), where the coarse root C:N ratio was greater under Low N (Organic N), especially at lower depths. Similarly, there was an overall crop ($F=62.5$, $p<0.001$) and N level ($F=10.4$, $p<0.002$) effect for fine root C:N ratio, where IWG had a significantly greater C:N ratio at all depths under Low N (Organic N) and greater C:N ratio in subsurface depths under Mid N and High N (Figure 3.4). In addition, there was a significant crop by N level interaction because IWG was more affected by N level compared to wheat (Figure 3.4).

Particulate organic matter C

There were no significant differences in large or medium POM-C concentrations between the two crops (Figure 3.5, $F=0.5$ and $p=0.5$ and $F=0$, $p=0.9$, respectively) or across N levels

(F=0.3, p=0.8 and F=0.6,p=0.9, respectively). The large POM-C concentrations were greatest in the top 0-10 cm of soil in both crops compared to other depth intervals. Mean IWG large POM-C concentrations at the surface depth were 3.6 ±0.4, 3.8 ±0.8, and 4.0 ±0.7 g C kg soil^{-1} for Low N (Organic N), Mid N, and High N respectively. Wheat POM C concentrations at 0-10 cm depth ranged from 3.6 ±1.3 to 4.7 ±0.7 g C kg soil^{-1}, with greater concentrations found in the Low N system.

Medium POM-C was greater than the large POM-C. At 0-10 cm, IWG medium POM-C ranged from 10.9 ±1.4 to 11.2 ±0.6 g C kg soil^{-1} across N levels, with the Mid N system having the lowest concentrations. Surface soil concentrations were very similar in wheat systems where concentrations ranged from 9.8 ±1.6 to 12.6 ±1.9 g C kg soil^{-1}, again with concentrations slightly higher in the Low N (Organic N) system. Pairwise comparisons reveal that large POM-C concentrations below the 10 cm depth interval were statistically similar to one another ($p>0.05$). In contrast, medium POM-C fractions significantly decreased by depth to 40 cm ($p<0.0001$).

POM-C content accounts for the weight of the fraction, C concentration, and length of depth interval. There was no difference in large or medium POM-C content between the two crops throughout the soil profile to 1 m (Figure 3.6, F=0 and p=0.9 and F=0.11, p=0.7, respectively). Approximately 40% of POM-C was found in the top 0-10 cm for both crops. POM-C below 20 cm was evenly distributed throughout the soil profile in the large fraction, but steadily decreased by depth in the medium fraction (Figure 3.6). In addition, POM-C content was statistically similar across N levels for both large and medium fractions (F=0.8 and p=0.5, F=1.6, p=0.2; respectfully).

Power analysis

I conducted a post-hoc power analysis for two different scenarios. First, I used the observed difference between wheat and IWG in the large POM-C at 0-10 cm depth and simply increased the number of replicates. Second, I hypothesized a 15% difference in C between wheat and IWG, while keeping the number of replicates at n=4. The power values calculated for both scenarios are shown in figure 3.7. For scenario one, a total of 52 replicates were needed in order to achieve 78% power. For scenario two, a 15% increase in the difference between wheat and IWG large POM-C with four replicates was needed to achieve 84% power.

DISCUSSION

Above and belowground C differed considerably between the annual wheat and perennial IWG systems, with root C contents up to 15 times greater in IWG. However, despite greater root C in the IWG system, I did not detect any differences in labile or recalcitrant soil C pools, as measured by POM-C between wheat and IWG four years after establishment.

Crop C and POM-C fractions

Perennial crops are often touted for their greater and more extensive root systems compared to annual crops (Glover et al., 2007), which was true for this study; I found that total coarse and fine root C stores of IWG were between 6 and 15 times greater than root C stores of wheat. The magnitude of differences in root C between IWG and wheat is on par with other studies comparing annual and perennial crops (Jarchow et al., 2012; Anderson-Teixeira et al., 2013). My findings are also consistent with expectations that perennial grains will have greater root C at subsurface depths (Glover, 2010). Significant differences between the two crops were

detectable to 70 cm depth and demonstrate that perennials are capable of placing greater amounts of root C deeper in the soil profile compared to annual crops. Although the majority of aboveground biomass is removed in both IWG and wheat, there is a portion of aboveground C that is left on the soil surface. Given that IWG has significantly greater straw C than wheat, there also could potentially be more aboveground C contributing to soil C stores in IWG systems compared to wheat.

However, despite greater overall aboveground C and up to 15 times more root C within the IWG systems compared to wheat, I did not find significant differences in the labile or recalcitrant soil C pools between the two crops at any depth. Averaging across N level, mean concentrations at the 0-10 cm depth for the large POM-C fraction was 4.1 g C kg soil^{-1} for wheat compared to 3.7 g C kg soil^{-1} for IWG ($p=0.5$). Surface mean medium POM-C averaged across N level was 10.4 and 10.5 g C kg soil^{-1} for wheat and IWG, respectively ($p=0.9$). These findings do not support the hypothesis that more labile and recalcitrant soil C will accumulate under IWG compared to annual cereals. Furthermore, I found similar POM-C concentrations across the three N levels, even though IWG grown under High N had more root C than the IWG grown with Low N (Organic N) and Mid N levels.

Given the widespread evidence for gains in soil C under perennial systems compared to annual row-crops, it is surprising that I did not find greater soil C under IWG compared to wheat even after 4 years. Zan et al. (2001) found that willow stands used for biofuel production had 15% more soil C compared to corn after four years of production. In a review of soil C under biofuels, Anderson-Teixeira et al. (2009) consistently found that crops like switchgrass and miscanthus on average accumulated 1 Mg ha^{-1} yr^{-1} in the top 30 cm after 5 years. McLauchlin et

al. (2006) found a linear increase in labile and recalcitrant soil C in grassland systems that were between 0 and 40 years post conversion.

Lack of increase in soil C under IWG

One reason for the lack of increase in soil C here might be length of time since conversion and/or establishment. Forest and grassland systems that had increased soil C, reviewed by Post and Kwon (2001), were between 8 and 126 years post-conversion from cropland. In studies that reported soil C accumulation in perennial grasses or cellulosic biofuels compared to annual cropping systems, perennial systems were typically 4-15 years old (Syswerda et al., 2010; Collins et al., 2010; Rehbein et al., 2015). For example, at the nearby KBS LTER site, Syswerda et al. (2010) found greater surface soil C concentrations 12 years post establishment in alfalfa compared to a conventionally managed corn-soybean-wheat system. Over a four-year period, Su (2007) detected C sequestration rates of 0.57 Mg C $ha^{-1}yr^{-1}$ following conversion to alfalfa. Rehbein et al. (2015) found a linear increase in soil C accumulation in both labile and recalcitrant pools in Miscanthus stands that ranged from 0-19 years post-conversion. In those stands, soil C accumulation in the coarse POM fraction accumulated within the first seven years and than reached saturation, while the silt and clay associated POM fractions continued to accumulate C over time. Nevertheless, in this study, I would expect to see an increase in at least the labile C pools after four years.

The labile C pool is comprised of recent inputs from aboveground litter and or root rhizodeposition, and thus it is especially surprising that I did not detect an increase in soil C within the Large POM-C fraction. This may be because under IWG the labile C pool could be lower quality due to slower root decomposition compared to wheat. The C:N ratios of both

coarse and fine IWG roots were significantly greater than wheat throughout the entire profile, which could lead to reduced turnover and smaller C contributions within the initial years of establishment. A higher C:N ratio within perennial roots compared to annual roots is common (Craine et al., 2003) and the greater C content could lead to longer root persistence. Over time, as the roots higher in C content turn over, gains in soil C might be detected under IWG.

Another plausible explanation for the lack of differences in soil C between the two crops is priming under IWG. The priming effect occurs when increased root exudates stimulate microbial activity, causing an increase in decomposition rates of older soil C (Cheng, 1999). Strickland et al. (2015) found a 21% decline in total soil C in established switchgrass stands mainly due to losses in POM-C. They attributed this loss of C to priming that occurred due to increased microbial activity. In the present study, omnivore nematodes were greater under IWG (Culman et al., unpublished), which could have led to increased decomposition.

A final explanation for a failure to detect differences in soil C under this IWG system could be due to limitations in methodology. Although the POM fractionation procedure has been widely used to detect system level differences in soil C in both labile and recalcitrant pools (Cambardella and Elliot, 1992; Rehbein et al., 2015), POM-C still reflects more of a recalcitrant or processed C compared to other methods like microbial biomass and permanganate oxidizable C (Culman et al., 2012). Sprunger (Chapter 4) found that long-term incubations that utilize the degradation of enzymes to determine soil respiration were more effective at detecting C dynamics across annual and perennial cropping systems compared to POM fractionation. However, C mineralization results from this same site show no difference between IWG and wheat four years after establishment (Culman et al., unpublished).

A power analysis further helps to explain the lack of significant soil C differences considering the Large POM-C at the 0-10 cm depth interval, which is where I most expected to see a difference between the two systems. The analysis revealed that 52 replicates would likely be needed to reach an acceptable probability (78%) of detecting a small significant difference ($p=0.05$) in large POM-C at that time. With four replicates in this study, alternatively, a 15% difference in surface soil C between wheat and IWG would be needed to achieve 84% power. Over time, then, soil C might accumulate sufficiently to reveal a 15% difference in POM-C. However, a long-term experiment would be required to capture such differences and in any case would take longer than the expected 3 year perennial grain rotation now projected (Wagonner, 1990; Culman et al., unpublished). This power analysis thus reinforces the fact that more time is needed in order to detect difference in C between wheat and IWG.

Vision of perennial grains as a tool for soil C accumulation

The concept of perennial grains as a means to increase yields while providing ecosystem services within agricultural landscapes has garnered much attention (Wagoner, 1990; Glover, 2007). In particular, proponents of perennial wheat development argue that a perennial version of wheat could lead to crops that are more productive with less need for fertilizers, that ameliorate erosion and reduce nitrate leaching, and that possess greater water use efficiency (Glover et al., 2010; Kell, 2011; Culman, 2013). Proponents especially tout the potential for soil C accrual throughout the soil profile due to deep roots (Crews and DeHaan, 2015; Asbjornsen et al., 2013). Critics dispute the claim that perennial wheat and IWG will be viable from a production standpoint and argue that increasing seed production while maintaining characteristics of a perennial system is insurmountable with current breeding efforts (Smaje, 2015).

Perennial wheat and IWG yields at KBS are 50% and 70% lower than annual winter wheat yields (Jaikumar et al. 2012; Culman et al., 2013). However, proponents argue that it could still be valuable to farmers who want to improve soil health and other ecosystem services (Adebiyi et al., 2015). Four years post establishment, I was unable to detect any gains in C accumulation under IWG compared to wheat in either labile or recalcitrant pools. Four years may be an insufficient amount of time to detect gains in C under IWG and given the large amount of belowground C content, soil C gains could eventually occur. However, because yields decline after three or four years, gains in C that fail to show up in this time period may never be realized before farmers rotate to another crop.

Soil C sequestration is not the only ecosystem service that IWG can provide. For example, Culman et al. (2013) found that IWG reduced nitrate leaching to up to 99% compared to wheat and Sprunger (Chapter 2) found that IWG improved crop-level N use efficiency by up to 42%. In addition, there is evidence that perennial roots persist even after a new annual crop is established (Dupont et al., 2014). Perennial roots could, therefore, contribute to soil C pools after conversion to an annual system. Nevertheless, the undetectable soil C accumulation within a short time period weakens the appeal of perennial wheat and IWG.

CONCLUSIONS

I measured labile and recalcitrant soil C pools in wheat and 4th year IWG across three N levels differing in rates and types of N. Coarse and fine root C were up to 15 times greater under IWG compared to wheat. However, I did not detect any soil C gains under IWG in either labile or recalcitrant pools. Due to the large C stores found in above and belowground biomass in IWG systems, it is reasonable to expect gains in soil C over time. However, the post-hoc power

analysis reveals that detecting a significant difference in C would require either a large number of replicate samples or a greater (15%) difference between wheat and IWG. Since yields of perennial IWG decline after two or three years, the insignificant soil C accumulation after four years weakens the appeal of perennial grain crops.

APPENDIX

Table 3.1 Grain and straw between wheat and IWG across three N levels (Low N (Organic N), Mid N, and High N). Comparisons of means within rows (among cropping system) followed by same lowercase letters are not significantly different. Different lower case letters denote significant differences between crops and across N levels.

	Grain		Straw	
	Wheat	IWG	Wheat	IWG
	Mg C ha $^{-1}$			
Low N (Organic)	1.28 (0.14)[a]	0.07 (0.002)[b]	2.34 (0.28)[b]	4.59 (0.41)[a]
Mid N	1.43 (191)[a]	0.11 (0.02)[b]	2.73 (0.24)[b]	5.190 (0.40)[a]
High N	1.39 (0.15)[a]	0.54 (0.01)[b]	2.42 (0.18)[b]	5.36 (0.25)[a]

Table 3.2 Coarse and fine root C contents between wheat and IWG across three N levels (Low N (Organic N), Mid N, and High N). Comparisons of means within rows (among cropping system) followed by same lowercase letters are not significantly different. Different lower case letters denote significant differences between crops and across N levels.

	Coarse Roots		Fine Roots	
	Wheat	IWG	Wheat	IWG
	Mg C ha $^{-1}$			
Low N (Organic)	0.29 (0.07)^{c}	1.74 (0.29)^{b}	0.063 (0.001)^{c}	0.24 (0.02)^{b}
Mid N	0.19 (0.06)^{c}	2.42 (0.13)^{a}	0.10 (0.01)^{c}	0.47 (0.09)^{a}
High N	0.16 (0.03)^{c}	2.42 (0.13)^{a}	0.11 (0.05)^{c}	0.47 (0.1)^{a}

Table 3.3 Carbon concentrations for coarse fine root biomass across N levels (Low N (Organic N), Mid N, and High N) at five depths. Comparisons of means within rows (among cropping system) followed by same lowercase letters are not significant. Different lower case letters denote significant differences between wheat and IWG.

		Coarse Root C concentration		Fine Root C concentration	
Management	Soil depth	Wheat	IWG	Wheat	IWG
	cm	g C kg^{-1}		g C kg^{-1}	
Low N (Organic N)	0-10	22.3 (0.9)[a]	31.1 (2.8)[b]	29.8 (1.5)[a]	28.7 (2.2)[a]
	10-20	27.6 (1.4)[a]	31.8 (0.4)[a]	29.1 (0.9)[a]	29.9 (1.7)[a]
	20-40	30.3 (0.8)[a]	36.1 (1.2)[a]	30.3 (2.5)[a]	35.5 (0.3)[a]
	40-70	21.9 (7.4)[a]	38.4 (0.6)[b]	30.8 (1.9)[a]	33.6 (1.1)[a]
	70-100	No Roots	28.7 (4.3)[*]	26.5 (4.9)[a]	28.8 (1.3)[a]
Mid N	0-10	16.8 (1.2)[a]	29.8 (2.8)[b]	28.9 (3.9)[a]	21.8 (3.2)[b]
	10-20	25.4 (2.1)[a]	30.9 (5.10)[b]	30.2 (1.2)[a]	17.4 (3)[b]
	20-40	24.2 (2.4)[a]	25.64 (2.4)[a]	29.4 (1.2)[a]	26.5 (3.2)[a]
	40-70	27.9 (5.6)[a]	29.7 (0.6)[a]	31.1 (1.0)[a]	32.7 (0.4)[a]
	70-100	No Roots	28.4 (1.4)[*]	32.8 (5.0)[a]	30.1 (1.1)[a]
High N	0-10	18.5 (2.5)[a]	33.3 (1.9)[b]	31.1 (1.6)[a]	16.9 (0.8)[b]
	10-20	21.9 (1.3)[a]	26.8 (3.3)[a]	24.4 (3.3)[a]	17.9 (1.6)[b]
	20-40	24.8 (2.7)[a]	33.7 (3)[a]	31.1 (2.3)[a]	29.1 (3.4)[a]
	40-70	20.1 (0.6)[a]	27.5 (2.8)[a]	28.9 (2.2)[a]	32.4 (1.1)[a]
	70-100	12.9 (0.8)[a]	22.6 (4.9)[a]	34.0 (1.7)[a]	30.4 (2.3)[a]

Table 3.4 Nitrogen concentrations for coarse fine root biomass across N levels (Low N (Organic N), Mid N, and High N) at five depths. Comparisons of means within rows (among cropping system) followed by same lowercase letters are not significant. Different lower case letters denote significant differences between wheat and IWG.

		Coarse Root N Concentrations		Fine Root N Concentrations	
Management	Soil depth	Wheat	IWG	Wheat	IWG
	cm	g N kg^{-1}		g N kg^{-1}	
Low N (Organic N)	0-10	0.89 (0.06)[a]	0.57 (0.03)[b]	1.3 (0.08)[a]	0.86 (0.09)[b]
	10-20	0.48 (0.1)[a]	0.43 (0.01)[a]	0.98 (0.02)[a]	0.77 (0.03)[b]
	20-40	0.59 (0.06)[a]	0.30 (0.03)[a]	0.95 (0.06)[a]	0.65 (0.06)[b]
	40-70	0.44 (0.05)[a]	0.26 (0.04)[a]	0.74 (0.09)[a]	0.49 (0.02)[b]
	70-100	No roots	0.33 (0.06)[*]	0.66 (0.08)[a]	0.55 (0.06)[a]
Mid N	0-10	0.8 (0.04)[a]	0.8 (0.02)[a]	0.99 (0.2)[a]	0.68 (0.1)[b]
	10-20	0.86 (0.04)[a]	0.62 (0.07)[b]	1.1 (0.05)[a]	0.64 (0.09)[b]
	20-40	0.7 (0.04)[a]	0.44 (0.02)[a]	1.0 (0.04)[a]	0.6 (0.04)[b]
	40-70	0.77 (0.06)[a]	0.46 (0.04)[b]	0.89 (0.08)[a]	0.66 (0.03)[b]
	70-100	No roots	0.48 (0.03)[*]	0.92 (0.09)[a]	0.64 (0.03)[b]
High N	0-10	0.85 (0.06)[a]	0.93 (0.01)[a]	1.09 (0.2)[a]	0.8 (0.02)[b]
	10-20	0.84 (0.07)[a]	0.68 (0.04)[a]	1.0 (0.09)[a]	0.59 (0.1)[b]
	20-40	0.81 (0.05)[a]	0.52 (0.05)[a]	1.1 (0.09)[a]	0.7 (0.06)[b]
	40-70	0.77 (0.05)[a]	0.54 (0.09)[a]	0.9 (0.05)[a]	0.76 (0.1)[a]
	70-100	0.38 (0.04)[a]	0.41 (0.04)[a]	0.84 (0.2)[a]	0.67 (0.06)[a]

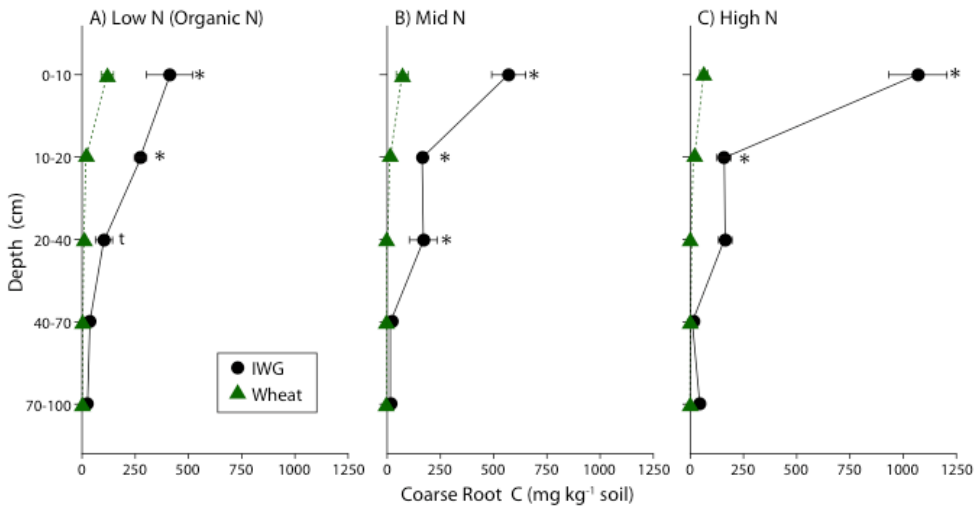

Figure 3.1 Coarse root C content for annual winter wheat (triangles) and IWG (circles) for three N levels (Low N (Organic N), Mid N and High N) at five different soil depths. Error bars represent the standard error of the mean and asterisks denote significance at $p<0.05$ and t denotes significance at $p<0.1$.

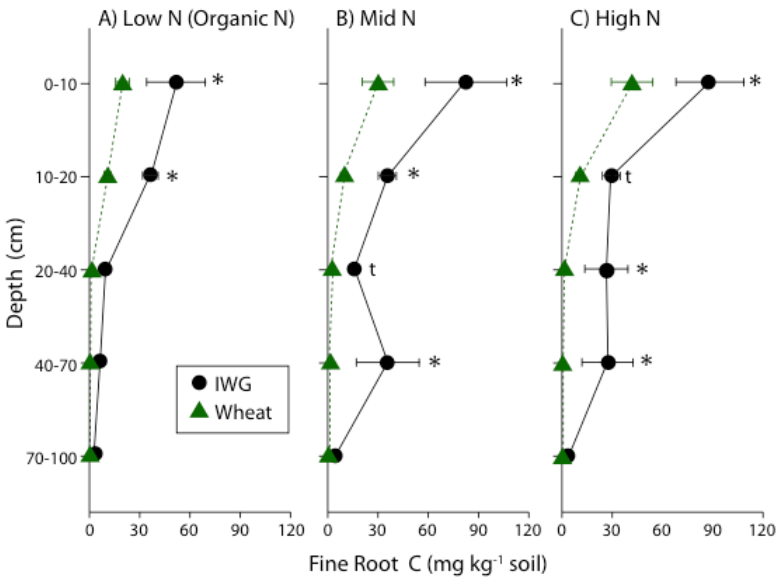

Figure 3.2 Fine root C in annual winter wheat (triangles) and IWG (circles) for three N levels (Low N (Organic N), Mid N and High N) at five different depths throughout the soil profile. Error bars represent the standard error of the mean and asterisks denote significance at $p<0.05$ and t denotes significance at $p<0.1$.

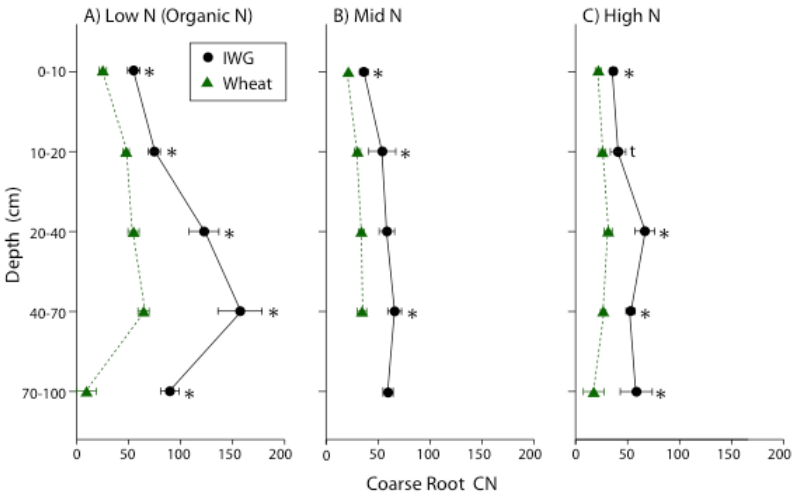

Figure 3.3 Coarse root C:N ratios for annual winter wheat (triangles) and IWG (circles) for three N levels (Low N (Organic N), Mid N and High N) at five different depths throughout the soil profile. Error bars represent the standard error of the mean and asterisks denotes significance at <0.05 and t

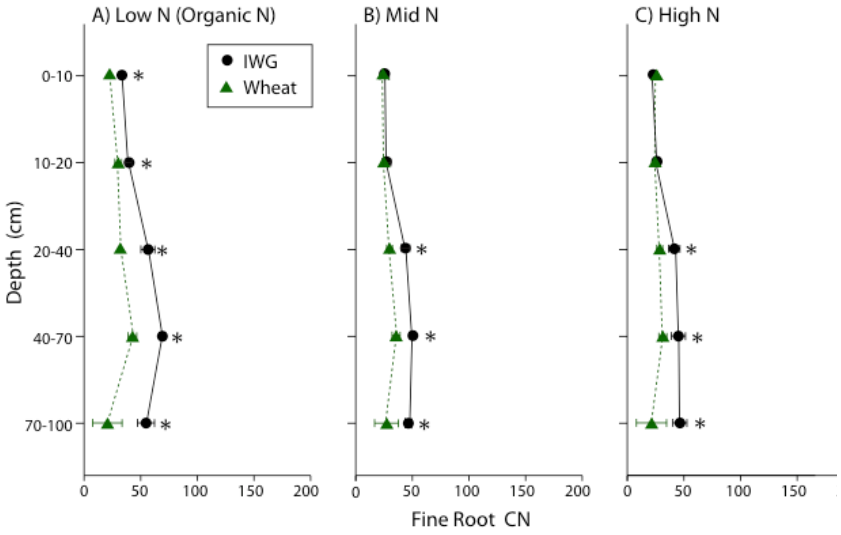

Figure 3.4 Fine root C:N ratios for annual winter wheat (triangles) and IWG (circles) for three N levels (Low N (Organic N), Mid N and High N) at five different depths throughout the soil profile. Error bars represent the standard error of the mean and asterisks denote significance at $p<0.05$ and t denotes significance at $p<0.1$.

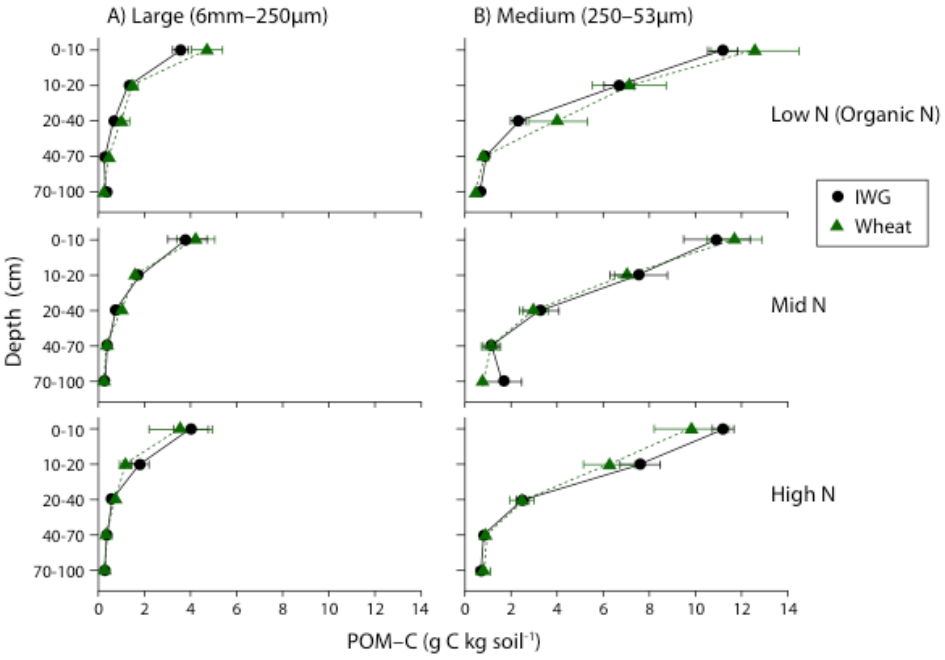

Figure 3.5 Large and Medium POM-C concentrations for annual winter wheat (triangles) and IWG (circles) for three N levels (Low N (Organic N), Mid N and High N) at five different depths throughout the soil profile. Error bars represent the standard error of the mean and asterisks denote significance at $p<0.05$ and t denotes significance at $p<0.1$.

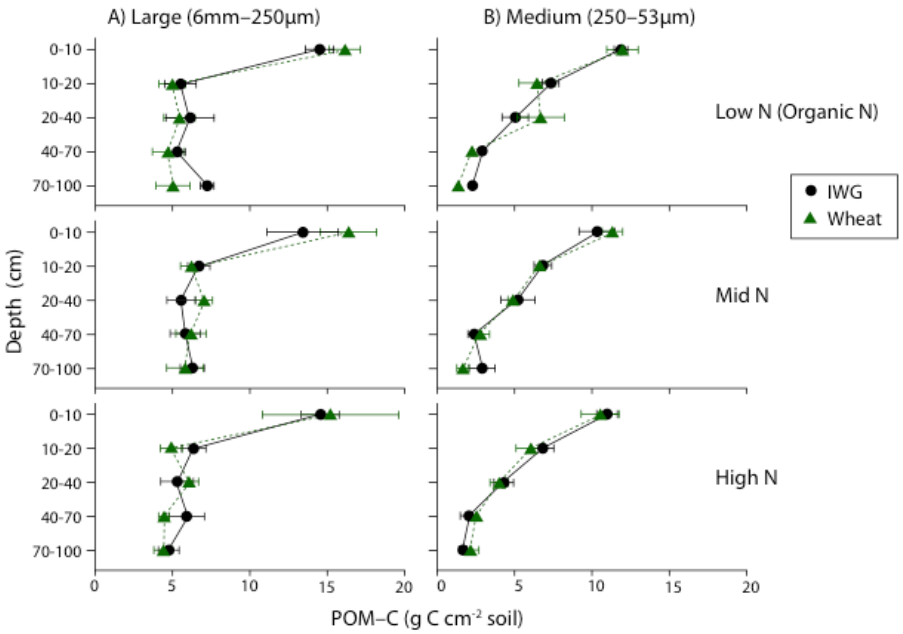

Figure 3.6 Large and Medium POM-C content for annual winter wheat (triangles) and IWG (circles) for three N levels (Low N (Organic N), Mid N and High N) at five different depths throughout the soil profile. Error

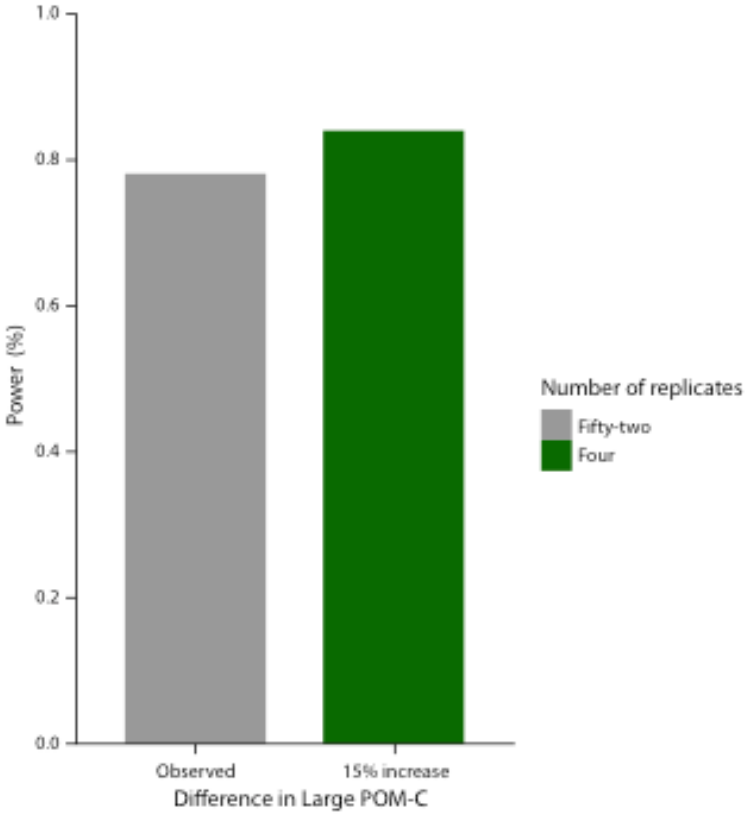

Figure 3.7 Probability (power) of detecting a statistically significant difference at (p=0.05) in surface (0-10 cm) POM-C between wheat and IWG for two scenarios: 1) increasing the number of replicates but keeping the observed difference in POM-C between wheat and IWG, and 2) keeping the same number of replicates (n=4) but using a hypothesized increase in POM-C (15 %).

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CHAPTER 4: CHANGES IN ACTIVE AND SLOW SOIL CARBON POOLS UNDER PERENNIAL BIOENERGY CROPS IN CONTRASTING SOILS

ABSTRACT

Differences in soil carbon (C) accumulation rates can markedly affect the sustainability of ecosystems managed for food and fuel production. I examined soil C accumulation and persistence in candidate biofuel cropping systems that differed in life histories (annual vs. perennial) and diversity (monoculture vs. polyculture) five years post-establishment, in ten replicated systems at both a moderate and high fertility site. I measured active, slow, and resistant C pools via long-term laboratory incubations and acid hydrolysis extraction. Cropping systems included four annual systems (no-till continuous corn and each phase of a corn-soybean-canola rotation), three monoculture perennial systems (switchgrass (*Panicum virgatum)*, miscanthus (*Miscanthus* × *giganteus*), and hybrid poplar (*Populus nigra* × *P*. maximowiczii 'NM6)), and three diverse herbaceous perennial systems (a five-species native grass assemblage, an early successional community, and a restored prairie). Replicate systems were sampled at both a moderate fertility site in southwest Michigan (Kellogg Biological Station; KBS) and a high fertility site in south central Wisconsin (Arlington; ARL).

Surface (0-10 cm) cumulative C mineralization was greatest in the diverse cropping systems at both sites; at KBS, the native grasses (65 μg C g^{-1} $soil^{-1}$) and early successional systems (64 μg C g^{-1} $soil^{-1}$) had significantly greater C mineralization fluxes compared to all the other systems ($p<0.05$). I found substantial differences in active C between the annual monoculture and the perennial polyculture crops but not between the annual and perennial monoculture crops. Active C pools under perennial polycultures were over 2.5 times greater than under continuous corn,

and among systems followed the rank order continuous corn (237 μg C g^{-1} soil) << early successional (500) < restored prairie (638) ≈ native grasses (656). Amongst the perennial monocultures, only the poplar system had 2.5 times more active C than the annual systems. System differences in the slow C pool were less apparent, and there were no significant differences among the systems in the resistant C pool.

At ARL, the more fertile site, the restored prairie system (75 μg C g^{-1} $soil^{-1}$) had significantly greater cumulative C mineralization than all other systems ($p<0.05$). Active C pools were similar to those at KBS, however, differences amongst systems were insignificant five years post-establishment, except the restored prairie and rotational corn had 3.4 times more active C than other systems. ARL accumulated significantly greater C in the resistant pool compared to KBS at every depth except 50-100 cm. Patterns of particulate organic matter carbon (POM-C) among systems were not consistent with long-term incubation results. These findings demonstrate that poplars and diverse perennial bioenergy systems are more effective at increasing C in the active pool than no-till annual crops and monoculture perennials, especially in less fertile soils. The fact that I did not find any differences in C accrual between monoculture perennials and no-till annuals suggests that no-till management may be equally advantageous to perenniality. Overall, these findings demonstrate that diverse perennial biofuels grown on marginal lands could lead to significant and rapid increases in C accumulation.

INTRODUCTION

Soil carbon (C) plays an important role at both local and global scales. At the local scale, C is crucial for improving soil structure, increasing biological activity, and increasing nutrient and

water availability, all of which lead to healthier and more productive soils (Lal et al., 2011; Seremesic et al., 2011). Globally, soil C has an important role in balancing the C cycle as soil C can serve as a source or a sink of atmospheric carbon dioxide (CO_2). When photosynthetic inputs exceed decomposition rates, soil C accumulates. However, when soil heterotrophs respire C at a faster rate than C accumulates, soil C is lost to the atmosphere as CO_2. Since soils hold twice the amount of C globally compared to the atmosphere (Swift, 2001), the stabilization of soil C pools is crucial for regulating atmospheric CO_2 concentrations.

Efforts to sequester soil C are motivated by the fact that anthropogenic activities have led to soil C losses of up to 100 Pg worldwide (Paustian, 2002). Replenishing the soil C pool could lead to atmospheric CO_2 mitigation as more C would be sequestered in the soil versus released to the atmosphere. Sequestering C in agricultural landscapes is especially attractive as it can enhance other ecosystem services like soil health and crop yields (Lal, 2011). Well-studied approaches to enhancing soil C consist of either slowing decomposition by converting to no-till management or increasing C inputs by adding manure, cover crops, or additional crop residue (Hutchinson et al., 2007; Jarecki and Lal, 2010; Johnston et al., 2009).

Planting perennial vegetation in place of annual crops is another strategy that could increase soil C by decreasing decomposition while simultaneously increasing organic matter inputs (Kell, 2011). Perennial species typically exhibit extensive root systems that can contribute large amounts of C belowground (Dupont et al., 2014). Furthermore, perennial crops are no-till by nature and thus decomposition is slowed as compared to annually tilled systems. Sperow et al. (2003) estimated that conversion from annual row crops to perennial vegetation could sequester 28 Tg C yr^{-1}. Yet another strategy for increasing soil C is enhancing plant diversity (Steinbeiss et al., 2008). For example, Fornara and Tilman (2008) found that grassland communities with

increased diversity sequestered 5 times more C compared to monoculture systems of the same species.

While conversion to perennial vegetation has proven to be effective at increasing total SOC, the proportion of C accruing in labile versus more recalcitrant pools is poorly known. The labile or active pool consists of freshly deposited material such as plant residue and root exudates, and typically has a mean residence time (MRT) of less than a year. In contrast, the slow pool is comprised of material that has been stabilized through physical and biochemical processes and has a MRT that ranges from a few years up to a decade, and the passive or resistant pool consists of non-hydrolyzable C that is closely associated with the inorganic fraction of soil and has a MRT of thousands of years (Paul et al., 2001; Wander, 2004).

Several techniques have been used to separate and quantify pools of soil C including biological, chemical, and physical methods. Previous research has shown that biological approaches via long-term incubations are particularly informative for discerning C accumulation in active and slow pools post disturbance or land conversion (Paul et al. 1999; 2001). For example, Paul et al. (1999) found that early successional systems accumulated more soil C in the slow pool compared to conventionally tilled annual crops, and overall, poplars were the most effective at stabilizing C. Collins et al. (2010) found that the slow C pool under five year-old switchgrass stands was 13% greater than in the nearby uncultivated native soils. Physical techniques, such as particulate organic matter (POM) size and density fractionation, can be used to isolate different physical fractions of soil C (Cambardella and Elliott; 2000). The lighter and larger POM fractions are typically more associated with labile pools of C compared to the more processed, heavier and smaller fractions, which represent a more stabilized pool of C (Six et al., 2000; Wander, 2004; Culman et al., 2012).

Here I utilize long-term incubations and POM fractionation to investigate C pools across eight potential biofuel cropping systems ranging in perenniality and diversity from continuous corn to restored prairie, and comparing equivalent cropping systems in two soils contrasting in fertility. I hypothesized that 1) perennial systems will have greater active and slow C pools compared to annual systems due to their more persistent roots, which contribute large amounts of C belowground; 2) in general, active C pools will accumulate faster at a more fertile site (Arlington; ARL) compared to a less fertile site (Kellogg Biological Station; KBS), because of higher C stocks and higher clay content; and 3) perennial systems higher in diversity will have more root production and thus greater soil C accumulation.

METHODS

Site description

Hypotheses were tested in the Biofuel Cropping System Experiments (BCSE) located at Arlington Agricultural Research Station (ARL) in Wisconsin, USA and the Kellogg Biological Station (KBS) Long-Term Ecological Research Site located in Michigan, USA. ARL has more fertile soils than KBS. Both sites are part of the U.S. Department of Energy's Great Lakes Bioenergy Research Center (GLBRC). Mean annual precipitation and temperature at ARL are 833 mm yr^{-1} and 7.4^{o}C, respectively. Soils are silty loam mesic Typic Argiudolls in the Plano Series (Sanford et al., 2012), with five horizons: Ap (0-23 cm), A (23-36 cm), Bt1 (36-48 cm), Bt2 (48-79cm), and Bt3 (79-109 cm). Prior to establishment in 2008 the surface (0-10 cm) pH was 6.6, total soil C was 22.4 g C kg^{-1} (Sanford et al., in press), and soil texture was 9% sand, 66% silt, and 25 % clay (http://data.sustainability.glbrc.org). Mean annual precipitation and temperature at KBS are 1005 mm yr^{-1} and 10.1^{o}C. Soils at KBS are well-drained loamy mesic

Typic Hapludalfs and primarily within the Kalamazoo and Oshtemo series with five distinct horizons: Ap (0-30 cm), E (30-41 cm), Bt1 (41-69 cm), 2 Bt2 (69-88 cm), 2E/Bt (88-152) (Robertson and Hamilton, 2015). In 2008 surface soils at KBS (0-10 cm) had a pH of 6.1, total soil carbon was 14.3 g C kg^{-1}, and texture was 63% sand, 31% silt, 6% clay (http://data.sustainability.glbrc.org). Prior to experiment establishment in 2008 both sites were under annual row crops.

Experimental design and systems

The BCSE is a randomized complete block design at each site with five replicate blocks consisting of nine biofuel cropping systems that include annual row crops, monoculture perennial crops, and diverse herbaceous perennial crops (Table 1). Four annual row crops consist of continuous corn *(Zea mays* L.) and each phase of a corn-soybean (*Glycine max* L.)- canola (*Brassica napus* L.) rotation. The perennial systems include three monocultures and three mixed plant assemblages. The perennial monoculture systems are switchgrass *(Panicum virgatum* L.), miscanthus *(Miscanthus × giganteus)* and hybrid poplars (*Populus nigra × P. maximowiczii* 'NM6'). The diverse perennial systems consist of a five species native grass mix (*Andropogon gerardii*, *Elymus canadensis*, *Panicum virgatum*, *Schizachrium scoparium*, and *Sorghastrum nutans)*, an early successional community, and an 18-species restored prairie consisting of C3, C4, and legume species.

Prior to planting, all plots were tilled with a chisel plow and secondary soil finisher in early spring 2008. The annual row crops were subsequently planted in late spring, and thereafter treated as no-till. Planting rates for corn and soybeans were 70,000 and 78,000 seeds ha^{-1}, respectively. Canola was planted at 4.5 kg ha^{-1}. The switchgrass, native grasses, and restored prairie systems were planted in the summer of 2008 with a brillion-type native plant seeder.

Seeding rates for switchgrass were 7.5 kg ha^{-1}. Planting densities for the native grasses ranged from 1.6 to 2.4 kg ha^{-1} and restored prairie planting densities ranged from 0.4 to 1.2 kg ha^{-1}. Both the miscanthus and the poplar systems were planted by hand in May 2008 at densities of 17,200 rhizomes ha^{-1} and 2,778 cuttings ha^{-1}, respectively. Miscanthus failed at ARL due to winterkill in 2009 and was replanted in spring 2010 (Sanford et al. in press). The early successional system reflects natural succession post cessation of agriculture and its composition reflects the soil seed bank and natural colonization.

Each plot within the BCSE is 27 m x 43 m (0.12 ha) and plots are separated by a 15 m-wide mowed alley. Nitrogen fertilizer application varied by cropping system. All corn systems received on average 167 kg N ha^{-1} y^{-1} as urea-ammonium nitrate at both ARL and KBS. Canola systems received 176 kg N ha^{-1} y^{-1} as urea-ammonium nitrate. The switchgrass, miscanthus, native grasses, and early successional systems each received 56 kg N ha^{-1} y^{-1} of ammonium nitrate. The poplars received a single pulse of ammonium nitrate fertilizer in 2010 at a rate of 155 kg N ha^{-1} at KBS and 210 kg N ha^{-1} at Arlington. The restored prairie and soybean systems were unfertilized.

Soil sampling

Intact soil cores were collected in November 2013 at both sites from blocks 1-3 with hydraulic direct-push soil samplers (Geoprobe; Salina, KS at KBS and Giddings; Windsor, CO at ARL). Three 100-cm deep cores (7.6 cm diameter) were taken at three designated sampling stations within each plot and divided into four different depths: 0-10 cm, 10-25 cm, 25-50 cm, and 50-100 cm. Cores within each plot were composited by depth interval and sieved to 4 mm.

Long-term incubations

Long-term laboratory incubations were used to estimate the turnover rates of the different soil organic C pools. The laboratory experiment was a two-site by ten-cropping-system full factorial design. Surface soils (0-10 cm) were analyzed from all systems and subsurface soils (10-25 cm, 25-50 cm, and 50-100 cm) were analyzed in the corn, switchgrass, native grasses, and restored prairie systems. Two analytical replicates were treated as subsamples. Twenty-five grams of fresh soil were placed in 237 mL glass Mason jars. I adjusted soils to 55% water-filled pore-space utilizing the methods described in Franzluebbers et al. (2000). I carried out a pilot study wherein surface soils from the continuous corn plots were incubated to determine optimal C mineralization rate over a range of water contents.

Throughout the experiment soils were kept in the dark at 25^{o}C. Soil moisture was adjusted once per week to maintain moisture between 45-55% throughout the course of the incubations. CO_2 measurements were taken 11 times over the course of 322 days, with more intensive sampling at the beginning (once per week) and less towards the end (once every 6 weeks). CO_2 production of each sample was determined by injecting 1 mL of headspace into a N_2 carrier gas that streamed through a LI-COR LI-820 infrared gas absorption analyzer (LI-COR Biosciences, Lincoln, NE). An initial CO_2 reading was taken immediately after jars were capped, followed by three subsequent readings separated by 40 minutes. CO_2 fluxes were calculated by regressing CO_2 respiration versus time (Robertson et al., 1999).

Acid hydrolysis

To determine the resistant or non-hydrolyzable C pool, I performed acid hydrolysis on soils after the last incubation (Paul et al., 1999; Collins et al., 2000; Sanford and Kucharik,

2013). Prior to hydrolysis, I used a dissecting scope (20x) to identify plant material from previously sieved (4 mm) soil. Next, I removed any plant material by hand and by flotation using a 5% NaCl solution. Two grams of soils were refluxed in 6N HCl (20 mL) at 116^{o}C for 16 hours. This process causes available C to be released as CO_2 and amino compounds, pectins, and cellulose to solubilize (Sollins et al., 1999). The remaining material is closely related to the parent material and was washed by centrifugation, dried, and ground for total C and N analysis with a CHNS Elemental Analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia, CA).

The three pool model

In order to determine active, slow, and passive pools I combined results from non-linear regression and acid hydrolysis and used a three-pool model with first-order kinetics:

Eq: 1

$$C_{t(t)} = C_a e^{-ka(days)} + C_S e^{-ks(days)} + C_r e^{-kr(days)}$$

where, $C_{t(days)}$ = total soil organic C; C_a, C_s, and C_r represent the C mass in active, slow, and recalcitrant pools and where, k_a, k_s, and k_r are decomposition rates for each fraction (Paul et al., 2000). Next, I determined the first order derivative of equation 1 to estimate C_a, k_a, k_s, and k_r via non-linear regression using the NLIN procedure in SAS 9.4 where the rate change of CO_2 evolution versus time was determined:

Eq: 2

$$\text{Total C mineralization} = C_a * k_a e^{(-k * days)} + C_s * k_s e^{(-ks * days)} + C_r * k_r e^{(-kr * days)}$$

where C_a= active C pool, and k_a is the decay constant for the active C pool; C_s =slow C pool, and k_s = the decay constant for the slow C pool; and C_r =passive or resistant C pool and k_r= the decay constant for the resistant C pool. Acid hydrolysis was used to determine the resistant pool. The

slow pool was calculated by subtracting the passive and active pool from total soil C (C_s=C_t-C_a-C_r). Mean residence times (MRTs) were calculated by taking the inverse of the decay constants for the active and slow pools (1/k). Laboratory estimated MRTs were scaled up to the field level by using a Q_{10} correction ($2^{\wedge(\text{lab-field mean temp})/10)}$ that utilizes the difference of laboratory temperature (25ºC) and field temperature at KBS and Wisconsin, 9.9ºC and 6.8ºC, respectively (http://data.sustainability.glbrc.org/protocols/122). Automated weather stations at both KBS and ARL were used to measure field temperatures. The preferred method for determining MRT of the resistant pool is through ^{14}C dating; prior analysis at the nearby KBS LTER site revealed that MRTs of the resistant soil C pools are thousands of years old (Paul et al., 2001).

Particulate organic matter

Physical size fractionation was used to determine particulate organic matter (POM), which has been shown to reflect both labile and more processed C pools (Cambardella and Elliot, 1992). I used 4 mm sieved soils so as not to disturb most soil aggregates (Ontle, 2013) and combined 10 g of air-dried soil with 30 mL of 0.05 sodium hexametaphosphate in 50 mL centrifuge tubes. Tubes were then placed on a shaker for 8 hours at 120 oscillations min^{-1}. Next, I separated three POM fractions, large (>500 μm), medium (250-500 μm), and small (53-250 μm), to capture both labile and more processed carbon pools. I used deionized water to pass the mixture of soil and sodium hexametaphosphate through stacked 500 μm, 250 μm, and 53 μm sieves. The materials that were retained on each sieve included fine roots and large sand particles. POM fractions were oven dried at 55°C until constant weight. Dried samples were then ground using a mortar and pestle and analyzed for C and N with a CHNSO Analyzer (Costech ECS 4010, Costech Analytical Technologies, Valencia, CA).

Statistics

Cumulative C mineralization, MRT, slow and active C pools, and POM were analyzed using Proc Mixed of SAS (version 9.4; SAS Institute, Cary, NC, USA). Site, biofuel cropping system, and depth were treated as fixed effects and block as a random effect. Depth was treated as repeated measures. Significant differences were determined at $\alpha = 0.05$ and means were compared with an adjusted Tukey's pairwise means comparison. The non-linear regression function in SAS (Proc NLIN, version 9.4; SAS Institute, Cary, NC, USA) was used to estimate the active pool (Ca) and the decay rates for the active, slow, and passive pools.

RESULTS

Surface cumulative fluxes

Cumulative CO_2 fluxes for surface soils (0-10 cm) differed by cropping system (Figures 4.1 and 4.2, $F=6.14$, $p=0.02$), but not by site ($F=2.93$, $p=0.2$). At ARL, fluxes ranged from 46.2 (±2.9, standard error of the mean) to 75.0 ±13.8 μg C g^{-1} soil day^{-1} (Figure 4.1). The restored prairie system had significantly greater fluxes compared to all the other systems. Switchgrass, early successional, and native grasses all had significantly greater C fluxes compared to the annual systems. Systems with the lowest fluxes consisted of the poplars, miscanthus and all four of the annual cropping systems. At KBS, cumulative fluxes ranged from 40 ±3.3 to 65 ±7.2 μg C g^{-1} soil day^{-1} (Figure 4.2). There were only two main divisions amongst the ten systems: the native grasses and early successional systems had significantly greater cumulative fluxes compared to others.

Subsurface cumulative fluxes

Subsurface cumulative CO_2 fluxes at 10-25 cm, 25-50 cm, and 50-100 cm depths amongst the corn, switchgrass, native grasses, and restored prairie systems yielded different trends at the two sites (Figure 4.4). At ARL, in the 10-25 cm depth all three perennial systems had significantly greater cumulative fluxes compared to the corn system ($p<0.05$), while there were no significant differences at 10-25 cm amongst the cropping systems at KBS. At the 25-50 cm and 50-100 cm depths, all four systems were statistically similar to one another at both sites. The interaction of site by cropping system by depth was significant ($F=3.0$, $p=0.003$), and was likely due to significantly different fluxes under switchgrass and corn between the two sites at 10-25 cm ($p<0.05$).

When averaging by site and cropping system, flux variability was lowest in the top surface depths, where mean coefficients of variation (CVs) were 0.13 ±0.2 and 0.11 ±0.2 for 0-10 cm and 10-25 cm, respectively. CV's increased at the 25-50 cm depth where the mean CV was 0.24 ±0.03. CVs were greatest at the deepest depth, where the mean CV was twice as high as at the surface (0.33 ±0.05).

Cumulative flux per soil C

Cumulative fluxes on a gravimetric basis did not differ between the two sites. Thus, I calculated cumulative fluxes expressed per total soil C and found significantly greater values at KBS (Figure 4.3, $F=154.7$, $p=0.006$). Fluxes ranged from 3.5 ±0.7 to 4.9 ± 0.3 mg C g^{-1} soil C day^{-1} at KBS compared to 1.9 ±0.2 to 3.3 ±0.5 mg C g^{-1} soil C day^{-1} at ARL. There was also an overall significant cropping system effect ($F=4.27$, $p=0.002$), with noteworthy trends among cropping systems at both sites. At KBS, the diverse perennial, corn, and miscanthus systems had significantly higher fluxes compared to the poplar, switchgrass, and the rotated corn, soybean,

and canola systems. Trends were very similar at ARL, where the restored prairie, switchgrass, native grasses, and miscanthus systems had significantly greater fluxes per total C compared to the annual and poplar systems (Figure 4.4).

Trends of surface fluxes over time

Most systems seemed to stabilize at a low CO_2 flux by incubation day 322 but a few systems had fluxes that were still decreasing (Figures 4.8-4.27). Furthermore, flux variability was greatest towards the end of the incubation for most systems. In general, the decline in CO_2 fluxes near day 100 differentiates the active and slow C pool. The stabilization of CO_2 flux, which for most crops is represented by an asymptotic line close to but not equal to zero, indicates the presence of the slow C pool.

The active C pool

The active C pool significantly differed by system (Figure 4.5, $F=6.8$, $p<0.0001$), but there was no site effect ($F=2.7$, $p=0.3$). At ARL, there were no distinct trends between the annual and perennial cropping systems, except for the restored prairie system, which had the largest active C pool (631 ± 134 μg C g^{-1} soil) compared to all other cropping systems except the soybean system. In addition, the poplar and native grasses had a larger active C pool compared to corn (Figure 4.5). At KBS, there was a clear difference between the diverse perennials plus the poplar system compared to the monoculture perennials and the annual systems (Figure 4.5): the diverse perennials had over twice the amount of active C compared to the other systems.

At ARL, the active C pool comprised between 1.9 and 2.7% of the total C pool (Table 4.1). The continuous corn system contained the greatest percentage of C in the active pool.

Proportionally, KBS stored more C in the active pool compared to ARL, where percentages of total C ranged from 1.7 % in the continuous corn to 5.7 % in the restored prairie of total C.

The slow C pool

Although the sizes of the slow C pool at KBS and ARL were statistically indistinguishable (Figure 4.6, $p=0.2$, $F= 2.7$), the proportion of C in the slow pool substantially contrasted for the two sites. The slow C pool at ARL accumulated between 27 and 43% of total C compared to KBS, which accumulated between 39 and 55 % (Tables 4.1 and 4.2). C accumulation rates significantly differed by cropping system ($F=6.9$, $p<0.0001$), however distinct trends were not as visible in the slow pool compared to the active pool. At ARL, the poplar and early successional systems had significantly greater accumulation in the slow C pool compared to the other systems (Figure 4.6). The next group consisted of the restored prairie, switchgrass and corn, which had significantly greater C compared to the native grasses, miscanthus and the other annual systems (Figure 4.6).

At KBS, the poplars had significantly greater C in the slow pool compared to all the other systems except the native grasses and early successional systems. Although not significant, the diverse perennials, with the exception of the restored prairie, tended to have greater accumulation in the slow C pool compared to the annuals and monoculture perennials.

The marginally significant site by cropping system interaction ($F=2.1$, $p=0.06$) was likely because the poplar and early successional systems at ARL had significantly greater C accumulation than the Poplar and early successional systems at KBS ($p=0.03$ and $p=0.005$).

Non-hydrolyzable (resistant) C pool

Only site and depth are presented in Figure 4.7 because I did not detect any differences amongst the systems for the resistant pool. The resistant C pool was significantly greater at ARL compared to KBS, accumulating 2.2 times more C in the 0-10 cm depth. On average, the resistant pool at ARL consists of 69% of the total C compared to 52% at KBS. Significant differences between the two sites were evident at every depth except 50-100 cm (Figure 4.7).

Mean residence time

The persistence or mean residence time (MRT) of the active C pool differed by cropping system (Tables 4.1 and 4.2, F=4, p=0.001) but not by site (F=2.7, P=0.2). At ARL, the soybean and poplar systems had the longest active C MRT at 63 ±10.8 and 58 ±6.0 days, respectively. The other systems had MRTs that ranged from 27-46 days, whereas Miscanthus had the shortest MRT. At KBS, the poplar system had an MRT of 78 ±18.8 days and was significantly greater than all of the other cropping systems except for the native grasses, which had an MRT of 69 ±13.4 days. Continuous corn had the shortest MRT of 33.4 ±6.7 days.

MRTs of the slow C pool did not differ by site (F= 1.3, p=0.4) or cropping system (F=1.06, p=0.4), although there were some noteworthy trends. For example, at ARL the longest MRTs for the slow C pool were 4.5 and 4.2 years for the poplar and early successional systems. At KBS, pairwise comparisons revealed that despite no overall cropping system effect, the native grasses had a significantly longer MRT of 7.9 ±4.5 years compared to all other systems (Table 4.2.)

Particulate organic matter fractions

Across the three POM size fractions, significant differences were more apparent by site than cropping system (Table 4.3-4.6). For example, at the 0-10 cm depth ARL had significantly greater POM-C concentrations (g C kg^{-1}) in the large and medium sized fractions (>500 μm and 125-500 μm) compared to KBS (Table 4.3, F=92.1, p<0.0001 and F=11.4, p=0.005). The opposite occurred in the small fraction (53-125 μm), where KBS had significantly greater POM-C concentrations (g C kg^{-1}) compared to ARL (F=564.9, p=0.002). Overall systems differences were only evident in the small fraction (F=17, p <0.0001) whereas the poplar system had significantly greater POM-C than continuous corn at surface depths. Differences were also visible at 25-50 cm depth at KBS, where continuous corn had POM-C concentrations of 14.9 g C kg^{-1}, compared to 9.4 g C kg^{-1} of the restored prairie systems and 8.1 of both the switchgrass and native grasses systems (Table 4.5). Overall, at the surface, ARL accumulated more C in the large and medium fractions where concentrations were up to 19 and 8 g C kg^{-1} in the large and medium size fractions, with lowest accumulation occurring in the 53-125 μm size class (2.6 g C kg^{-1}). At KBS, the opposite occurred, in that the large and medium fractions had POM-C concentrations that were substantially lower than the C in the smaller fraction. For example, the small fraction had concentrations of up to 3.9 g C kg^{-1} compared to 2.4 g C kg^{-1} and 1.4 g C kg^{-1} in the large and medium fractions, respectively.

DISCUSSION

Overall, diverse perennial systems had substantially greater C accumulation in the active pool compared to both annual systems and monoculture perennial systems, especially at KBS with its less fertile soils. Within monoculture systems, there were no active C pool differences

between annual and perennial crops. Differences in C accumulation between annual and perennial systems were less apparent in the slow pool. However, the poplar system had significantly greater slow C compared to all annual row crop systems at both ARL and KBS.

My first hypothesis that perennial systems have greater active and slow C pools was not supported because there were several perennial systems that had statistically similar C pools to the annual systems at both sites. In addition, I did not find any evidence for faster C accumulation at ARL compared to KBS; instead, I found that C accumulation under perennials was faster relative to annuals at KBS. However, the expectation that C accumulation would be greater in systems with more diversity was supported for the active C pool at both sites, though the trend did not hold for the slow C pool.

Active C pool

Five years after establishment at KBS, the lower fertility site, the diverse perennial and poplar systems had 2.5 times more accumulation in the active C pool at the 0-10 cm depth relative to the annual systems and monoculture perennials, which were statistically similar to one another. The similarity in active C accrual among annual systems and monoculture perennials is surprising because several studies have demonstrated greater C accumulation under switchgrass and miscanthus compared to corn (Liebig et al., 2004; Follett et al., 2012). One explanation for the lack of difference here could stem from the no-till management in the annual systems, which reduces soil C losses (Follett et al., 2012). Bonin and Lal (2012) also found similarities in C accumulation between corn and switchgrass, suggesting that no-till corn systems have the ability to accumulate similar amounts of C compared to monoculture perennials in surface soils. That the poplars at KBS behaved more like the diverse perennial systems than the other monoculture perennials is curious, but is probably because of greater diversity than the other monoculture

perennials. Although the poplar system was planted as a monoculture and its overall biomass is dominated by *Populus* sp., the understory nevertheless contains six different herbaceous species that provide 24% ground cover. Thus, while poplars are the dominant species, the system resembles a polyculture more than a true monoculture.

Greater C accumulation under perennials compared to annuals has been shown in several studies (Grandy and Robertson, 2007; Anderson-Teixeira et al., 2009; Collins et al., 2010). However, the fact that diversity plays a crucial part in these differences was unexpected and to my knowledge has only been shown in grassland and forest systems (Fornara and Tilman, 2008; Steinbeiss et al., 2008, He et al., 2013), and not before in intensive cropping systems. One factor that contributes to greater C sequestration under perennials relative to annuals are extensive root systems that have 3 to 8 times greater biomass (Dupont et al., 2014; Culman et al., 2010; Anderson-Teixeira et al., 2013) in addition to year-round ground cover. Root biomass differences among perennial crops have not been intensively studied and thus are less clear.

Why might diverse perennial systems accumulate more active C than monoculture perennial systems? One explanation is root productivity. Fine root production results from this site reveal that the diverse crops allocate more biomass to roots compared to monoculture perennials (Sprunger, Chapter 5). Since aboveground net primary productivity (ANPP) is equal among cropping systems, except for the miscanthus system, which has larger ANPP (Sanford et al., in press), I can conclude that greater belowground C inputs are the primary driver for enhanced C accumulation under the diverse perennial systems.

Reasons for greater root production under diverse cropping systems are poorly understood. However, a possible explanation for greater C accumulation in the restored prairie system could be a result of greater fine root production due to plant competition for nutrients or the 'functional

composition effect', where N fixation by legumes facilitates growth of C_4 grasses (Steinbeiss et al. 2008 and Fornara and Tilman, 2008). Given that the restored prairie has C3, C4, and legume species, the legumes could be facilitating increased nitrogen and stimulating more fine root production leading to greater belowground C. Greater C accumulation in the other diverse species systems where legumes are absent (poplar, native grass, and early successional systems) could be consistent with principles put forth by de Kroon et al. (2012), who argue that root foraging activity will be intensified in mixed species systems, where competitive root networks are established due to greater nutrient demand.

At ARL, differences were much less visible between the annual and perennial systems, with only the restored prairie accumulating more active C than the majority of the other systems. One reason for less differentiation at ARL compared to KBS could be that the mollisols found at ARL are extremely high in soil organic matter. For example, 0-10 cm depth baseline soil C at ARL was 22.4 g C kg^{-1} compared to 14.3 g C kg^{-1} at KBS. Thus, Arlington soils could be approaching C saturation, which implies that the system does not have the capacity to stabilize additional C inputs as soil C (Stewart et al., 2007). Sandier soils such as those at KBS may be able to build C at a quicker rate after disturbance or changes in management because they are less likely to be close to their maximum C storage capacity (Anderson-Teixeira et al., 2009; Johnston, 2011). Clay soils, on the other hand, will build C at a much slower rate as C approaches equilibrium (West and Six, 2007). Surface soils at KBS are 63% sand compared to 25% sand at Arlington.

Although the active C pool turns over rapidly, increases in the active C pool will eventually result in greater accumulation of C in more recalcitrant pools if management remains the same. For example, as the active C pool increases, a greater proportion of C will transfer into the more

recalcitrant pools of C through physical breakdown of organic material and microbially mediated processes (Grandy and Neff, 2008). This filtering effect of molecular C compounds is driven by selective microbial degradation, whereby more recalcitrant pools accumulate in the slow and passive pools of C.

Slow C pool

Differences between the annual and perennial systems in the slow C pool were much less pronounced at KBS; only the poplars had significantly greater slow C pool accumulation compared to other systems. Although not significant, the native grasses and early successional systems had slightly greater slow C pool accumulation compared to the annuals and monoculture perennials, following trends visible in the active C pool. There were no clear trends between annuals and perennials at ARL, but the poplar and early successional systems had substantially greater C compared to all other systems. The restored prairie system had a lower asymptote compared to the other systems at both ARL and KBS (see appendix), which could reflect more C accumulation in the active and slow C pools and less in the passive pool.

At both sites, the poplars had twice as much slow C as the other systems. Poplars are the only woody species and previous experiments at the nearby KBS LTER site have also shown that poplars are effective at sequestering C. In the first ten years of establishment, the KBS LTER poplar system added between 32 to 44 g C m^{-2} y^{-1} to the total surface soil C pool (Robertson et al., 2000). Twelve years post establishment, Grandy and Robertson (2007) found that poplars accumulated 37% more total C relative to conventional row crops in the top 5 cm. My findings demonstrate that in the first five years of establishment poplars are accumulating twice as much C in both the active and slow pool in the top 10 cm of soil relative to no-till corn.

Given that the aboveground biomass is removed post harvest in the poplar systems, this accumulation of C in the slow pool is likely due to coarse and fine root production and turnover. However, fine root production results from this site (Sprunger, Chapter 5) show that poplars produced fewer fine roots compared to the other polyculture systems. Thus, this belowground C accumulation could be a function of quality rather than quantity. Results from a decomposition experiment in Quebec showed that hybrid poplar roots have a high lignin to N ratio, which could lead to reduced microbial activity and slow the overall rate of decomposition (Camire et al., 1991). Although not always significant, poplars tended to have longer mean residence times in both the active and slow C pools compared to other systems, which corroborates findings of Paul et al. (1999).

The slow C pool can be altered by management but is generally associated with more stabilized pools of C, which greatly influences long-term C sequestration (Wander et al., 2004). The slow C pool is also largely influenced by physical protection (Grandy and Robertson, 2007), which will give systems with more extensive roots an advantage for building C over time, since roots play an important role in regulating aggregate formation and physico-chemical protection of soil organic matter (Rasse et al., 2004). Because of this association with more recalcitrant forms of C, it can take several years for gains in the slow C pool to be detectable. Thus, it is reasonable to expect detectable increases in the slow C pool over longer periods of time in these perennial cropping systems.

Passive C pool

I determined the resistant pool by conducting acid hydrolysis analysis on post-incubation soils. Although I did not detect any differences between the two sites for active and slow C, I found substantial differences between ARL and KBS within the resistant C pool. The fact that I

did not detect any differences between systems is not surprising, given that C in the resistant pool is typically associated with inorganic materials in soil and is generally not influenced by short-term management or biological activity (Wander, 2004). I did not calculate MRT for the resistant C pool because ^{14}C dating can provide a more accurate MRT for this C pool than those determined from the decay rate constants. Paul et al. (1999) found that MRTs for soil C in soils high in clay like those found in ARL were about 2840 years and prior ^{14}C dating at the KBS LTER site showed an MRT of 1435 years. The high clay content at ARL is likely the reason for high C stabilization in the resistant pool (Collins et al., 1999).

The amount of total C found in the resistant pool also differed between the two sites. At KBS, the resistant pool accounts for 52% of total C. Nearly identical percentages have been reported from work at the KBS LTER. For example, Paul et al. (1999) found that the resistant pool was 56% and 53% of the total C pool for corn and never-tilled systems. In contrast, 69% of C at ARL is stored in the resistant pool. Thus, while C accumulation in the active C pool is occurring quicker under diverse perennials at KBS, ARL is more effective at stabilizing C overall, which is also supported by the amount of C that is respired per gram of total C.

Little evidence for active C at lower depths

Patterns amongst corn, switchgrass, miscanthus, and restored prairie in the top two depth strata (0-10 cm and 10-25 cm) differed between the two sites. In the 0-10 cm depth at Arlington, cumulative fluxes increased with diversity, whereby corn had the lowest fluxes and restored prairie had the greatest fluxes. At ARL, the significantly greater C fluxes in the 10-25 cm stratum for all three perennial systems compared to corn supports the hypothesis that perennial systems have greater C accumulation compared to annual systems. However, I found no evidence for increasing C fluxes with greater diversity at 10-25 cm, which refutes my diversity hypothesis. At

KBS in the 0-10 cm depth, cumulative flux patterns did not increase with diversity, with the greatest cumulative flux found in the native grass system, followed by the restored prairie. The corn and switchgrass had the lowest fluxes and were statistically similar to one another. At the 10-25 cm layer I found no differences between the four systems. The fact that I detected differences below the surface layer at ARL suggests that soil C is more evenly distributed between 0-10 cm and 10-25 cm compared to KBS and is a reflection of the deep A horizon often found in mollisols, which extends to 36 cm at ARL. Below the 10- 25 cm stratum, I did not detect any differences at either site.

There are two plausible explanations for this lack of difference in cumulative C at depth. First, C fluxes were substantially smaller at subsurface depths due to inherently lower C concentrations, and second, fluxes were more variable at depth, suggesting that larger sample sizes are needed in order to detect differences at subsurface horizons (Kravchenko and Robertson, 2010; Syswerda et al., 2011). Non-linear regression was only reported for surface depths because active C pool (C_a) estimates were highly variable at 10-25 cm and I did not detect an active C pool in the 25-50 cm and 50-100 cm depths. In fact at KBS, the acid hydrolysis and total C concentrations were not significantly different from one another, indicating that C at lower depths is largely comprised of the resistant C pool.

Particulate organic matter patterns

In general, POM results did not correspond to patterns of C mineralization from the long-term incubation. On the other hand, POM results were more similar to acid hydrolysis findings, with large site differences but no noteworthy differences amongst the systems. One reason for this difference is methodology, because flux data are very sensitive to changes in management (Culman et al., 2013), while physical size fractionation techniques and acid hydrolysis are

associated with organic and inorganic material and as a result reflect more recalcitrant C. Thus, POM appears not to reflect short-term changes in soil C cycling in these systems.

Management implications

My results demonstrate that diverse perennial cropping systems could be used to increase soil C in low fertility soils and marginal landscapes in particular, which has important implications at multiple scales. In terms of energy policy, cellulosic biofuels grown on marginal lands do not compete with food production (Robertson et al., 2008), have a large climate benefit (Gelfand et al., 2013), can produce biomass yields comparable to corn (Bonin and Lal, 2012; Sanford, in press), and also provide additional ecosystem services such as reduced nitrate leaching (Smith et al., 2013) and biodiversity benefits such as pollination and biocontrol (Werling et al., 2014). To my knowledge, these findings are the first to report that polyculture second-generation biofuels are more effective at accumulating C than monoculture perennials in moderate fertility environments. Relative to corn, polycultures accumulated over twice as much C in the active pool. Furthermore, these findings further suggest that restoring prairies in both high and low fertility soils leads to substantial short-term C sequestration.

Finally, my results support the notion that C can be accumulated more rapidly in soils lower in fertility. Soil C stocks continue to decline globally and strategies are needed in order to replenish the total C pool. This work demonstrates that diverse systems could be used as a means to sequester C over short and long-term time frames.

CONCLUSIONS

1. Soil C gains in the active pool occurred more quickly at the low fertility site (KBS) compared to the high fertility site (ARL).
2. Five years post-establishment, the perennial polyculture systems at KBS, the lower fertility site, had 2.5 times more C accumulation in the active pool compared to no till annual row crops and monoculture perennial systems.
3. Annual row crops and monoculture perennial systems had similar rates of active C accumulation, demonstrating a no-till advantage rather than perenniality per se.
4. At ARL, the site higher in fertility, differences in the active C pool between annual and perennial systems were only evident in the restored prairie system, possibly because of C saturation due to soils high C soils.
5. Differences between annual and perennial cropping systems were much less pronounced in the slow C pool. However, at both sites, the poplar system had the highest slow C pool accumulation.
6. ARL, the site higher in fertility, had significantly greater C accumulation in the resistant pool compared to KBS at every depth interval except 50-100 cm.
7. Patterns of particulate organic matter concentrations did not correlate with long-term incubation results, where large site differences were visible in each fraction. This indicates that biological fractionation is more sensitive to management and crop effects than are physical and chemical fractionations.

APPENDIX

Table 4.1 Mean Residence Times for surface soils (0-10 cm) of ten biofuel cropping systems at ARL for the active and slow C pool.

	Active C			Slow C		
System	% of Total	Lab MRT	Field MRT	% of Total	Lab MRT	Field MRT
	%C	days	days	C	years	years
Corn	2.7	41.7 (3.8)[b]	147.4 (13.4)	27.1	2.1 (0.5)[a]	7.4 (1.6)
Corn-Soybean-Canola	2.1	38.7 (8.3)[b]	136.6 (29.3)	19.6	2.1 (0.3)[a]	7.4 (0.9)
Soybean-Corn-Canola	2.2	62.7 (10.8)[a]	221.2 (38.0)	27	2.6 (0.7)[a]	9.1 (2.5)
Canola-Corn-Soybean	2.6	46.3 (7.9)[ab]	163.6 (28)	24	3.2 (0.3)[a]	11.3 (1.2)
Switchgrass	2.6	31.4 (1.4)[b]	111.0 (5.0)	30.2	2.3 (0.5)[a]	7.88 (1.8)
Miscanthus	2.5	27.0 (4.0)[b]	95.5 (14.2)	16.8	1.4 (0.3)[a]	4.9 (0.9)
Poplar	2.1	57.5 (6.0)[a]	202.9 (21.1)	43.9	4.5 (0.3)[a]	15.7 (0.9)
Native Grasses	2.8	39.3 (7.6)[b]	138.6 (26.9)	25	2.1 (0.13)[a]	7.4 (0.5)
Early Successional	1.9	35.0 (5.1)[b]	123.5 (17.9)	41.9	4.2 (1.2)[a]	14.6 (4.4)
Restored Prairie	2.0	39.3 (4.6)[b]	138.6 (16.2)	32.4	2.8 (0.3)[a]	9.9 (0.9)

Table 4.2 Mean Residence Times for surface soils (0-10 cm) of ten biofuel cropping systems at KBS for the active and slow C pool. Asterisks represents (n=1).

System	Active C			Slow C		
	% of Total	Lab MRT	Field MRT	% of Total	Lab MRT	Field MRT
	C	days	days	C	years	years
Corn	1.7	33.4 (6.7)[c]	94.9 (19.1)	41	3.1 (0.5)[b]	8.8 (1.5)
Corn-Soybean-Canola	4.4	27.3*	77.7*	47	3.1*	9.0*
Soybean-Corn-Canola	3.3	51.5 (9.4)[bc]	146.2 (26.6)	44	2.5 (0.1)[b]	7.2 (0.4)
Canola-Corn-Soybean	3.3	33.9 (1.7)[bc]	96.5 (4.7)	40	2.7 (0.2)[b]	7.7 (0.6)
Switchgrass	3.4	54 (12.8)[bc]	153.0 (36.2)	41	3.1 (0.3)[b]	8.9 (0.9)
Miscanthus	4.0	36.8 (4.1)[bc]	104.4 (11.9)	41	2.2 (0.8)[b]	6.4 (0.8)
Poplar	4.9	78.2 (18.8)[a]	222.2 (53.3)	54.5	3.3*	9.4
Native Grasses	5.4	69.2(13.4)[ab]	196.4 (38.1)	45.5	7.9 (4.5)[a]	22.6 (12.8)
Early Successional	4.0	40.1 (7.9)[bc]	113.7 (22.6)	48	2.9 (0.8)[b]	8.1 (2.4)
Restored Prairie	5.7	55.8 (1.9)[b]	158.4 (5.3)	38.7	3.6 (0.5)[b]	10.1 (1.4)

Table 4.3 Surface soil (0-10 cm) particulate organic matter C concentrations (means and standard errors) for ten biofuel cropping systems at ARL and KBS.

	ARL			KBS		
System	Large (>500 μm)	Medium (125-500 μm)	Small (53-125 μm)	Large (>500 μm)	Medium (125-500 μm)	Small (53-125 μm)
			g C kg^{-1}			
Corn	11.6 (0.5)[b]	6.6 (1.7)[a]	1.5 (0.2)[a]	1.5 (0.2)[a]	0.6 (0.08)[a]	2.4 (0.08)[b]
Corn-Soybean-Canola	11.4 (2.6)[b]	6.5 (2.0)[a]	2.6 (1.4)[a]	2.1(0.2)[a]	0.9 (0.05)[a]	2.8 (0.1)[ab]
Soybean-Corn-Canola	15.3 (3.9)[ab]	5.9 (2.5)[a]	1.7 (0.6)[a]	1.3 (0.12)[a]	0.8 (0.08)[a]	2.9 (0.2)[ab]
Canola-Corn-Soybean	8.9 (3.8)[b]	3.9 (0.2)[a]	1.6 (0.2)[a]	2.3 (0.2)[a]	0.9 (0.1)[a]	3.4 (0.5)[ab]
Switchgrass	11.6 (0.9)[b]	6.4 (0.4)[a]	1.4 (0.3)[a]	2.4 (0.2)[a]	0.8 (0.1)[a]	2.9 (0.3)[ab]
Miscanthus	15.7 (0.9)[b]	8.4 (1.2)[a]	1.3 (0.3)[a]	1.4 (0.2)[a]	0.8 (0.04)[a]	2.6 (0.2)[ab]
Poplar	13.5 (1.6)[a]	7.6(1.1)[a]	1.4 (0.1)[a]	1.4 (0.2)[a]	1.4 (0.3)[a]	3.9 (0.7)[a]
Native Grasses	13.8 (2.3)[ab]	7.7 (1.9)[a]	1.2 (0.2)[a]	1.3 (0.4)[a]	1.2 (0.3)[a]	2.7 (0.4)[ab]
Early Successional	15.1 (1.1)[ab]	7.5 (0.6)[a]	1.7 (0.2)[a]	1.9 (0.2)[a]	1.1 (0.08)[a]	3.2 (0.3)[ab]
Restored Prairie	19.1 (3.8)[a]	7.3 (2.0)[a]	1.5 (0.3)[a]	0.51(0.1)[a]	0.8 (0.08)[a]	2.8 (0.05)[ab]

Table 4.4 Particulate organic matter C concentrations from 10-25 cm, 25-50 cm, 50-100 cm depths (means and standard errors) for four biofuel cropping systems at ARL and KBS.

	ARL			KBS		
System	Large (>500 μm)	Medium (125-500 μm)	Small (53-125 μm)	Large (>500 μm)	Medium (125-500 μm)	Small (53-125 μm)
			g C kg^{-1}			
10-25 cm						
Corn	8.3 (1.2)[a]	2.2 (0.5)[a]	1.3 (0.4)[a]	0.4 (0.1)[a]	0.2 (0.02)[a]	1.2 (0.06)[a]
Switchgrass	9.5 (0.56)[a]	2.5 (0.6)[a]	0.9 (0.3)[a]	0.6 (0.1)[a]	0.3 (0.05)[a]	1.05 (0.2)[a]
Native Grasses	4.4 (0.06)[b]	1.6 (0.08)[a]	0.9 (0.3)[a]	0.5 (0.1)[a]	0.4 (0.04)[a]	1.4 (0.1)[a]
Restored Prairie	6.7 (0.84)[b]	2.5 (0.56)[a]	0.8 (0.1)[a]	1.0 (0.6)[a]	0.2 (0.06)[a]	0.9 (0.1)[a]
25-50 cm						
Corn	2.7 (0.7)[ab]	1.3 (0.2)[ab]	0.3 (0.05)[a]	6.8 (0.3) [a]	5.3 (0.6)[ab]	14.9 (0.2)[a]
Switchgrass	4.6 (0.7)[a]	2.7 (0.7)[a]	0.2 (0.02)[a]	6.3 (0.2)[a]	5.4 (0.52)[ab]	8.1 (0.1)[c]
Native Grasses	2.4 (1.2)[ab]	2.0 (0.9)[ab]	0.2 (0.05)[a]	8.7 (2.9)[a]	7.2 (2.9)[a]	8.1 (1.5)[c]
Restored Prairie	0.5 (0.4)[b]	0.3 (0.1)[b]	0.1 (0.008)[a]	7.0 (3.2)[a]	3.9 (1.3)[b]	9.4 (0.9)[b]
50-100 cm						
Corn	1.3 (0.2)[a]	0.6 (0.2)[a]	0.4 (0.2)[a]	0.3 (0.1)[a]	0.2 (0.09)[a]	0.4 (0.08)[a]
Switchgrass	0.9 (0.6)[a]	0.3 (0.2)[a]	0.1 (0.04)[a]	0.4 (0.06)[a]	0.6 (0.3)[a]	0.5 (0.1)[a]
Native Grasses	0.2[*]	0.6 (0.3)[a]	0.1 (0.008)[a]	0.4 (0.1)[a]	0.3 (0.08)[a]	0.6 (0.3)[a]
Restored Prairie	0.3 (0.2)[a]	0.2 (0.04)[a]	0.1 (0.009)[a]	0.3 (0.06)[a]	0.2 (0.03)[a]	0.3 (0.1)[a]

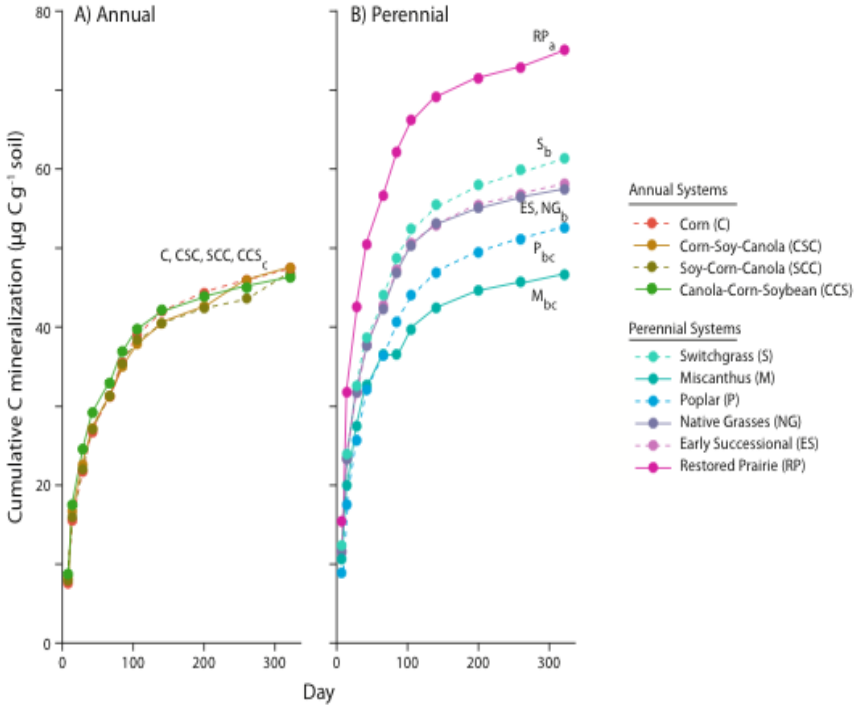

Figure 4.1 Cumulative C mineralization from surface soils (0-10 cm depths) over the course of 322 day incubations for ARL (n=3). Systems with different lowercase letters are statistically different from one another ($p < 0.05$).

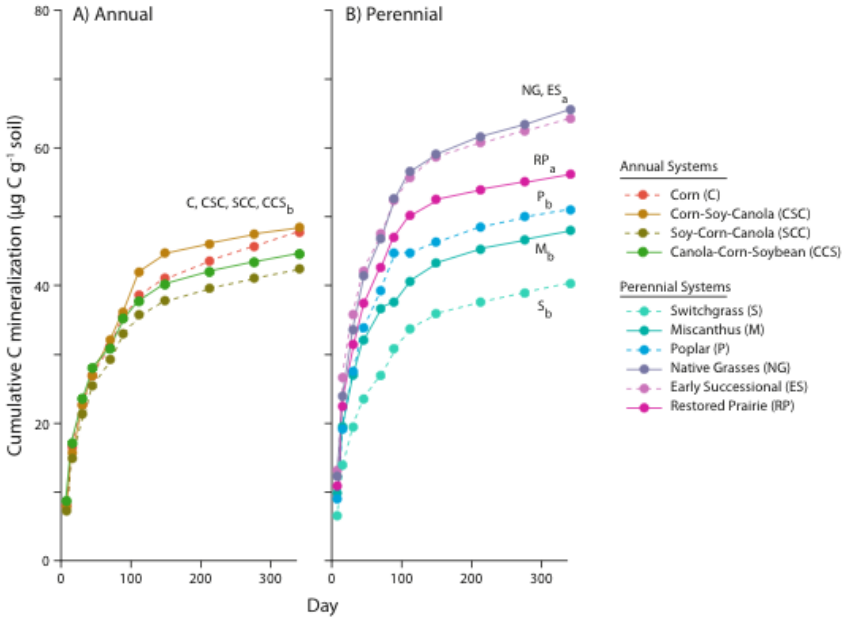

Figure 4.2 Cumulative C mineralization from surface soils (0-10 cm depths) over the course of 322 day incubations for KBS (n=3). Systems with different lowercase letters are statistically different from one another ($p < 0.05$).

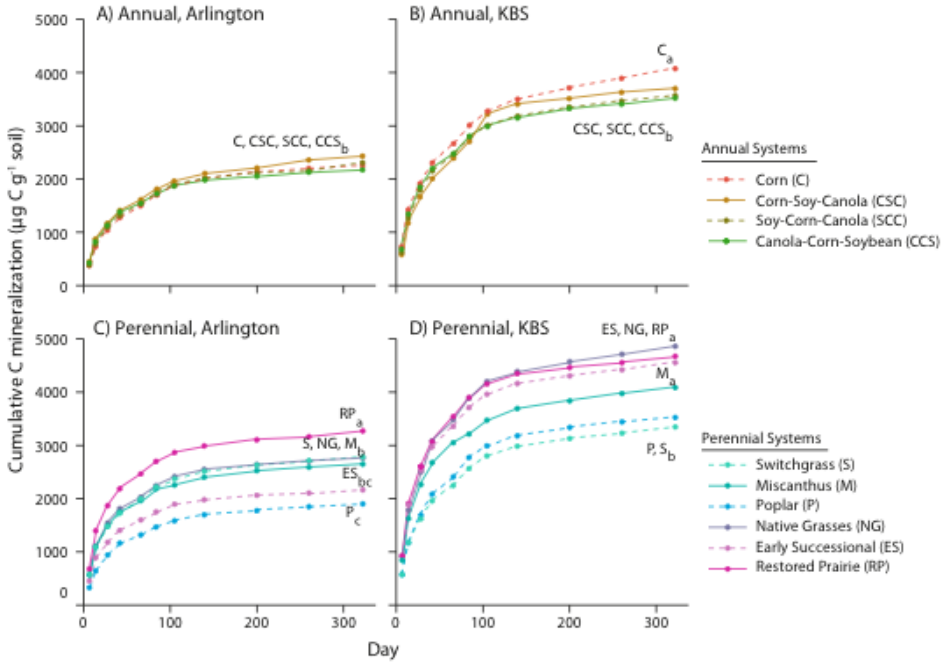

Figure 4.3 Cumulative C mineralization for corn, switchgrass, native grasses, and restored prairie gradient at 0-10 cm, 10-25 cm, 25-50 cm, and 50-100 cm depths. Within each site and depth interval, systems with different lowercase letters are statistically different from one another ($p < 0.05$).

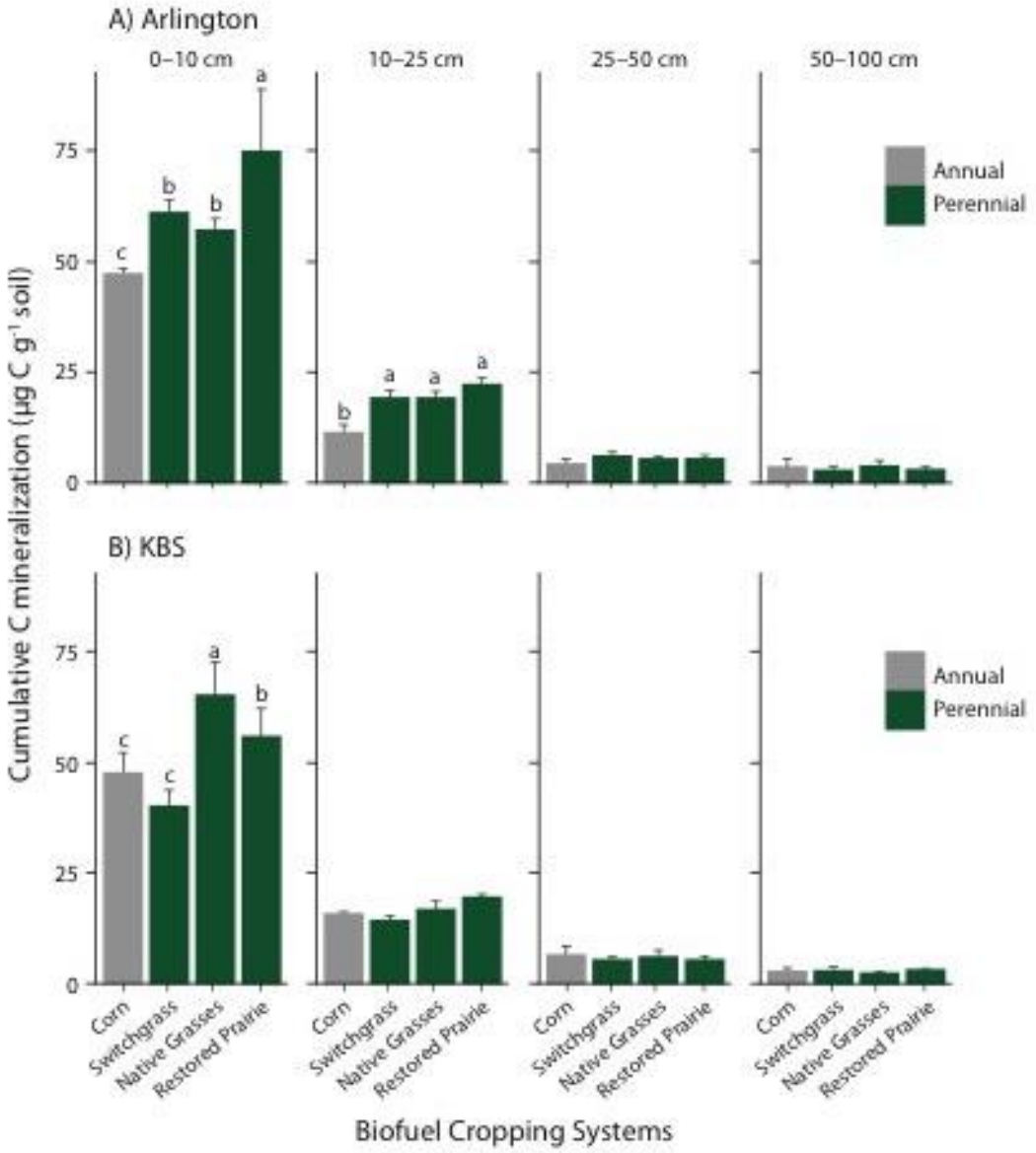

Figure 4. 4 Cumulative C mineralization per gram of soil C from surface soils (0-10 cm depths) over the course of 322 day incubations for ARL and KBS (n=3). For each site, systems with different lowercase letters are statistically different from one another ($p < 0.05$).

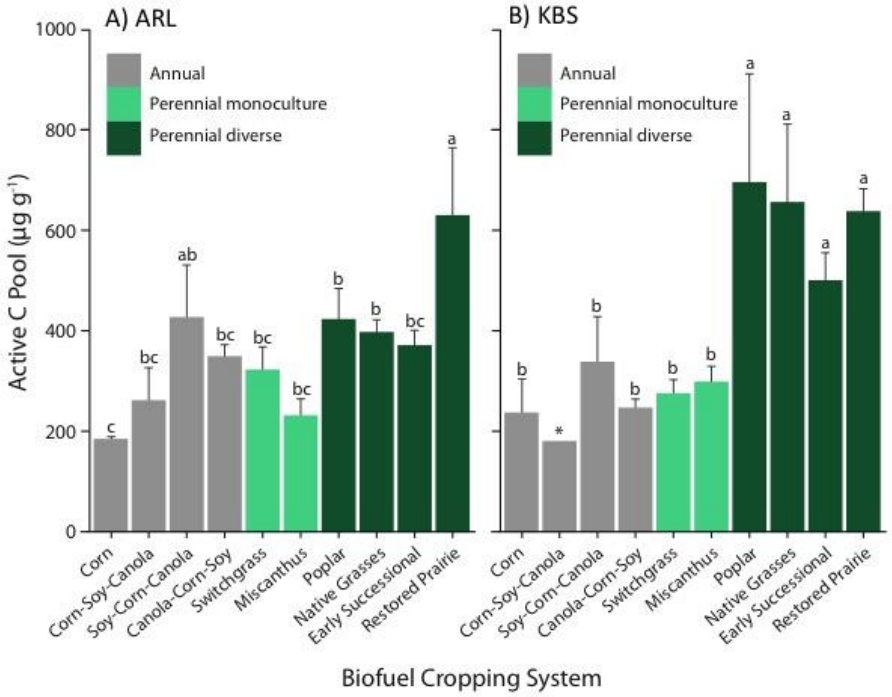

Figure 4.5 The active C pool for surface soils (0-10cm). Within each site, systems with different lowercase letters are statistically different from one another ($p < 0.05$). Bars with no letters were not significantly different from one another. Asterisk represents (n=1). Bars are means ± SE.

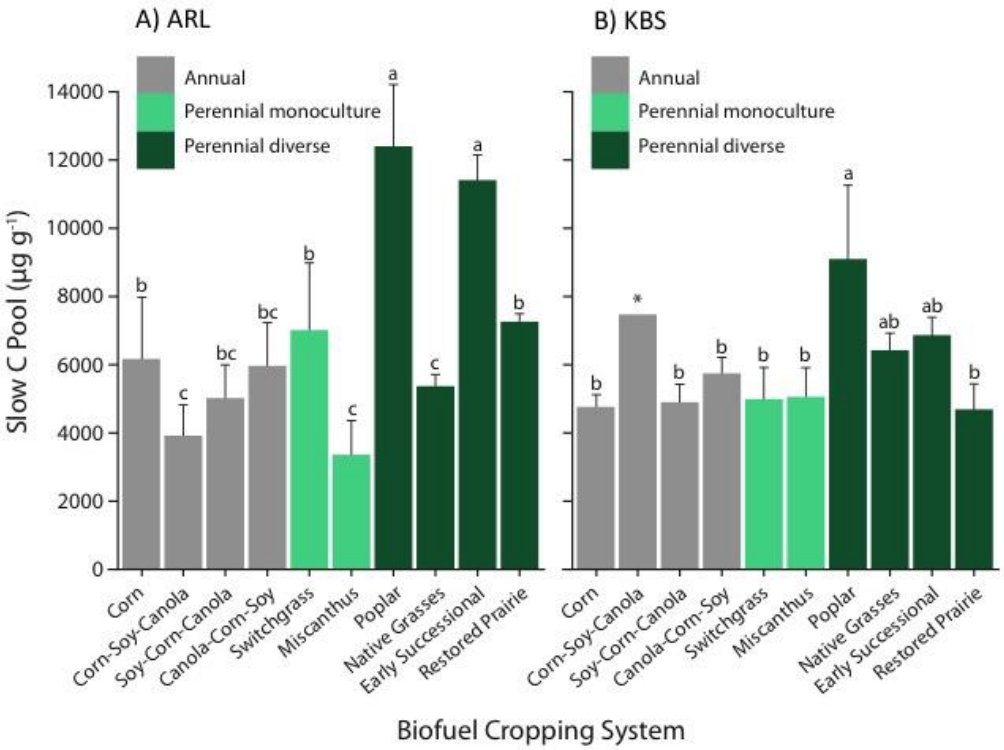

Figure 4.6 The slow C pool for surface soils (0-10cm). Within each site, systems with different lowercase letters are statistically different from one another ($p < 0.05$). Bars with no letters were not significantly different from one another. Asterisk represents (n=1). Bars are means ± SE.

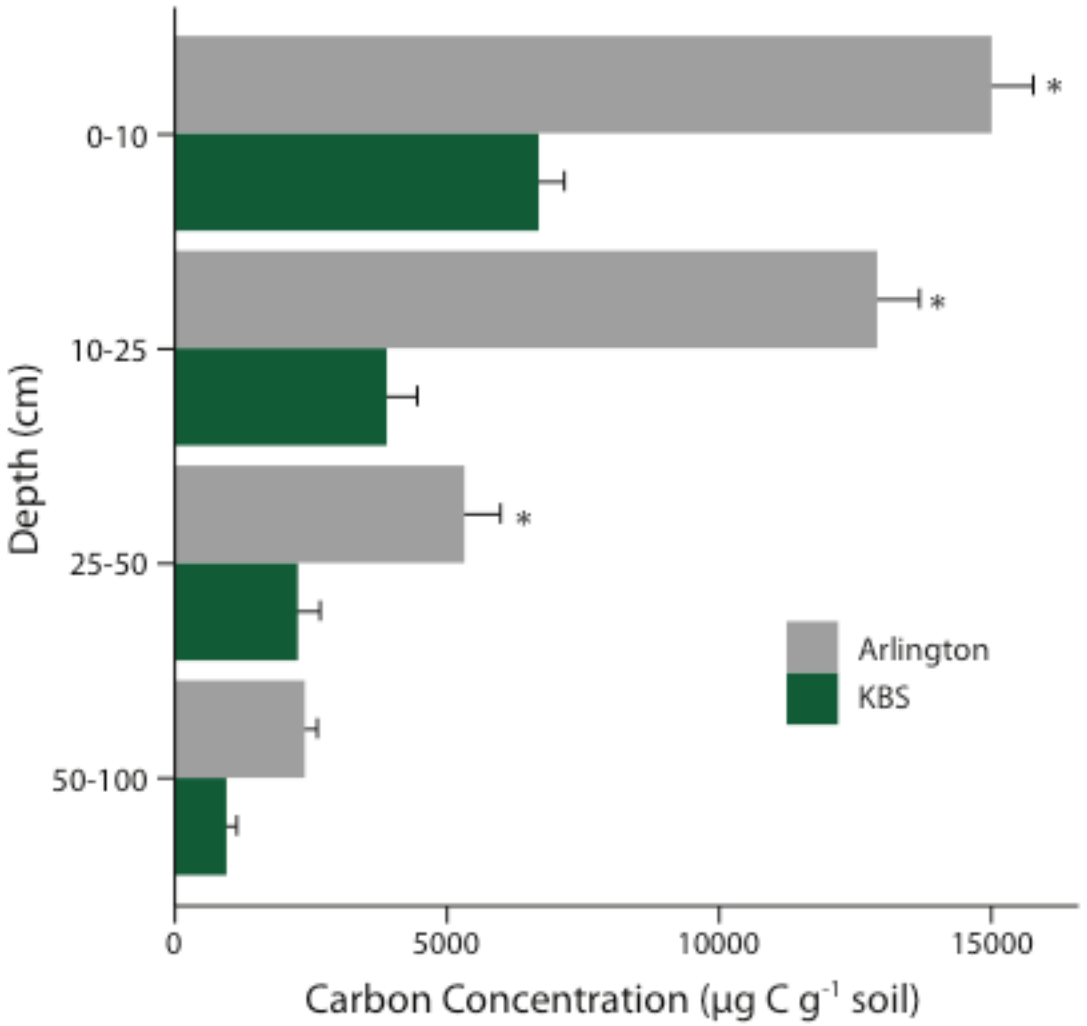

Figure 4.7 The resistant C pool determined by acid hydrolysis and averaged across cropping system. For each depth interval, asterisks represent statistically significant differences across site ($p < 0.05$). Bars with no asterisk were not significantly different from one another. Bars are means ± SE.

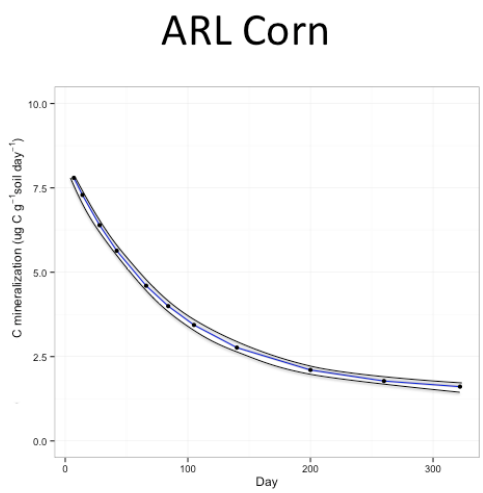

Figure 4.8 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the continuous corn system at ARL. Shaded bands represent standard error from the mean.

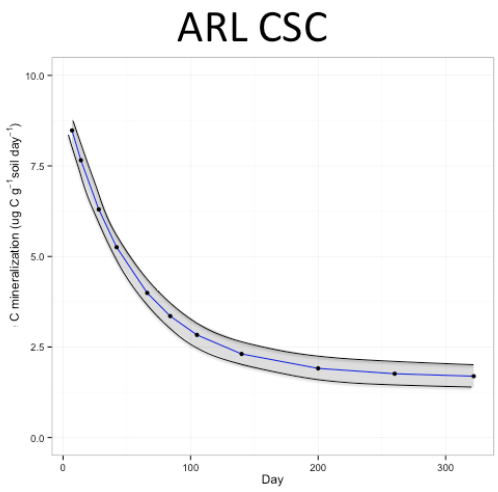

Figure 4.9 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the corn-soybean-canola system at ARL. Shaded bands represent standard error from the mean.

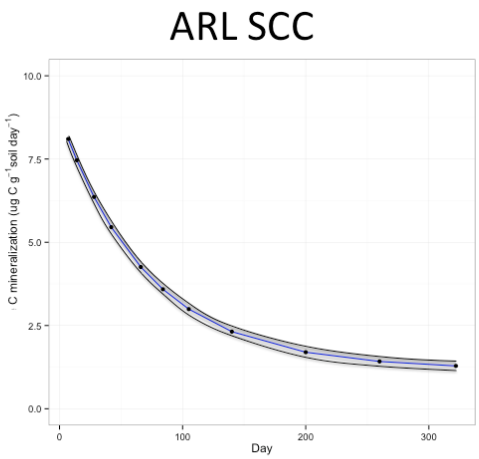

Figure 4.10 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the soybean-corn-canola system at ARL. Shaded bands represent standard error from the mean.

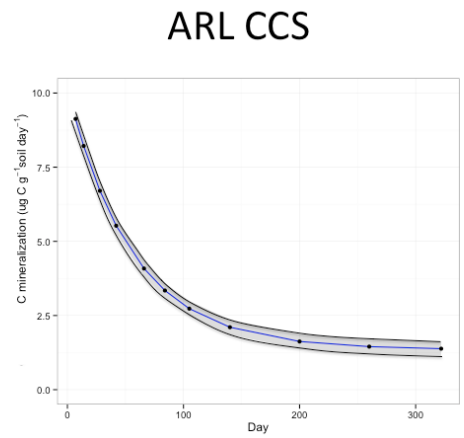

Figure 4.11 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the canola-corn-soybean system at ARL. Shaded bands represent standard error from the mean.

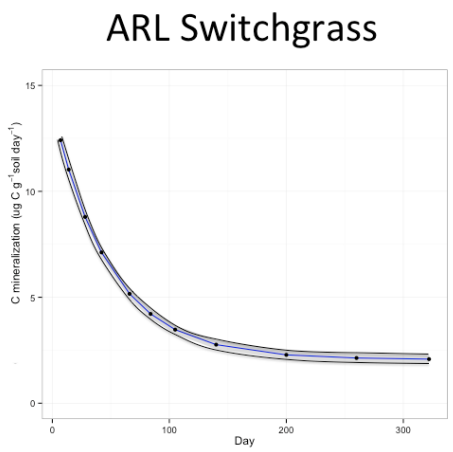

Figure 4.12 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the switchgrass system at ARL. Shaded bands represent standard error from the mean.

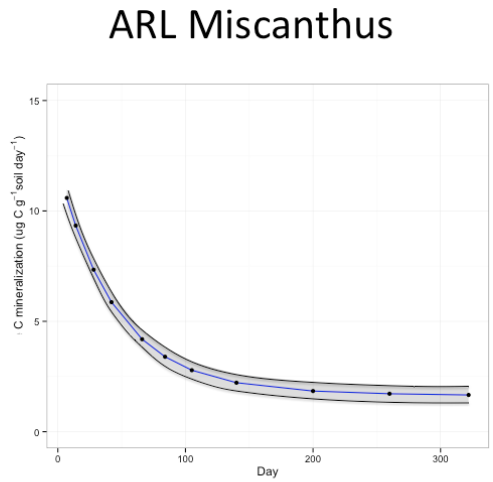

Figure 4.13 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the miscanthus system at ARL. Shaded bands represent standard error from the mean.

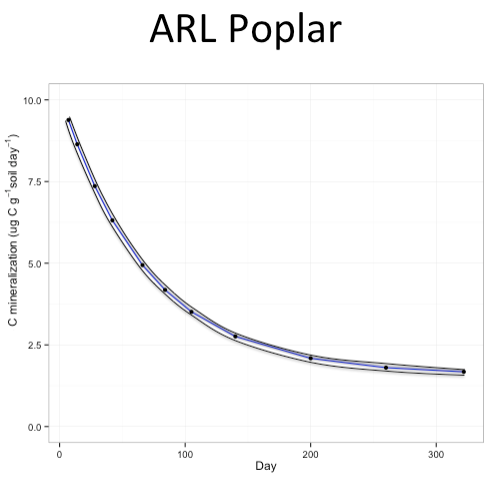

Figure 4.14 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the poplar system at ARL. Shaded bands represent standard error from the mean.

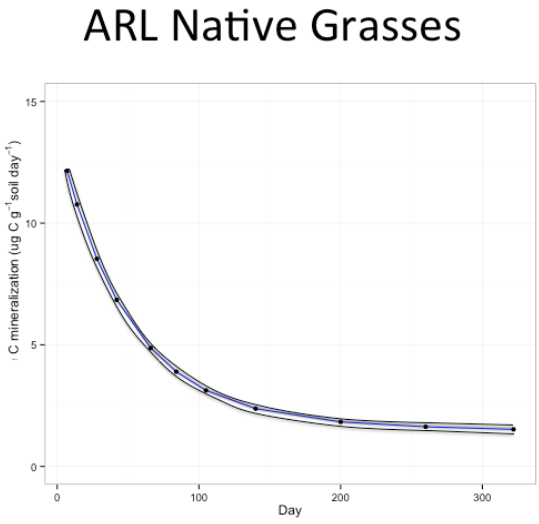

Figure 4.15 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the native grass system at ARL. Shaded bands represent standard error from the mean.

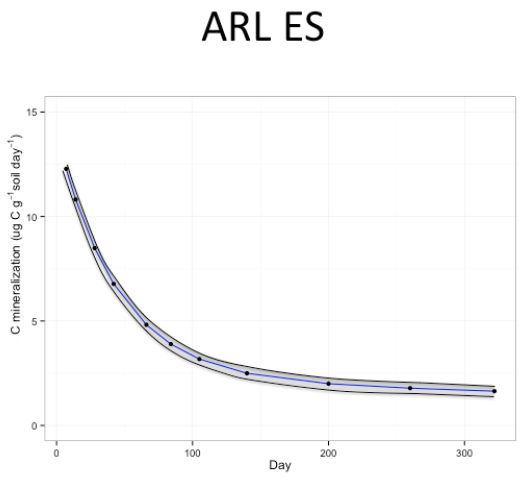

Figure 4.16 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the early successional system at ARL. Shaded bands represent standard error from the mean.

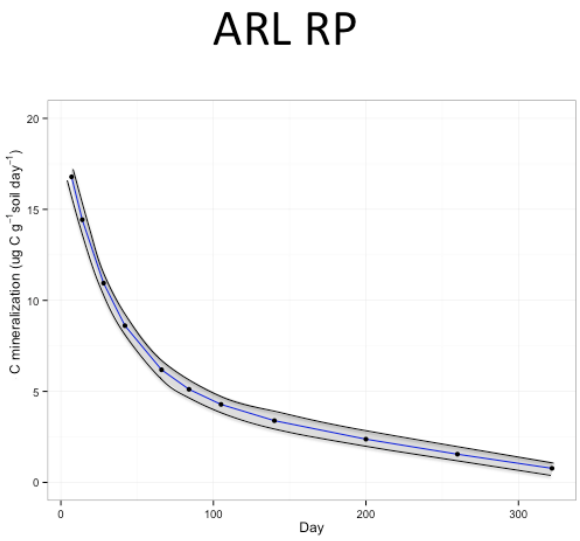

Figure 4.17 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the restored prairie system at ARL. Shaded bands represent standard error from the mean.

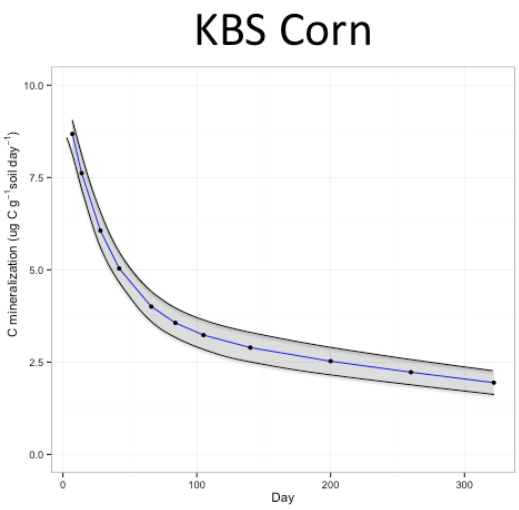

Figure 4.18 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the continuous corn system at KBS. Shaded bands represent standard error from the mean.

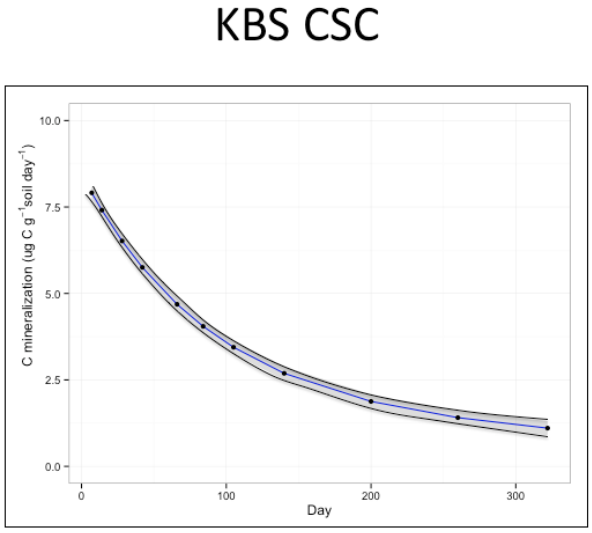

Figure 4.19 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the corn-soybean-canola system at KBS. Shaded bands represent standard error from the mean.

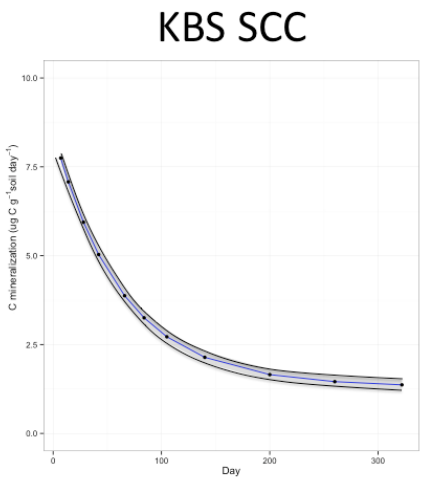

Figure 4.20 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the soybean-corn-canola system at KBS. Shaded bands represent standard error from the mean.

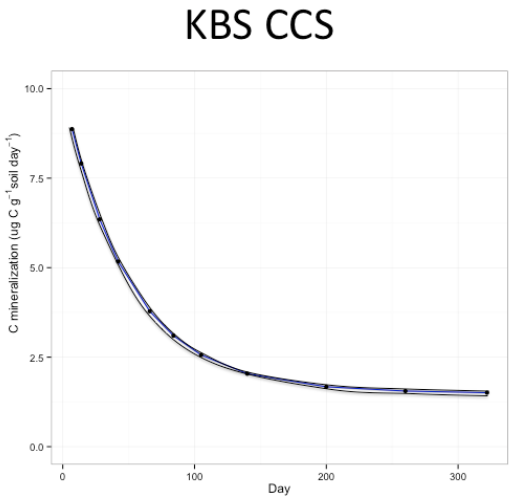

Figure 4.21 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the canola-corn-soybean system at KBS. Shaded bands represent standard error from the mean.

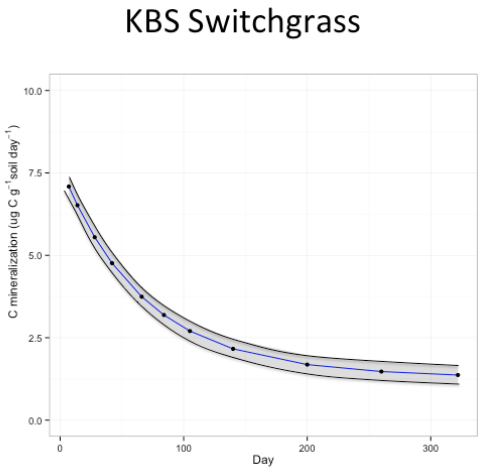

Figure 4.22 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the switchgrass system at KBS. Shaded bands represent standard error from the mean.

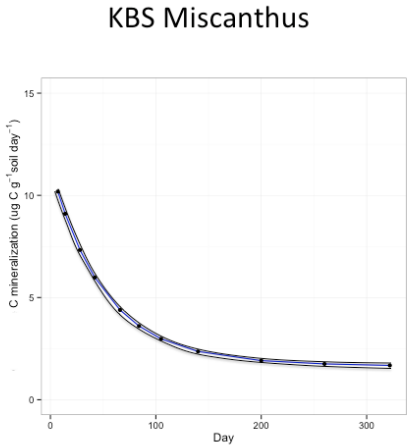

Figure 4.23 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the miscanthus system at KBS. Shaded bands represent standard error from the mean.

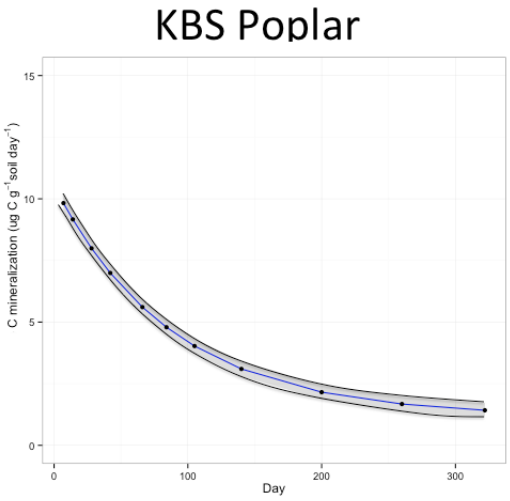

Figure 4.24 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the Poplar system at KBS. Shaded bands represent standard error from

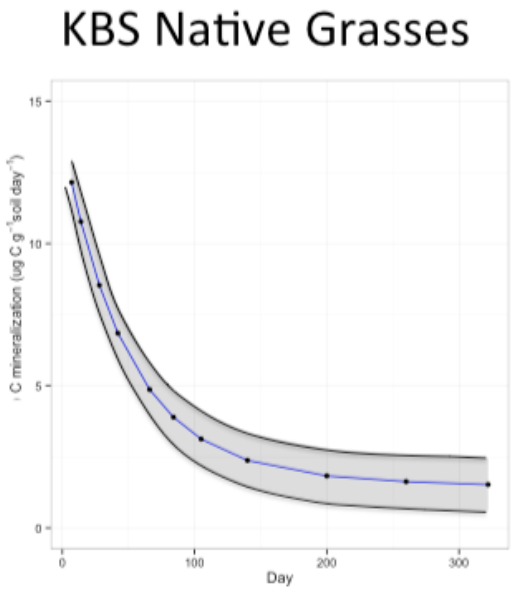

Figure 4.25 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the native grass system at KBS. Shaded bands represent standard error from the mean.

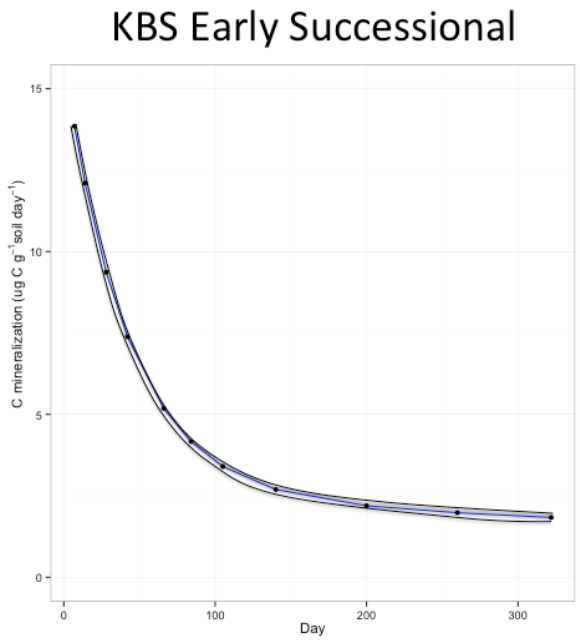

Figure 4.26 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the early successional system at KBS. Shaded bands represent standard error from the mean.

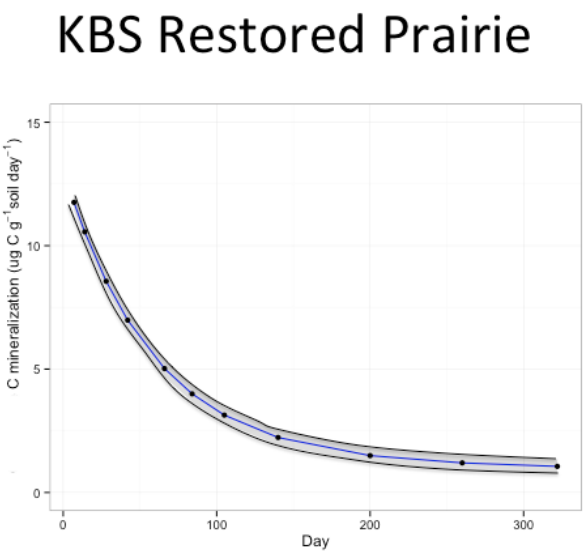

Figure 4.27 Predicted mean C mineralization over the 322 day incubation period (dotted curve) for the restored prairie system at KBS. Shaded bands represent standard error from the mean.

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CHAPTER 5: PLANT DIVERSITY INFLUENCES FINE ROOT PRODUCTION AND BIOMASS ALLOCATION AMONG PERENNIAL BIOFUEL CROPPING SYSTEMS IN CONTRASTING SOILS OF THE UPPER MIDWEST, USA.

ABSTRACT

Fine roots play a key role in the global carbon (C) cycle because much of the C accumulating in soil is the result of fine root production and turnover. Here I explore the effect of perennial plant diversity on fine root production, timing of peak fine root production and plant biomass allocation to fine roots over a three-year period at each of two sites in the upper Midwest, USA. Six perennial cropping systems were established in 2008: switchgrass (*Panicum virgatum)*, miscanthus (*Miscanthus* × *giganteus*), hybrid poplar (*Populus nigra* × *P.* maximowiczii 'NM6.), a five-species native grass assemblage an early successional community, and a restored prairie. The site in southwestern Michigan is on a moderately fertile Alfisol soil (Kellogg Biological Station; KBS) and the other site is in south central Wisconsin on a highly fertile Mollisol soil (Arlington; ARL). From 2011-2013, two sets of in-growth cores were deployed each spring and extracted during 'mid-season' and 'late season'. Averaging across the three years at KBS, I found that the restored prairie and the native grasses had the greatest mid-season fine root production (2.58 ± 1.2 and 2.2 ± 0.4 g m^{-2} day^{-1}, respectively, $p<0.05$, $n=5$). Switchgrass, Early Successional, and Miscanthus followed at 1.4 ± 0.5, 1.09 ± 0.2, and 1.06 ± 0.1 g m^{-2} day^{-1}, respectively. Poplar had the lowest fine root production, averaging 0.9 ± 0.3 g m^{-2} day^{-1} across the three years. Similar trends were visible at ARL, where the native grasses and the restored prairie systems had significantly greater mid-season fine root production compared to the other cropping systems ($p<0.05$). In general, diverse cropping systems allocated more

biomass to fine root production compared to the monoculture systems ($p<0.05$, $n=5$). Timing of peak fine root production differed by year and site, likely the result of climate differences. Overall, results suggest that systems with higher species diversity have greater fine root production and allocate a relatively greater amount of biomass to fine roots compared to monoculture systems, which could have important implications for C sequestration.

INTRODUCTION

Fine roots represent 33% of global net primary productivity (Jackson et al., 1997) and play a key role in the global carbon (C) cycle because the majority of C accumulating in the soil is the result of fine root production and turnover (Haynes and Gower et al., 1995). Fine roots turn over at least once per year, a frequency that has a direct effect on soil C cycling since a portion of the C from senesced roots is incorporated into soil organic matter (Kumar et al., 2006). As roots senesce, C enters the soil organic matter pool, which holds twice the amount of C as the atmosphere (Swift, 2001). Across different ecosystems fine root turnover can account for 30-80% of organic C inputs into soil (Kalyn and Van Rees, 2006). Furthermore, C derived from roots has been found to persist longer in soil compared to C derived from aboveground material (Rasse et al. 2005; Kong and Six, 2010).

Thus, it is important to better understanding fine root production and how it influences C sequestration (Gill and Jackson, 2000) and to determine strategies that might promote root production and C sequestration in various ecosystems (Glover et al., 2010; Kell et al., 2011). One strategy could be to increase crop diversity. The benefits of biodiversity for aboveground production are well known and have been demonstrated in a variety of natural (Tilman, 1996, Hooper and Vitousek, 1997; Catovsky, 2002) and managed (Smith et al. 2008; Werling et al.,

2014; Fraser et al., 2015) ecosystems. Several have hypothesized that biodiversity could also have a positive effect on belowground production. Hooper and Vitousek (1997), for example, suggest that root production should be greater in more diverse cropping systems due to plant complementarity effects, or differences in phenology and nutrient demand. de Kroon et al. (2012) hypothesized that pathogens constrain root growth in monocultures such that root growth is enhanced in mixed species communities.

Empirical evidence, however, is scant and often conflicting. For example, Fornara and Tilman (2008) found that high diversity grasslands on sandy soils in northern U.S. stored five times more C than monoculture systems due to greater belowground net primary productivity (BNPP), standing root biomass, and more roots below 60 cm. Bessler et al. (2009), on the other hand, found that belowground biomass and root production remained the same across increased species richness in a similar long-term grassland biodiversity experiment in Europe. Increased fine root production has also been documented in forest systems, where mixed forest stands have greater standing fine root biomass and production compared to monoculture stands (Liu et al., 2014). In contrast, others working in forest systems have found no difference in root production with increased plant species diversity (Domisch et al., 2015; Jacob et al., 2013).

In this study I used ingrowth cores to explore patterns of fine root production across six perennial biofuel cropping systems that vary in species diversity. Specifically, I test the hypothesis that fine root production is greater in more diverse cropping systems compared to monocultures. I contrast these responses in two different soil types, a moderately fertile Alfisol in southwest Michigan and a very fertile Mollisol in south central Wisconsin. I further test the consistency of these relationships across three growing seasons, including a drought year.

METHODS

Site description

This study was conducted at the Great Lakes Bioenergy Research Center's Biofuel Cropping System Experiment (BCSE) co-located at the W.K. Kellogg Biological Station (KBS) Long-Term Ecological Research site in southwest Michigan (42°24'N, 85°24'W) and at the Arlington Agricultural Research (ARL) station in south central Wisconsin (43°18'N, 89°21'W). Mean annual precipitation and temperature are 1005 mm and 10.1°C at KBS and 833 mm and 7.4°C at ARL. Soils at the KBS site are moderately fertile, fine-loamy mixed, semiactive, mesic Typic Hapludalfs primarily of the Kalamazoo and Oshtemo series (Robertson and Hamilton, 2015): Ap (0-30 cm), E (30-41 cm), Bt1 (41-69 cm), 2 Bt2 (69-88 cm), and 2E/Bt (88-152). Surface (0-10 cm) pH is 6.1 and total soil carbon is 1.25 g kg^{-1} (Sanford et al., in press) and are 63% sand, 31% silt, 6% clay (http://data.sustainability.glbrc.org). Soils at the ARL site are highly fertile, silty loam, mesic Typic Argiudolls in the Plano series (Sanford et al, 2012) with five horizons: Ap (0-23 cm), A (23-36 cm), Bt1 (36-48 cm), Bt2 (48-79 cm), and Bt3 (79-109 cm). Surface (0-10 cm) soils at ARL are 9% sand, 66% silt, and 25 % clay (http://data.sustainability.glbrc.org) and in 2008, pH was 6.6 and total soil carbon was 2.2 g kg^{-1} (Sanford et al., in review). Prior to 2008, both sites were under annual row crops.

Experimental design and systems

The BSCE was established in the fall of 2008 replicated at each site as randomized complete block designs with five replicate blocks. Treatments are biofuel cropping systems that include annual row crops, monoculture perennial grasses, and diverse perennial grasses and forbs. In this study I sampled the perennial cropping systems, which includes three monoculture systems and three diverse systems. Amongst the monocultures are switchgrass *(Panicum*

virgatum), miscanthus *(Miscanthus × giganteus)*, and hybrid poplars (*Populus nigra × P.* maximowiczii 'NM6). The three diverse systems include a native grass assemblage with five species (*Andropogon gerardii*, *Elymus canadensis*, *Panicum virgatum*, *Schizachrium scoparium*, and *Sorghastrum nutans)*, an early successional community that represents the seed bank and natural colonization since establishment at the beginning of the experiment, and a restored prairie system planted with eighteen different native C3, C4, and legume species (http://data.sustainability.glbrc.org/protocols/144). Dominant species in the early successional community during this study at KBS included *Conzya canadensis* and *Setaria faberi*; and at ARL, included *Lactuca serriola*, and *Elymus canadensis*. Dominant species in the restored prairie during this study at KBS included *Elymus canadensis*, *Sorghastrum nutans*, *Andropogon gerardii;* and at ARL, included *Elymus canadensis*, *Ratibida pinnata*, *Monarda fistulosa*, *and Symphyotrichum novae-angliae*. The Shannon-Weiner diversity index for each system is presented in Table 5.1.

Field preparations in the Spring of 2008 included chisel plowing and secondary tillage (Sanford et al., in press). The BCSE consists of 27 m x 43 m (0.12 ha) plots, separated by 15-m wide mowed alleys planted in turfgrass. The switchgrass, native grasses, and restored prairie systems were planted in the summer of 2008 with a brillion-type native plant seeder. Seeding rates for switchgrass were 7.5 kg ha^{-1}. Planting densities for the native grasses ranged from 1.6 to 2.4 kg ha^{-1} and restored prairie planting densities ranged from 0.4 to 1.2 kg ha^{-1}. Both the miscanthus and the poplar systems were planted by hand in May 2008 at 17,200 rhizomes ha^{-1} and 2,778 cuttings ha^{-1}, respectively. Miscanthus failed at ARL due to winterkill in 2009 (Sanford et al., in press) and was replanted in Spring 2010. Nitrogen fertilizer (56 kg N ha^{-1} y^{-1} as ammonium nitrate) was applied to switchgrass, miscanthus, and early successional systems each

June beginning in 2009 and in 2010 for native grasses. The poplars received a single application of ammonium nitrate fertilizer in 2010 at a rate of 155 kg N ha^{-1} at KBS and 210 kg N ha^{-1} at ARL. The restored prairie system was unfertilized. Weeds were controlled with herbicide application in swtichgrass, miscanthus, hybrid poplar, and native grass systems. Harvest for switchgrass, miscanthus, native grass, early successional and restored prairie systems occurred in late October at ARL and early November at KBS. Poplars were harvested in December of 2013 at ARL and January of 2014 at KBS. More extensive details on agronomic practices can be found in Sanford et al. (in press).

Fine root production

In-growth cores were used to estimate fine root production. The in-growth cores were constructed of 2 mm #5 plastic mesh plastic stapled to form a cylinder 5 cm in diameter x 13 cm long (KBS) or 15 cm long (ARL) and closed at the bottom with plastic caps. Cylinders were filled with soil from cores taken to a depth of 15 cm at ARL and 13 cm at KBS. At KBS, soil cores were taken from individual BSCE plots and sieved to 2 mm in the field. The same procedure was used at ARL, except soil cores were taken from a fallow plot adjacent to the BSCE. At each site, in-growth cores were filled with soil from the site mixed with sand in a 3:1 ratio. Cores were vertically inserted in 5 cm diameter holes to 13 cm depths at KBS and 15 cm depths at ARL at six locations per plot within the BSCE experiment.

Installation of cores at both sites typically occurred in mid to late April every year (Figure B.1). The in-growth cores were harvested twice within each growing season, once near the end of July, hereafter referred to as mid-season, and a second time following harvest near the end of October, hereafter referred to as late season.

Following removal, cores were sieved and washed free of soil. Remaining roots (both live and dead) were then dried at 60ºC for two days and weighed. Fine root production values were calculated as:

Total fine root biomass / number of days in the field.

To estimate root production between the mid-season and late-season samplings I subtracted mid-season biomass from late-season biomass.

Aboveground net primary production

Aboveground Net Primary Production (ANPP) was determined from maximum aboveground biomass as detailed in Sanford et al. (in press) for the herbaceous perennial crops. In brief, ANPP for switchgrass, native grasses, early successional, and restored prairie systems was determined in mid-August when the crops reached physiological maturity. At three pre-determined stations, 2.0 x 0.5 m quadrats were placed in an east-west direction, except for Miscanthus for which a 1.5 x 0.6-m quadrat was used. Within quadrats, plant biomass was clipped to ground level. Biomass was dried at 60ºC for a minimum of 48 hours. The dry weight was then determined and recorded. For poplars, tree biomass in each plot was determined in December 2011 and 2012 by measuring basal diameter and applying an allometric equation relating diameter to mass. To determine the equation, five trees per plot were harvested and weighed after measuring basal diameter. Lastly, basal diameter was regressed against mass for all trees. In winter 2013, the entire poplar plots at KBS were harvested and biomass was calculated by weight. Poplar ANPP values in 2013 at ARL are not available because the trees were infected with a fungal disease.

Fine root BNPP:ANPP index

Since I calculated fine root production on a per day basis, and because there is no daily measurement for ANPP, I established an arbitrary fine root BNPP: ANPP index to compare belowground fine root production per aboveground net primary production.

Fine root BNPP:ANPP Index= Fine root production (g m^{-2} d^{-1}) /Aboveground production (g m^{-2} y^{-1})

where, fine root BNPP is fine root production estimated as described above. The index is a ratio that can be used to make relative comparisons for rates of belowground allocation across the six systems.

Root depth distribution

The standing stock of live and dead root biomass was determined at the end of the growing season in late November for select systems. Root biomass was assessed by taking deep core samples with a hydraulic direct-push sampler to a depth of 1 m (Geoprobe®; Salina, KS at KBS and Giddings® probe; Windsor, CO at ARL). Cores were taken at three locations in each plot (center and adjacent to plant as well as the interstitial space in cases where plant distribution was clumped or in rows). Cores were then divided into four different depth strata (0-10, 10-25, 25-50, and 50-100 cm). Roots were washed free of soil over a 2 mm sieve and dried at 60^{o}C over a two-day period, then weighed.

Statistics

Mid-season fine root production and the BNPP:ANPP index were transformed to reduce heterogeneity of variance. I utilized a square-root transformation and back-transformed after statistical analyses. Thus, geometric means are reported for mid-season biomass and the

BNPP:ANPP index. To back transform the standard error, I calculated a 95% confidence interval of the transformed data and then back transformed the interval (Bland and Altman, 1996).

Data were analyzed using Proc Mixed of SAS (version 9.4; SAS Institute, Cary, NC, USA). Cropping system and depth were treated as fixed effects and block as a random effect. For mid-season fine production, fine root:ANPP index, and the difference between late and mid season production, year was treated as a repeated measure. Significant differences were determined at p=0.05 and means were compared with an adjusted Tukey's pairwise means comparison.

RESULTS

Precipitation

At ARL cumulative precipitation during the time that the in-growth cores were installed (April-Oct/Nov) was 451, 491, and 546 mm for 2011, 2012, and 2013, respectively (Figure 5.1A). ARL always received more rain in the first part of the growing season compared to the later portion. At KBS, in both 2011 and 2013, precipitation was above average between April and late October. In 2012, KBS had a drought early in the growing season, with only 152 mm by mid season (Figure 5.7).

Fine root production

Mid-season fine root production significantly differed across the six cropping systems at both ARL and KBS (Figures 5.2 and 5.3; ARL, F=3.7, p=0.01; KBS, F=4.8, P=0.003). Fine root production also significantly varied from year to year (ARL, F=5.3, p=0.009; KBS, F=12.8, p<0.0001), although trends amongst the different cropping systems were similar each year.

At ARL, the native grass and restored prairie systems typically had the greatest amount of fine root production (Figure 5.2). In 2011, fine root production ranged from 0.52 to 1.40 g m^{-2}

day^{-1}, and the native grass and restored prairie systems had significantly greater fine root production compared to the miscanthus and poplar systems (Figure 5.2, $p<0.05$). In 2012, the native grass system had significantly greater ($p<0.05$) fine root production compared to all three monoculture systems with a mean of 1.65 g m^{-2} day^{-1} compared to switchgrass (1.26 g m^{-2} day^{-1}), miscanthus (1.18 g m^{-2} day-1), and poplar (1.18 g m^{-2} day^{-1} Figure 5.2). Similarly in 2013, the native grass and restored prairie systems had significantly greater fine root production compared to miscanthus and poplar systems. In 2012 and 2013, production across the monoculture systems was approximately even. Averaging across year, the native grass system produced the greatest amount of fine roots (2.3 ± 0.2 g m^{-2} day^{-1}), while the miscanthus system produced the lowest at 1.2 ± 0.13 g m^{-2} day^{-1}.

There was a strong year effect at ARL ($F=5.3$, $p=0.0009$), which was likely caused by the variability in fine root production amongst the monoculture perennials, as the diverse fine root production was relatively consistent across the three years (Figure 5.2). For example, the poplar system fine root production significantly increased in 2012 and 2013 ($p<0.05$), while fine root production of switchgrass and miscanthus tended to decrease over time. The diverse cropping systems stayed remarkably stable over the three years, except for the 2013 early successional system, which was lower in 2012 by 40%.

At KBS, the native grass and restored prairie systems also produced the greatest amounts of fine roots, except in the case of restored prairie in 2011 (Figure 5.3). In 2011, the native grass system produced significantly more fine roots than all other systems except for the early successional system. In 2012 and 2013, the restored prairie system had greater fine root production than all other systems, except native grasses and switchgrass in 2013. In all years, the poplar and miscanthus systems had the lowest fine root production, except for poplars in 2013.

Averaging across years, the restored prairie system produced the greatest amount of fine roots with (2.6 ± 1.2 g m^{-2} day^{-1}) followed by native grasses (2.2 ± 0.4 g m^{-2} day^{-1}), while the poplar system produced the lowest at (0.9 ± 0.3 g m^{-2} day^{-1}).

In general, fine root production at KBS was greatest in 2013, when production ranged from 1.0 to 4.8 g m^{-2} day^{-1}, followed by 2011 (Figure 5.3). Lowest production occurred in the drought year 2012 when values ranged from 0.8 to 1.3 g m^{-2} day^{-1}. Differences over time were especially evident for the switchgrass and restored prairie systems, which had significantly greater root production in 2013 compared to prior years ($p<0.05$).

Fine root BNPP:ANPP index

The fine root BNPP:ANPP index significantly differed by cropping system at both sites (Figures 5.4 and 5.5; ARL, $F=30$, $p<0.0001$; KBS, $F=16.1$, $p<0.0001$). A significant year effect was only evident at ARL ($F=72$, $p<0.001$). However, there was a significant crop by year interaction at both sites (ARL, $F=5.1$, $p<0.0001$; KBS, $F=2.1$, $p=0.05$).

At ARL the fine root BNPP:ANPP index in the restored prairie system was significantly greater than in all three monoculture systems (Figure 5.4, $p<0.05$). In 2011, BNPP:ANPP indices ranged from 5.4 to 27.3, where the restored prairie had the greatest index and miscanthus had the smallest index. In 2012 and 2013, the diverse perennials always had significantly greater indices compared to the monocultures with the exception of the native grasses (index= 44.5) in 2012, which were not significantly different from the poplars (index=30.9).

Fine root BNPP:ANPP indexes greatly varied from year to year at ARL. Averaging across cropping system, indexes in 2012 were 59% greater than in 2011 and 71% greater than in 2013.

With the exception of switchgrass and miscanthus systems, all system indices were significantly greater in 2012, compared to the other two years ($p<0.05$).

At KBS, the diverse perennial systems (native grasses, early successional, and restored prairie) always had significantly greater fine root BNPP:ANPP indexes than miscanthus and poplar systems, except in 2013, when the early successional system had a lower index (Figure 5.5, $p<0.05$). The switchgrass system had a significantly greater index compared to the other monocultures, except in 2012. In 2011 and 2012, the native grass system was the only diverse system that had a significantly greater index than switchgrass. Amongst the diverse perennial systems, there were no significant differences, except in 2013, when the early successional system had a substantially lower index compared to the native grass and restored prairie systems. Averaging across years, the restored prairie system had the greatest index of 23.6 ± 4.3, while the miscanthus system had the lowest index of 4.6 ± 0.8.

There was no overall year effect at KBS ($F=0.3$, $p=1.3$), as Fine root BNPP:ANPP indices remained relatively stable over the three years for all systems. However there was a significant interaction ($F=2.1$, $p=0.05$), likely caused by certain crops that had indexes that fluctuated through time. For example, pairwise comparisons revealed that switchgrass in 2013 had significantly greater indices than in 2011 and 2012 ($p=0.02$ and 0.03, respectively).

Late season vs. mid-season fine root production

I calculated the difference between late season fine root production and mid-season fine root production to reveal the seasonal pattern of fine root production in a given growing season. Peak fine root production did not differ among cropping systems at either site (Table 5.2, ARL, $F=2.0$, $p=0.1$; KBS, $F= 1.1$, $p=0.3$). However, there were noteworthy differences through time, as the year effect was marginally significant at both sites (ARL, $F=2.7$; KBS, $F=2.8$, $p=0.07$

p=0.08). At ARL, all cropping systems exhibited peak biomass in by the middle of the growing season in both 2011 and 2012. However, in 2013 the switchgrass, miscanthus, native grasses, and restored prairie systems had greater root production in the later part of the growing season. In contrast, peak production at KBS tended to occur at the later part of the growing season for almost every crop, especially in 2012. Exceptions where peak fine root production occurred in the middle of the growing season included the poplar and native grass systems in 2011, and the restored prairie system in 2011 and 2013.

Root depth distribution

Root biomass was strongly concentrated at surface depths for the switchgrass and miscanthus systems (Figure 5.6A). For example, 77% and 78% of total miscanthus root biomass were found in the top 10 cm at ARL and KBS, respectively. The switchgrass system root distribution at ARL was very similar to that of the miscanthus system where 77% of total root biomass was found in the top 0-10 cm depth. Switchgrass distributions at KBS were more even in the top two depth intervals, where 67% of total root biomass was found in the top 0-10 cm depth interval, 79% was found in the top 0-25 cm depth interval, and 89% was found in the top 0-50 cm depth interval. Total root biomass between 50 and 100 cm depths ranged from 4-9% for both systems at both sites, except in the switchgrass system at KBS, where 11% of total root biomass was found below 50 cm.

Root biomass distribution in an identical poplar system near the BSCE at the KBS LTER site (Robertson and Hamilton, 2015) was more evenly distributed throughout the soil profile, with a greater relative percentage of deeper roots compared to the miscanthus and switchgrass systems (Figure 5.6B). For example, 57% of total root biomass was found in the top 10 cm, 68% in the top 0-25 cm, and 85% in the top 0-50 cm.

DISCUSSION

The native grasses and the restored prairie systems consistently produced greater amounts of fine roots compared to the monoculture systems (especially poplar and miscanthus) at both ARL and KBS, while in the early successional systems fine root production was generally more similar to the monoculture systems. At ARL, the native grass, early successional community, and restored prairie systems all allocated greater fine root production per aboveground net primary productivity (BNPP:ANPP) compared to the monoculture perennials in 2012 and 2013. At KBS, the restored prairie and native grass systems had greater BNPP:ANPP indices compared to the monoculture perennials in 2011 and 2012. In contrast, the early successional community typically had greater BNPP:ANPP than the miscanthus and poplar systems but had statistically similar indexes to switchgrass. Measuring total root biomass to one meter revealed that 56% of roots were in the top 0-10 cm for the poplar system and almost 80% for the switchgrass and miscanthus systems. Thus, the 15 cm deep in-growth cores used in this study sufficiently captured the majority of fine root production in the switchgrass and miscanthus systems and over half in the poplar systems.

Diversity influences mid-season fine root production and allocation

In general, the native grasses and restored prairie systems produced more fine roots than miscanthus, switchgrass, and poplar systems at both sites over all years. Fine root production in the early successional system was more similar to that in monoculture systems. Thus, while not all of the diverse cropping systems differed from the monocultures, the mixed grass systems consistently produced more fine roots. Although I could find no other studies that compared fine root production between monoculture and diverse perennial cropping systems, a few studies have

reported enhanced root production under more diverse forest and grassland ecosystems (Steinbeiss et al., 2008; Fornara and Tilman, 2008; Brassard et al., 2013; Gamfeldt et al., 2013).

Enhanced root production within the native grass and restored prairie systems was likely driven by one or two dominant species rather than species richness. For example, *Elymus canadensis* was dominant in both the native grasses and restored prairie systems except in 2013 at ARL. *Elymus canadensis* and *Luctuca serriola* were dominant in the early successional system but species abundance was more evenly distributed compared to the mixed grass systems and thus a dominant species was not as easily identifiable. Similar species distributions were evident at KBS where the early successional system was dominated by species like *Conyza canadensis* rather than the *Andropogon gerardii* or *Elymus canadensis* dominants found in the native grass and restored prairie systems.

One explanation for lower fine root production in the early successional system is the greater presence of annual species like *Conyza canadensis* compared to perennials such as *Elymus canadensis*, which tend to produce a greater amount of roots (Sainju et al., 1998). At ARL, annuals comprised 6% of total plant composition in the native grasses, 33% in the early successional community, and less than 1% in the restored prairie system. At KBS, annuals accounted for 1% of the native grass system, 79% of the early successional community, and 3% of the restored prairie system. Thus, my findings suggest that diverse systems have greater fine root production, except where annuals are dominant.

Even though I did not compare the two sites statistically due to slight differences with the in-growth cores and pseudoreplication concerns, I found similar trends at both locations in terms of root production across the six different cropping systems. This suggests that these diverse

perennial systems produce larger amounts of fine roots than monoculture systems, regardless of soil type and climate.

These results are consistent with Fornara and Tilman (2008) who found greater fine root production with increased diversity in a long-term biodiversity grassland experiment at the Cedar Creek LTER in northern Minnesota, USA. Furthermore, these results support the diversity-productivity hypothesis and the plant complementarity effect hypothesis, both of which posit that systems with more diversity will have greater root production due to differences in rooting depths caused by a variation in phenology and plant resource demand (Tilman et al., 1996 and Hooper and Vitousek, 1997). For example, the system greatest in diversity in this experiment was the restored prairie, which consists of C3 forbs and grasses, C4 grasses, and legumes. The different plant functional groups could dictate when different species reach peak biomass, which could ultimately lead to greater plant nutrient demand. This in turn enhances belowground competition resulting in greater fine root production (Fornara and Tilman, 2008).

Belowground allocation

The fine root BNPP:ANPP index is an indication of investment in belowground versus aboveground production. Although there were a few exceptions, I generally found that the fine root BNPP:ANPP index was greater in diverse cropping systems compared to the monoculture systems, which suggests that at both sites, plants in diverse perennial systems allocated a relatively greater amount of biomass to roots compared to plants in the monoculture perennial systems. This trend contrasts with Bessler et al. (2009), who found a decrease in root:shoot ratios with increased diversity at an experiment in Germany. Bessler et al. (2009) suggest that the plant complementarily effect led to more available N in the diverse cropping systems, causing a reduction in belowground biomass and greater allocation to aboveground.

Implications for carbon sequestration

The greater fine root production and relative biomass allocation to fine root production within the diverse systems could have important implications for C sequestration as fine roots are a primary contributor to soil C inputs (Haynes and Gower, 1995). For example, despite the fact that fine roots often make up less than 5% of total biomass, fine roots account for nearly 50% of cycled C in certain ecosystems (Meier and Leuschner, 2008). Fine roots contribute to C accumulation and stabilization through chemical and biophysical processes. While both above and belowground plant litter represents new sources of C, fine roots are composed of complex structures that are more recalcitrant to microbial decomposition compared to aboveground litter (Rasse, 2005). As fine roots decompose, microbial communities selectively degrade the labile forms of C leaving more complex materials behind that subsequently transition into more recalcitrant pools of C (Grandy and Neff, 2008). As a result, a primary contributor to the more recalcitrant C pools are microbial biomass and by-products such as polysaccharides and lipids that interact with silt and clay fractions and ultimately stabilize soil C (Paul et al., 2015). Thus, the diverse systems that have greater fine root production will likely have greater C accumulation and C stabilization over time.

Furthermore, in Sprunger (Chapter 4), I found that the diverse perennial systems had 2.5 times more active C accumulation compared to the switchgrass and miscanthus systems, indicating that greater fine root production in theses systems contribute to C accumulation. Enhanced C sequestration with diversity and perenniality has also been demonstrated in other environmental settings (Fornara and Tilman, 2008, Collins, 2010, Kong and Six, 2010). For example, Steinbesis et al. (2008) reported that soil C storage increased with species richness in large part due to enhanced root biomass with greater diversity in native grass systems.

Timing of peak fine root production

I quantified the difference between late and mid season fine root production to determine peak production. A positive value indicates greater root production later in the growing season, whereas a negative number indicates greater root production in the first part of the growing season.

I found that the majority of systems over the three years had peak production in the middle of the growing season at ARL. This suggests that roots were decomposing and turning over in the later stages of the growing season. At KBS the opposite trend occurred, with the majority of crops producing the greatest amount of fine roots in the later half of the growing season. A particularly noteworthy trend at KBS was that the greatest amount of late season production occurred during 2012. This was possibly caused by the drought that KBS experienced in the early part of the 2012 growing season, which likely slowed root production. However, when increased rainfall occurred in the second part of the growing season, fine root production was stimulated, which has been shown in several studies (Steinemann et al., 2015; Fiala et al., 2009; Pavon and Briones, 2000). In addition, contrasting trends occurred at the two sites, suggesting that peak fine root production occurrence is also affected by soil type. For example, ARL has greater N availability than KBS and there is some evidence that fine root turnover is faster in systems with greater N accumulation (Brassard et al., 2009).

It has been a long-standing view in the literature that peak belowground production occurs in the middle of the growing season, with enhanced root decomposition and turnover in the later stages of the growing season (Domisch et al., 2015). For this reason, it has been an accepted practice for investigators to sample root production once within the growing season (Solly et al., 2013, Wang et al., 2013; Ravenek et al., 2014). Others have promoted using sequential coring or

the maximum-minimum method for quantifying fine root biomass (Nadelhoffer and Raich, 1992, Brassard et al., 2011) to capture changes in fine root production throughout the growing season. My results suggest that the former approach could lead to unrealistic measures of fine root production. For example, at KBS, had I only sampled during the middle of the season, I would have underestimated fine root production. My results demonstrate that it is imperative to sample fine root production at least twice to capture peak production.

Root depth distribution

One potential limitation of this study is that the in-growth cores were only 15 cm deep. Thus, I was not able to capture dynamics of root foraging at greater depths. However, Bessler et al. (2009) found that species richness and diversity do not affect root biomass at lower depths. Furthermore, similar research at the KBS site measured fine root biomass to a depth of 1 m in an annual and perennial system over a three year period, and did not detect any differences in fine roots in any of the three years, including the drought year (Sprunger, Chapter 2). Finally, deep cores from both the KBS and ARL sites demonstrate approximately 80% of root biomass is found in the top 10 cm for switchgrass and miscanthus and 57 % for the nearby Poplar system. Thus, it seems reasonable to conclude that the in-growth cores captured the majority of the fine root production in these systems, and that in-growth cores are certainly sufficient to make valid cross-system comparisons.

CONCLUSIONS

Overall, I found that the native grasses and restored prairie systems had greater mid-season fine root production at both fertile (ARL) and moderately fertile (KBS) sites, which suggests that more diverse systems produce more fine roots regardless of soil type. Fine root

production in the early successional system was more similar to the monoculture systems compared to other diverse perennial systems, which suggests that dominance by annuals reduces system-level fine root production. Over the course of three years, it was clear that the diverse perennials allocated more biomass to fine root production compared to the perennial monoculture stands. These findings are consistent with other studies that demonstrate that biodiversity plays a key role in fine root production, which in turn has important implications for soil C accumulation. Precipitation distribution across the growing season and soil type seemed to influence peak fine root production, while cropping system did not. At ARL, peak production was typically greatest in the middle of the growing season, when the majority of the precipitation events occurred. In contrast, rainfall patterns were more variable over the three years at KBS, where fine root production was typically greatest in the later part of the growing season. Fine root production should be quantified more than once during the growing season to obtain more accurate estimates of fine root peak production. Finally, results underscore the importance of plant diversity for promoting soil C sequestration in biofuel and other managed perennial communities Table 5.2 Difference between end of season fine root production and mid-season fine root production at ARL and KBS. Numbers represent the mean and standard error (in parentheses) for each system. A positive number indicates greater root production at in the later part of the growing season and a negative number indicates greater root production during the middle of the growing season.

APPENDIX

Table 5.1 Shannon-Weiner diversity index for native grass, early successional, and restored prairie systems at KBS and ARL for years, 2011, 2012, and 2013.

		Year		
Location	System	2011	2012	2013
ARL	Native Grasses	1.09	1.34	1.23
	Early Successional	1.96	1.97	1.94
	Restored Prairie	1.75	1.92	2.23
KBS	Native Grasses	1.56	1.79	1.42
	Early Successional	1.48	2.40	2.10
	Restored Prairie	2.04	2.24	2.20

Table 5.2 Difference between end of season fine root production and mid-season fine root production at ARL and KBS. Numbers represent the mean and standard error (in parentheses) for each system. A positive number indicates greater root production at in the later part of the growing season and a negative number indicates greater root production during the middle of the growing season.

		Year		
Location	System	2011	2012	2013
ARL	Switchgrass	-0.3 (1.3)	-0.4 (0.7)	1.1 (0.9)
	Miscanthus	-0.4 (0.8)	-0.5 (1.1)	1.4 (0.9)
	Poplar	-0.3 (0.2)	-2.0 (0.7)	-3.1 (1.7)
	Native Grasses	-2.1 (0.9)	-1.5 (1.5)	0.4 (1.1)
	Early Successional	-1.8 (0.6)	-1.3 (0.6)	-0.1 (0.5)
	Restored Prairie	-0.5 (0.7)	-1.0 (0.8)	-0.4 (1.6)
KBS	Switchgrass	0.5 (0.8)	1.4 (0.5)	0.2 (1.1)
	Miscanthus	0.6 (0.6)	0.8 (0.4)	2.3 (0.7)
	Poplar	-0.4 (0.5)	1.1 (0.5)	0.3 (0.7)
	Native Grasses	-0.02 (0.3)	2.2 (0.6)	3.0 (1.6)
	Early Successional	1.3 (0.5)	0.3 (0.2)	0.2 (0.6)
	Restored Prairie	-0.3 (0.4)	2.1 (0.4)	-2.4 (2.1)

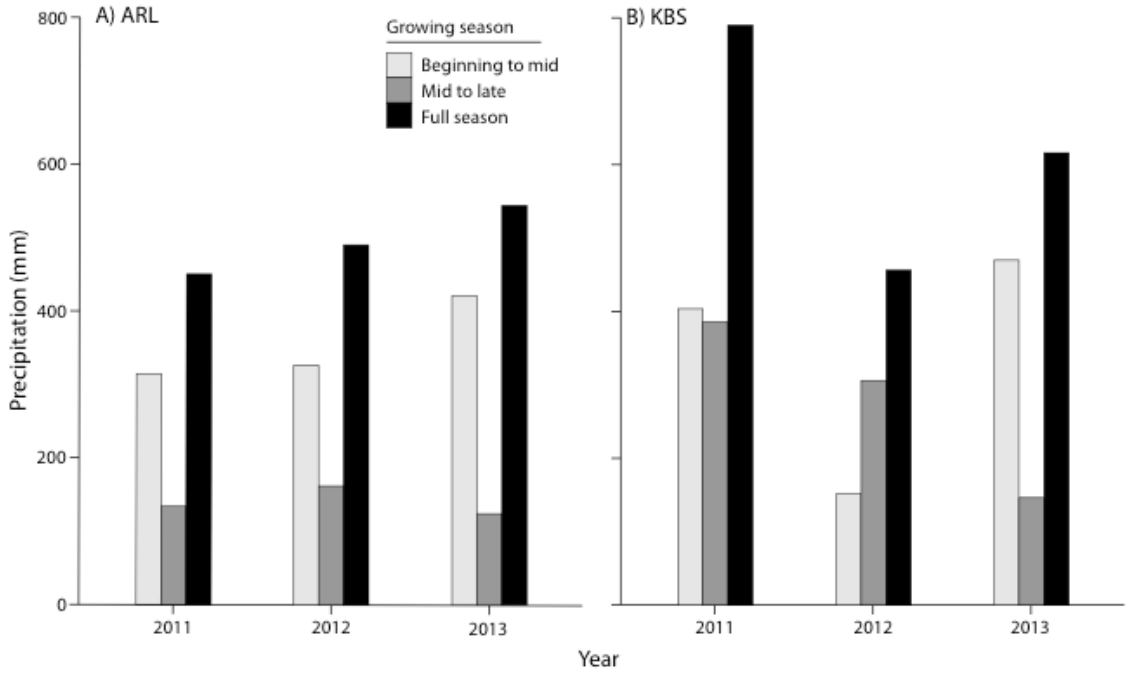

Figure 5.1 Precipitation during two different intervals of the growing season for which in-growth cores were installed. Beginning to Mid season started when the cores were installed in mid April and ended when the first set of cores were removed during the middle of the growing season. The Mid to Late season interval covers the length of time between the mid season core removal and the date when the second set of cores were removed at the end of the growing season.

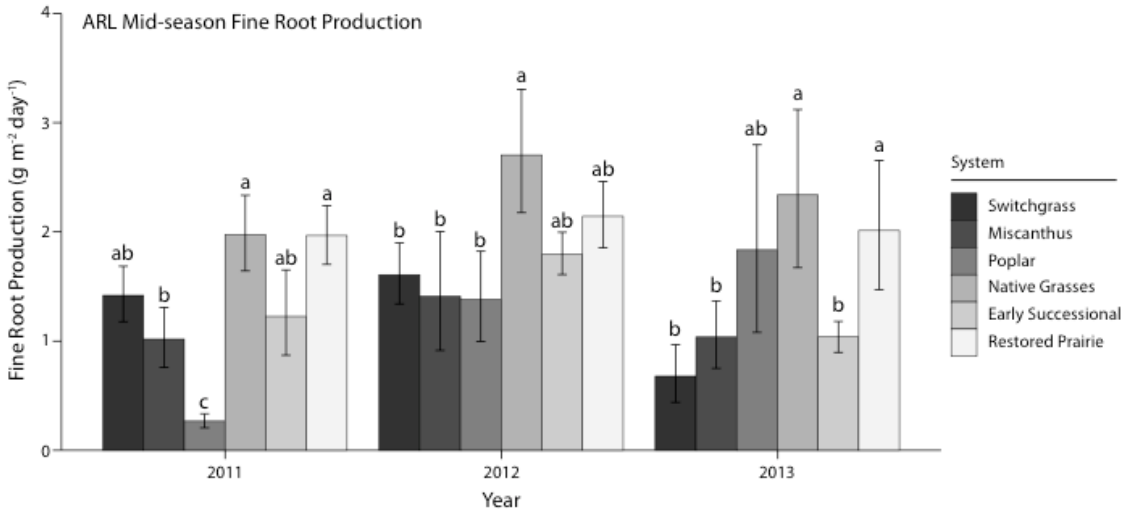

Figure 5.2 Mid-season fine root production (geometric mean) for six perennial cropping systems ranging in diversity (switchgrass, miscanthus, poplar, native grasses, early successional, and restored prairie) at ARL in 2011, 2012, and 2013. Error bars represent back transformed 95% confidence intervals. Different letters within a given year denote a significant at $\alpha=0.05$.

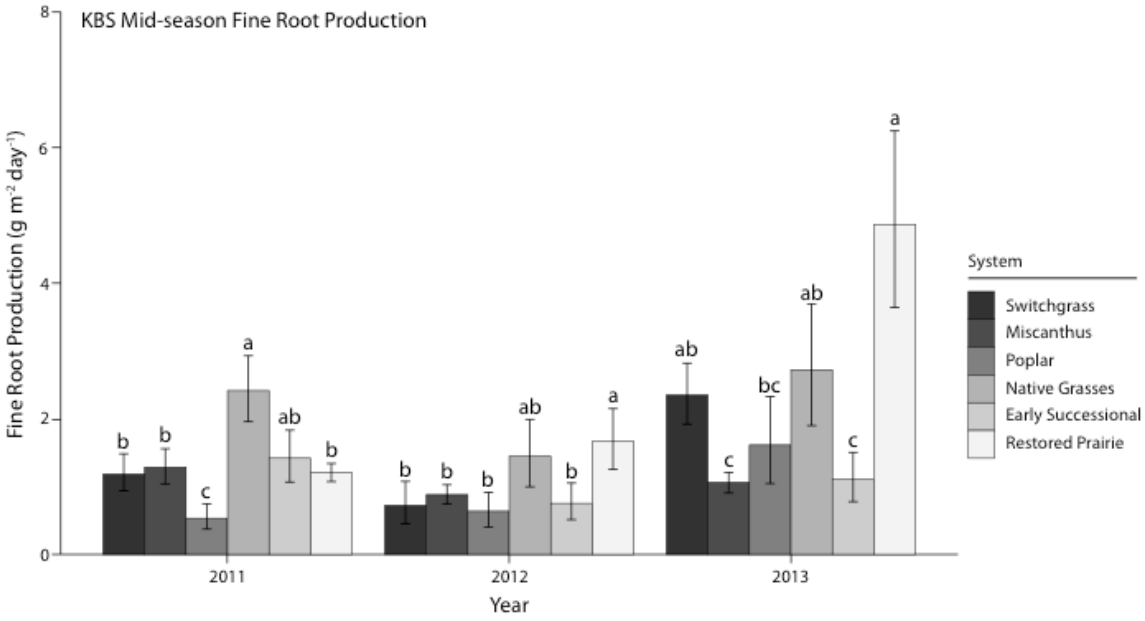

Figure 5.3 Mid-season fine root production (geometric mean) of six perennial cropping systems ranging in diversity (switchgrass, miscanthus, poplar, native grasses, early successional, and restored prairie) at the KBS in 2011, 2012, and 2013. Error bars represent back transformed 95% confidence intervals. Different letters within a given year denote a significant at α=0.05.

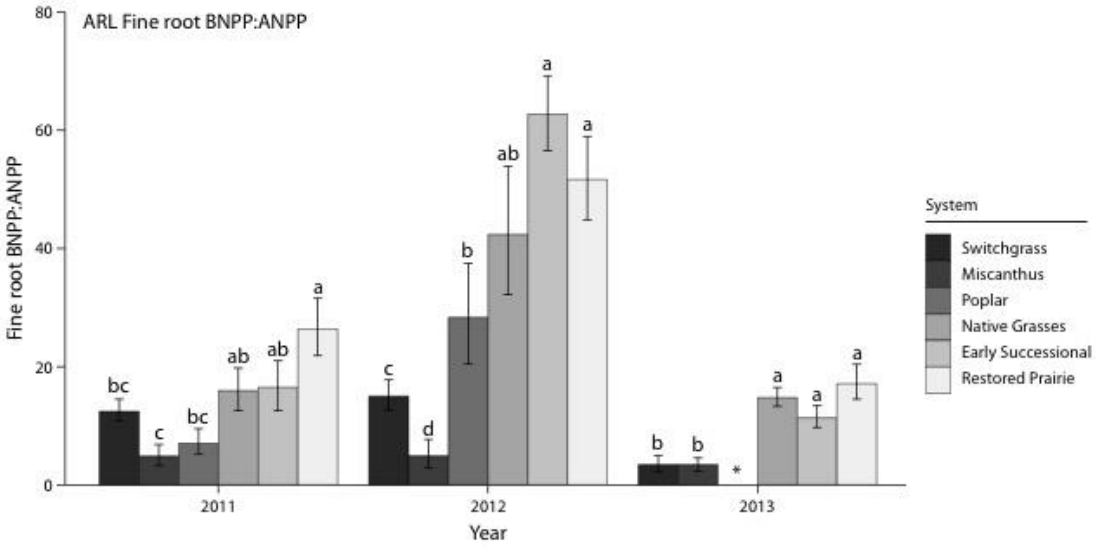

Figure 5.4 Fine root BNPP:ANPP Index (geometric mean) of six perennial cropping systems ranging in diversity (switchgrass, miscanthus, poplar, native grasses, early successional, and restored prairie) at ARL in 2011, 2012, and 2013. Fine root BNPP:ANPP indices are the ratio of fine root production to ANPP. Error bars represent back transformed 95% confidence intervals. Different letters within a given year denote a significant at α=0.05.

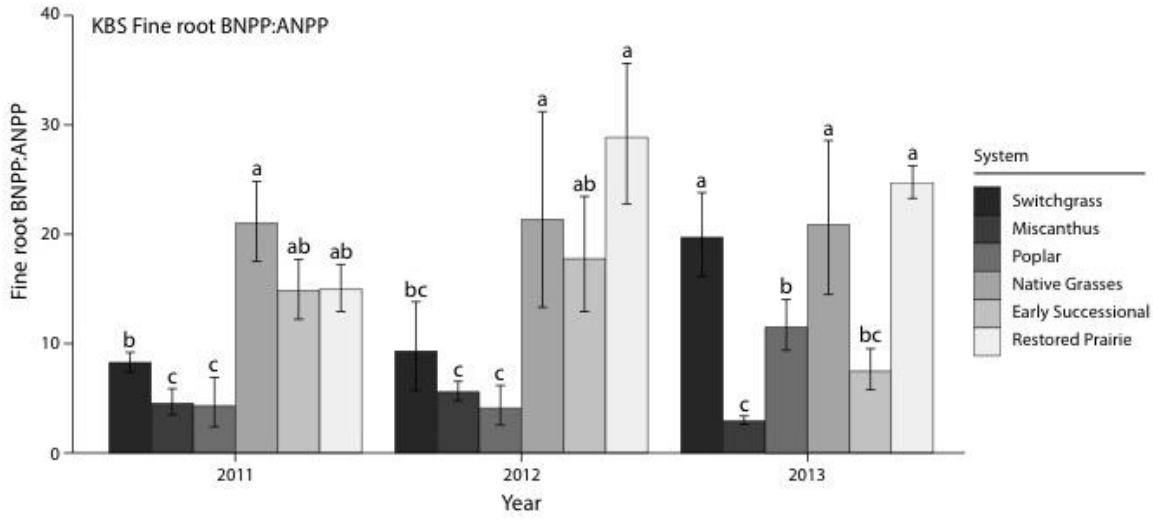

Figure 5.5 Fine root BNPP:ANPP Index (geometric mean) of six perennial cropping systems ranging in diversity (switchgrass, miscanthus, poplar, native grasses, early successional, and restored prairie) at KBS in 2011, 2012, and 2013. Error bars represent back transformed 95% confidence intervals. Different letters within a given year denote a significant at α=0.05.

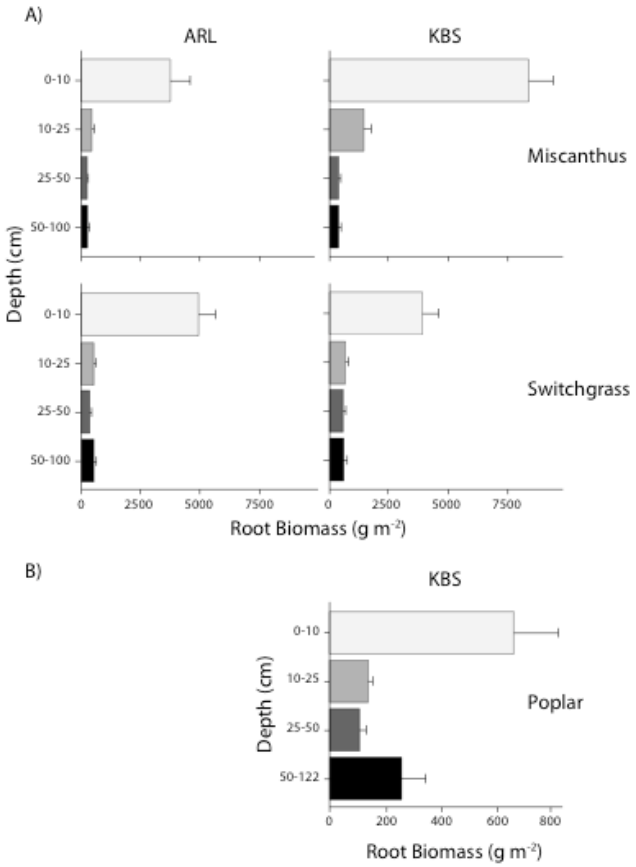

Figure 5.6 A) Miscanthus and Switchgrass root biomass distribution averaged across three years (2011, 2012, and 2013) to one meter at KBS and Arlington. B) Poplar root biomass distribution to 1.22 meter from the nearby Long-Term Ecological Research experiment at KBS.

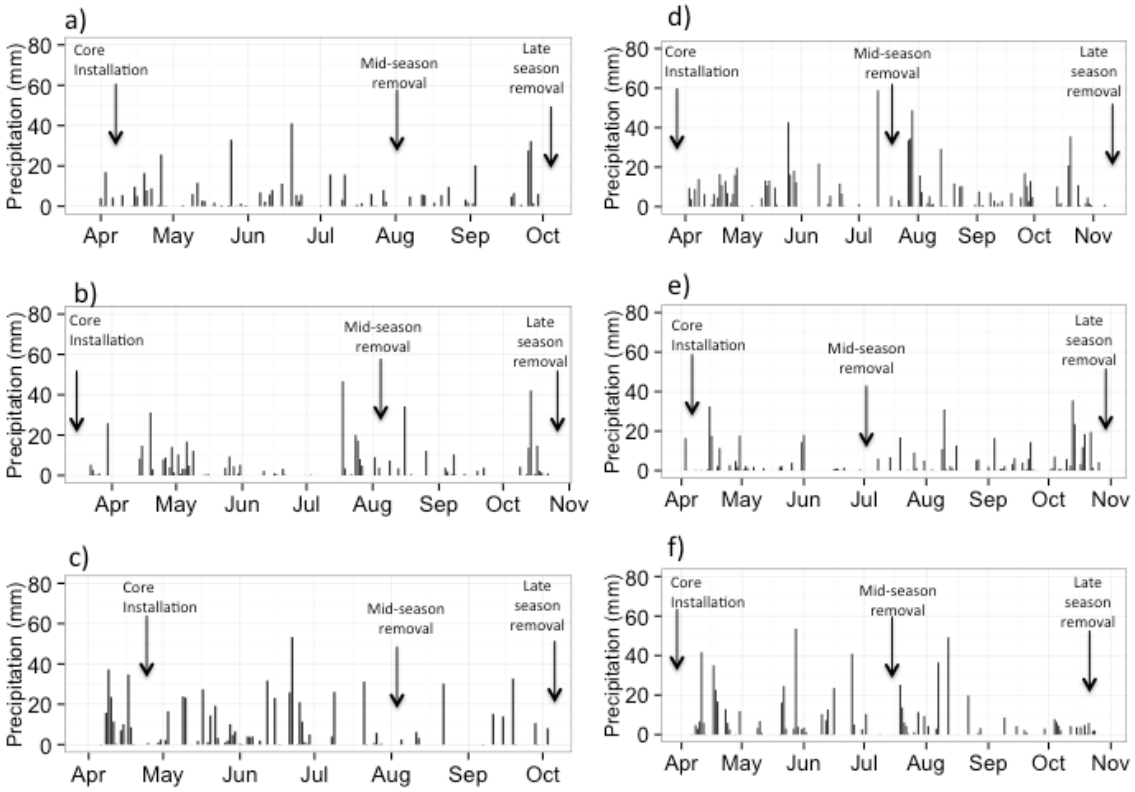

Figure 5.7 Timing of in-growth core installation and precipitation at ARL and KBS during 2011, 2012, and 2013. Both sets of cores were installed in late March or early April. The first set of cores (Mid-season) were typically removed in mid July or early August and the second set of cores (late season) were removed in late October or early November. Arrows indicate when cores were installed and removed. a)=ARL 2011, b)ARL 2012, c) ARL 2013, d) KBS 2011, e) KBS 2012, f) KBS 2013.

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CHAPTER 6: DO TOTAL SOIL CARBON TESTS MEET FARMER MANAGEMENT NEEDS? MEASURES OF ACTIVE CARBON VERSUS STATIC SOIL ORGANIC MATTER POOLS

ABSTRACT

Farmers are dependent on soil testing for management decisions that influence farm profitability and soil health. Providing farmers with tests that are more management sensitive could improve economic returns and on-farm environmental performance. Here I compare two soil carbon (C) measures on 52 Michigan farmer fields: total soil organic matter (SOM) and active C. Total SOM is widely accessible to farmers via university and commercial laboratories, while C mineralization (active C) is not yet commercially available. I used quantitative field-based research and qualitative approaches to determine the effectiveness of total SOM versus active C for deciphering differences in soil C across fields identified by farmers as Best performing, Worst performing, a field of their choice, as well as a non-row crop area. Farmer descriptions of fields were typically based on yield, SOM levels, compaction, and water holding capacity. After soil sampling, laboratory analyses and field observations, I held individual meetings with each farmer to determine field history, management practices, and soil testing history and to explain soil test results. Active C tests detected significant differences between the Best vs. Worst fields (t-test= 5.8; $p<0.0001$), while total SOM tests were statistically similar for the Best and Worst fields (t-test=2.8, $p=0.07$). The average coefficient of variation for between the Best and Worst fields for Active C was 0.30 ± 0.03 and was substantially greater than the CV for SOM (0.05 ± 0.01), suggesting that active C is a more sensitive test than total SOM. Additionally, the level of agreement found among my field observations, farmer perceptions of

soil health and active C test results were strong. The active C tests are more management sensitive and better support farmer perceptions of SOM than do results from the total SOM tests. University and commercial laboratories should consider offering active C tests to provide farmers test results that better inform short-term SOM management decisions.

INTRODUCTION

Soil health is interchangeable with soil quality, and represents the capacity of soil to sustain plant and animal productivity, maintain or enhance environmental quality, and promote plant, animal, and human health (Doran, 2002; Magdoff and Van Es, 2009). The most effective way to improve soil health is to increase soil organic matter (SOM) because SOM can help to improve physical, chemical, water retention, and biological soil parameters (Robertson and Grandy, 2006). For example, SOM leads to the formation of more soil aggregates (Tisdall and Oates, 1982), which increases nutrient retention, improves soil physical structure, and alleviates compaction (Jarecki and Lal, 2010). SOM also enhances microbial activity, which is important for nutrient cycling and crop productivity (Rees et al., 2005).

For this reason, farmers rely on SOM indicators as an overall measure of soil health and for predicting agronomic performance (Magdoff and Van Es, 2009). However, SOM is often insensitive to changes in management because it is a large and dynamic pool that consists of carbon (C) that varies in persistence and decomposition (Wander, 2004). For instance, the smaller and more labile SOM pool consists of recently deposited material that typically decomposes within a year, fluctuates with crop growth, is sensitive to changes in management, and is a predictor of long-term C sequestration (Franzluebbers, 2000). However, the larger and

older pools of C persist in the soil for thousands of years and are insensitive to new management practices.

The most widely available SOM indicator for farmers is total SOM, which is largely comprised of the older and more recalcitrant pools of C. As a result detecting changes in SOM after the introduction of new management practices can take many years. Thus, the length of time needed to detect changes in total SOM, has motivated researchers to develop methods that isolate the younger and more active portions of the SOM pool (Culman, 2013). Since the active C pool represents a large nutrient reservoir that influences other chemical, physical, and biological soil properties, measuring only this pool could be informative for farmers as they consider adopting a variety of different management practices to improve soil health and boost yields.

An increasingly common indicator that is used to determine the active portion of SOM is C mineralization, which can provide an estimate of available C by measuring microbial respiration (Paul et al., 2000). Culman et al. (2013), for example, detected different C mineralization rates amongst monoculture and rotated cropping systems and differences between conventional and organic management. C mineralization was the best of six labile C and nitrogen (N) measures for predicting agronomic performance. However, it is not yet clear if C mineralization (active C) can detect on-farm differences in soil C that will inform farmers about soil health.

Farmer Participation

Since the early 1980s there has been a large effort to conduct on-farm research because solving agronomic and soil quality issues on-farm can lead to more realistic results compared to experiments at research stations (Thompson and Thompson, 1999; Wander and Drinkwater,

2000). Furthermore, there has been a strong effort to introduce different soil quality indicators that are sensitive to changes in management to extension educators and farmers (Doran and Parkin, 1996). However, farmer knowledge is a key component for making on-farm research successful and applicable when evaluating soil quality with new soil tests (Gruver and Weil, 2007; Leibig, 1996). For example, in an attempt to match soil quality indicators with farmer perceptions in the Mid-Atlantic region of the U.S., Gruver and Weil (2007) found strong agreement between farmer perceptions of soil quality and soil quality indicators when comparing fields that farmers had identified as having good versus poor soils. The microbial biomass, anthrone reactive C, and macro aggregate tests had higher levels of agreement with farmer ratings than did soil pH and micronutrient content.

Little is known about the effectiveness of active C for informing farmers about soil health and whether it corresponds with farmer perceptions and investigator field observations. In this study, I use techniques from action research and soil science to explore farmer perceptions of soil C, to investigate the underlying reasons behind adopting certain management practices to improve soil health, and to determine if farmers have access to tests that meet management needs. I combine soil testing with investigator field observations and meetings with farmers to ask: Does the active C test better reflect farmer perceptions of yield expectations and soil health on each field than does total SOM?

METHODS

Participant selection

This study was grounded in a participatory action research framework where Michigan farmers, MSU Extension staff, and MSU researchers worked together to determine if different

soil tests corroborated with farmer perceptions of soil health. Thirteen farms across three counties in Michigan, USA participated in this study (Table 6.1). The three counties included Isabella (43º60'N, 84º76'W), Presque Isle (45º42'N, 83º81'W), and Van Buren (42º21'N, 85º89'W) and represent three different geographical parts of Michigan, as well as different soil types. Farmers were chosen by Michigan State University (MSU) extension agents based on willingness to participate in interviews and workshops in exchange for free soil testing. While it is likely true that the farmers who participated in this study were more invested in soil testing than the average farmer, the overall goal of this research is not to generalize farmer responses to a larger farmer population. Instead, I intend that results from this study can inform researchers and extension staff on how farmer perceptions of soil quality are reflected in different soil tests and whether this information can be used to strengthen soil testing and farmer management decisions.

On-farm soil sampling and field observations

Initial farm visits consisted of meeting with farmers, field observations, soil sampling, and when possible, concise interviews with farmers regarding farm history and soil and crop information. At each farm, I asked farmers to select four fields to include for sampling: a best-performing field (Best), a worst-performing field (Worst), a field of their choice (Choice), and a non-row crop or unmanaged area (NRC). Soils were sampled at the end of May and early June, 2014, spanning three weeks. Five samples were randomly taken from each field. The first sample was taken 4.5 meters from a field edge, while subsequent samples were taken every 4.5 meters by walking diagonally across each field. Samples were taken by digging 15 cm deep pits between cultivated rows and extracting 4 cm wide slices of soil. Next, a trowel was used to cut a rectangular block of soil (15 cm by 10 cm by 4 cm). The five soil samples were composited by

field and mixed thoroughly and then put through a 2 mm sieve. In addition, 30 g were kept for the C mineralization test while 40 g were sent to the MSU soil analysis lab for the total SOM test. At each of the five sampling points, two penetrometer readings were also taken at 15 cm and 46 cm depths to determine surface and subsurface compaction. Field observations consisted of taking photographs of the crop and soil and making descriptive field notes on the condition of crops and soils.

Laboratory analyses: total soil organic matter (SOM) and active C

The Soil and Plant Nutrient Laboratory at MSU utilizes loss on ignition to determine percent SOM. Samples (40 g) were oven dried for 48 hours at 105º C, weighed, then heated in a muffle furnace at 550º C for five hours. SOM was determined by difference in weight: where $SOM_{L.O.I} = (DW_I - DW_o)/DW_I \times 100$; DW_I = Oven dry soil weight (dried at 105ºC) and DW_o = Soil weight after ignition at 550ºC.

Active C was determined via short-term C mineralization incubations. Ten grams of soil were placed in a 237 mL Mason jar, re-wetted and then incubated for 24 hours at 25ºC. Two analytical replicates were analyzed per field. Soils were adjusted to 50% water-filled pore-space utilizing the methods described in Franzluebbers et al. (2000). Following the 24-hour incubation, each Mason jar was capped tightly with a lid fitted with a rubber septum. A time zero CO_2 reading was taken immediately following capping, by injecting 0.5 mL of headspace into a LI-COR LI-820 infrared gas absorption analyzer (LI-COR Biosciences, Lincoln, NE). Three subsequent readings were taken over 90 minutes and a flux was calculated by regressing the change in CO_2 versus incubation period (Robertson et al., 1999).

Individual farmer meetings and qualitative analyses

During the first part of each farmer meeting I asked farmers to describe each of their target fields including characteristics and challenges of each. Next, I presented both the MSU test results (total SOM) and the active C results to the farmer. The last part of the meeting was unstructured, which allowed for more in depth questioning on farm history, management decisions, and soil testing. Each meeting took place in the winter following my sampling and lasted up to two hours. All meetings were recorded and notes were expanded within 24 hours of each meeting. Recordings were transcribed and analyzed for emerging themes and concepts by reading through transcriptions, writing text summaries, and coding transcripts within Nvivo 10.2 (QSR International, Burlington, MA). To compare test results to field observations and farmer experiences, I wrote summary memos of field characteristics and farmer descriptions and then constructed data matrix displays (Tables 6.3-6.5) with extracted text combined with active C results to examine common concepts and themes.

Statistics

Paired t-tests were used to compare active C and total SOM results between different fields. I calculated the coefficient of variation (CV) to determine the variability (n=4; four fields per farm) of total SOM compared to active C.

RESULTS

Variability in total SOM and active C across farmer fields

Averaging across the 13 farms, variability was lower for total SOM, with an average CV of 0.05 ± 0.01 compared to an average CV of 0.30 ± 0.03 for Active C between the Best and

Worst fields (Table 6.2). In addition, there was always more variability in active C between the other paired fields compared to total SOM (Table 6.2).

Differences between Best and Worst fields: total SOM versus active C

Total SOM was statistically similar in all row-crop field comparisons across the three counties (Table 6.2). SOM was slightly higher in the Best field, with a mean of 38.5 ± 0.4 g SOM kg soil^{-1}, across the 13 fields compared to the Worst field (37.4 ± 0.3 g SOM kg soil^{-1}), however, there were no significant differences between the two types of fields (t-test=2.8, p=0.07). Percent difference in total SOM between the Best and Worst fields ranged from 1.2 to 6.7 across the 13 different farms (Figure 6.3). I found significant differences in active C between the Best and Worst fields (t-test= 5.8, p<0.0001), where active C was greatest in the Best fields with a mean of 43 ± 0.3 μg C g^{-1} soil day^{-1} and significantly lower in the Worst fields (28.2 μg C g^{-1} soil day^{-1}). Percent difference in active C between the Best and Worst fields ranged from 13 to 60% (Figure 6.1).

Penetration resistance

Penetration resistance for the subsurface 15-46 cm depth interval was twice as high as the penetration resistance in the surface 0-15 cm depth interval for the Best, Worst, Choice, and NRC fields (Table 6.3). At the 0-15 cm interval the Choice field (mean, 32.9 ± 4.4 psi) had significantly greater penetration resistance compared to the Best field (mean, 21.9 ± 4.9; t-test=-2.3; p=0.04). There was a marginally significant difference between the Best and the Worst fields (t-test= -2.0; p=0.06), where the Worst field had a mean of 30.1± 6.1. The Choice and Worst fields were statistically similar to one another (t-test= -0. 5; p=0.6). Among the different pairings at the subsurface 15-46 cm interval, the Best (58.5 ± 4.7) versus Worst (66.2 ± 5.2) field

comparison was the only pairing that showed significant differences from one another (t-test= -2.5; p=0.03).

Field observations versus active C

During the field observations and soil sampling, I measured compaction and took notes on soil structure and other field characteristics (6.4). When describing the Best fields, I used phrases and characteristics such as, "good soil structure," "dark top-soil," "earthworm activity," and "good aggregation." The Worst fields were more commonly described as, "poor soil structure, "sandy," "sloped/hilly," "extremely compacted," "cracked surface," and "evidence of drainage problems." There were, however, characteristics that overlapped between the Best and Worst fields in certain cases.

In almost every case, my field observations of the Best field compared to the Worst field agreed with the active C test, which showed rates to be highest in the Best field (Table 6.2). For example, I utilized phrases such as, "earthworm activity, dark in color, and good structure" to describe the Best field at Farm No. 3. In contrast, I described the Worst field at Farm No. 3 as "sandy, windswept, and rocky surface" (see appendix).

Farmer perceptions of soil health versus active C

The characteristics that farmers used to describe their Best field were strikingly similar across the different farms and counties (Table 6.5). Eleven out of the thirteen farmers mentioned yield or 'consistent production' when asked to describe the qualities of their Best field. Soil health and organic matter were also important characteristics for the Best field, as 70% of the farmers mentioned soil structure, quality, and/or soil nutrients. For example, farmer No. 7 answered, "better organic matter, higher earthworm population, and less disease pressure… it's

always higher in yield." Five farmers explained that consistent manure additions were what made a given field their best field, as illustrated by farmer No. 4, who explained differences on the basis of "[high] production and the soil structure; we've been applying manure over the last several years."

When describing the Worst field, all of the farmers mentioned something negative about soil quality. Farmer No. 13 stated, "the SOM levels are problematic" and farmer No. 1 said, "the SOM is not as high as the [Best] field." Yield or crop conditions were mentioned by 53% of the farmers but were often in conjunction with soil health indicators. For instance, farmer No. 5 described the Worst field as, "one of our lowest yielding fields…with [high] compaction…there is something in that ground and I don't know what it is." Farmer descriptions greatly contrasted between the Best and Worst fields, which mirrored results from the active C test, where the Best fields had greater active C than the Worst fields (Table 6.2).

The importance of SOM and associated challenges

There were several themes and concepts regarding SOM that emerged during the meetings with farmers. First, most farmers explicitly expressed the importance of SOM and all of the farmers mentioned challenges associated with building SOM. Second, farmers noted different motivations for increasing SOM. Third, farmers reported a suite of management practices to address SOM, which were largely chosen based on cost. Finally, farmers connected soil test results to management strategies and expressed future interest in soil health testing.

Every farmer explicitly stated the importance of SOM. For example, farmer No. 5, said, "I mean your organic matter is one of the most important things in my opinion." Other farmers described how SOM is related to other important soil properties; for example, farmer No. 6 stated,

> The big thing is to get that organic matter up, because then, especially my soil, if you can get that organic matter up, then you could retain the moisture, then you can work with the fertilizers, then you got everything going for you, except the sunlight. And them soils, they'll produce, I've seen them produce, just as good as anything else, but everything's got to be right.

The importance of SOM was further illustrated when farmers described the time and effort needed to increase SOM. Farmer No. 2 explained, "it's not an overnight fix on that ground, we fixed up a lot of ground that has not been into farming over the years and we tried to build that ground up." All farmers voiced frustrations with building SOM. At least two farmers stated that the SOM test results would not change in their lifetime. For example, Farmer No. 2 said, "I won't live to see [SOM] change." Others mentioned frustrations in building SOM over time. Farmer No. 1 stated,

> I pulled some of my tests in 2006 and 2000 and you look down through there, some of the [SOM] are higher, some are lower and when you get down, I don't know—am I gaining, am I loosing, I don't know, you know?

Despite frustrations with building SOM, farmers described continuous efforts to improve SOM. For instance, farmer No. 12 explained, "I don't know how to fix it, I tried actually. What I've been doing, I'm trying to build organic matter, maybe I'm doing it the wrong way. It's been corn for 15 years straight." Finally, the majority of farmers mentioned that SOM was one of the first indicators they looked for when receiving soil test results. Farmer No. 9 stated, "typically I'm looking for how much phosphorous and potash we're moving, and to see is my organic matter moving in the right direction."

Motivations for building SOM

Farmers offered different reasons for desiring greater SOM levels on their fields. Most farmers associated SOM with high yields. For example, farmer No. 5 stated, "I mean organic matter is very important in growing the crop." However, nearly 50% went further and mentioned that it was part of their job. For example Farmer No. 6 stated, "you're supposed to take care of the land, that's what you're taught, especially a farmer." In addition, over half of the farmers explained that farm success was dependent on healthy soils. Farmer No. 7 explains,

> I want to get better at this…I learned a long time ago it's not what I'm growing above the ground, it's what I got going on below the ground. I mean, this is my future…so I'm trying to get better with the soil here.

Management practices used to build SOM

The farmers in this study utilize a wide variety of management practices to address SOM (Table 6.7). Every farmer incorporated crop rotations into their farm operation. Several farmers noted that adding wheat, alfalfa, or oats into a rotation helped to build SOM or humus. Farmers often qualified this thought by mentioning the root systems of rotational crops, for example, "[alfalfa] has a lot more roots there to hold everything" (Farmer No.11). Farmer No. 6 stated, "when you're harvesting [corn], it looks like [a] desert, just sand…but that wheat, I think, you got that root structure and you get all that straw back into that ground."

Cover crops were the second most common practice used to build SOM, but also posed the greatest challenges for farmers. Farmer No. 7 mentioned that cover crops brought his SOM up from 0.8%. Farmer No. 13 had used cover crops in the past and had great expectations for cover crops during the upcoming growing season: "I want to see what these cover crops are going to do, because we can stop burning up the carbon, we can start sequestering, and get those

numbers up a little bit." Two farmers expressed frustrations with cover cropping, for example, Farmer No. 2 said, "I remember when we had a radish pea [it] didn't amount to nothing…. it won't be used again, I know that." This experience however, did not discourage farmer No. 2 from growing cover crops as he went on to explain that he would try a different type of radish cover in the upcoming year. When I asked what his expectation for the radish cover was, he said, "hopefully we gain some soil tilth and water holding [capacity]." Farmer No. 3 said he no longer used cover crops, "when we planted wheat, we used to put clover in the spring…but we don't do that no more because that got kind of pricey."

Sixty-three percent of the farmers applied manure to increase SOM, but the amount and availability of manure varied for each farm. For example, some farms had cattle as part of their operation and had excess manure that was applied to almost every field: "if you've got cattle you can make some major differences on organic matter in the matter of ten to fifteen years" (Farmer No. 5). In contrast, other farmers only had enough manure to add it to problem areas. Farmer No. 3 explained,

> If I could just get that sand[y] [spot] to grow something but I am [not getting crops to grow there]; it seems like I got cow manure from farmer No. 2 one year, spread it on [the sandy spot] to see if that would bring [SOM] back….the spot got smaller; [SOM] will raise some.

Some farmers mentioned that there are trade-offs associated with manure application, especially in terms of cost and compaction. As farmer No. 4 explained, "I mean, I am gonna pay somebody to truck [manure] over there and they're gonna pound the heck out of [the ground], getting [manure] on there." Other farmers were concerned with increased phosphorus levels.

Nearly half the farmers in this study incorporated perennial crops such as alfalfa in their operation. The length of time that alfalfa was left to grow ranged from 4 to 12 years. Several farmers mentioned that the deep roots were the main benefit of the crop. For example, farmer No. 7 said, "[alfalfa] is chock full of roots…to me, that's what I want…I want them roots decaying." Other farmers described using alfalfa to revitalize certain fields. For example, Farmer No. 6 described how he has used alfalfa to improve soil quality over time,

> I had some fields that just wouldn't grow nothing… there were spots, a couple of acres… I left that alfalfa out there, we sold the hay off it, I don't know, three, four years and them spots aren't there no more.

Forty-six percent of farmers actively used no-till as a strategy to increase SOM. Farmer No. 13 explained that building SOM was the main reason why he switched to no-till, "if we can no-till, we can build up that organic matter."

Only 30% of the farmers mentioned residue management as part of their approach for building SOM. Farmer No. 7 was very adamant about keeping residues on certain fields, especially after wheat harvest,

> You got guys that come along to buy straw. I won't sell the straw, I want to put that straw back into the field… when we started raising that wheat, I think that was the biggest change [in SOM], the biggest help.

Farmer No. 1 mentioned that he uses residue management as a strategy because it's easier than crop rotations or changing tillage practices.

The least common approach for building SOM was utilizing probiotics and amendments such as Sumagrow® and gypsum. Farmer No. 5 explained, "I mean your organic matter is one of the most important things...and that's why we tried this Sumagrow®, is it's your biological activity… that's what breaks all this stuff down so it's useable." The farmer further explained that

they were only adding Sumagrow® to one field initially, “we are going to continue with those spots and see what happens…most of our ground is in pretty good shape I feel… our ground has pretty big pools… we don't want to destroy that [with] somebody's snake oil.” Farmer No. 4 described a particular field where both soil quality and yield were down. He explained that he spent a large amount of money on gypsum in hopes of turning that field around,

> Gypsum…it makes that soil bond…[salesmen] want you to do it annually, that’s not a let’s put it on and see what it does; it’s a put it on and it’s gonna be a couple years before you even know if it works….but you’re several thousand dollars in debt before that happens.

Linking soil tests to management and expressed interest in soil health testing

The final theme that emerged is that farmers mainly had a positive view of active C and its ability to aid in understanding SOM trends on their fields. An extension of this theme was that farmers raised important questions about active C and gave critical feedback that will be crucial for making soil health testing even more applicable in the future.

When viewing the active C and total SOM results side by side, farmers immediately comprehended that the two tests were illustrating different trends across the fields. For example, farmer No. 12 said of active C, “it's an eye opening…it’s a different way to look at it.” Other farmers were genuinely shocked by the results, for example the farmer who added gypsum to his field (Farmer No. 4) was surprised when he saw significantly lower C fluxes compared to the other fields, “in the tests that we have, you know like you could look at this booklet that I got right here and you have [total SOM results] right here, [SOM] doesn’t look like it’s a problem”, he goes on to say,

> I’ve thought about putting more organic matter, matter of fact, I’ve thought about trying to find somebody that puts [manure] on this field… but then I go back and

> look at this, like this number [total SOM] we weren't looking at that number [active C]. Looking at this [total SOM], you'd say why would I do that…

This farmer is frustrated because his problematic field (Choice field) and Best field have equal total SOM values. However, results from the active C test reveal that active C values are much lower in the Choice field compared to the Best field. Past total SOM test results have stopped him from adding manure, as he states, "it's like what am I going to benefit from [adding manure], right?" Instead, his approach has been to invest a large sum of money into gypsum application.

Several farmers had questions about the active C test. More than one farmer asked, "What is the average value?" Or "What's the county average?" Or " How does my C flux compare to the other farms?" Farmer No. 1 questioned how active C could be useful if it varies, "that's what we need is some sort of a stable number, where as this [active C] you know, can move up and down too much." Other farmers wanted to know how they could raise active C rates in the Worst field to be on par with the Best field.

At the end of each meeting, farmers were asked about the value of active C and soil health testing and if being a participant in the study was useful. All farmers expressed future interest in soil health testing. Famer No. 12 explained, "It's neat. I'm glad that I got involved and I think it's going to help us." In a similar sentiment, farmer No. 6 stated, "I think the more information that we all can get, it's something that we all need to improve the soils and to make it better for the next generation." Some farmers expressed frustration that active C and other soil health tests are not widely available. For example, Famer No. 7 exclaimed, "this is not only my opinion, but other growers, this is where MSU gets kicked in the you know what…"

The real validation that farmers were interested in soil health testing is that twelve out of the thirteen farmers asked, "Are you coming back to sample again next spring?"

DISCUSSION

SOM is the most common indicator that farmers use to gauge soil health (Granastein and Bezdicek, 1992; Gruver and Weil, 2007). In this study, I combined quantitative field based research with field observations and meetings with farmers to determine if two soil C indicators (total SOM and active C) were able to detect differences amongst farmer fields and reflect farmer perceptions of SOM. The active C test proved to be more effective at detecting differences across farmer fields compared to the total SOM test. Furthermore, there were substantial differences in active C between the Best and Worst fields and more variation in active C between the different paired fields (Table 6.2). Active C also corresponded better to investigator field observations and farmer perceptions of soil health than did total SOM. Active C is a more sensitive test that reflects farmer experiences with yield and soil health and should be commercially available at soil testing facilities.

Active C vs. SOM test results

Variability in active C between the different paired fields was substantially greater than the variability in SOM, which indicates that the active C test was more capable of detecting differences among farmer fields. In particular, I found significant differences in active C between the Best and Worst fields, but found no significant differences in SOM. These findings concur with Culman et al. (2013) who also found significant differences in active C amongst different rotational crops and management practices but not in total soil C. Our findings contrast with Gruver and Weil (2007), however, who detected significant differences in total C between farmer chosen fields varying in soil quality. One explanation for this difference could be that Gruver and Weil (2007) used a combustion analyzer to detect total C (Islam and Weil, 1998),

which is more sensitive than the loss on ignition method that was conducted at the MSU Plant and Soil testing laboratory (Abella and Zimmer, 2007).

Neither the active C test nor the total SOM test found differences between the Best and Choice and the Worst and Choice fields (Table 6.2). This is likely because the Choice fields were more intermediate in performance as noted by farmer descriptions and reported in investigator field notes (Tables 6.4 and 6.6). For example, seven farmers classified the Choice field as a problematic field, four farmers characterized it as a better performing field, and two farmers classified the Choice field as an average field (Table 6.6). The wide range of performance amongst the different Choice fields is reflected in the higher SEs, CVs and insignificant t-test results between Choice and Best and Choice and Worst field comparisons (Table 6.2).

Do active C test results support field observations and farmer perceptions?

Results from the text summary analysis demonstrates that the active C test strongly supports field observations and farmer perceptions of soil health, especially when deciphering between the Best and Worst fields. In contrast, total SOM values were statistically similar across farmer fields and therefore did not support investigator field observations or farmer perceptions of SOM. Gruver and Weil (2007) also show that soil C indicators strongly correlate with farmer perceptions of soil quality, however, in contrast to this study, they found that total C had just as strong a correlation with farmer perceptions as other labile soil C indicators. In this study, the lack of distinction between the Worst and Choice fields in field observations and farmer experience was also reflected in the active C test results, where results between the two fields were statistically similar (Table 6.2).

Every farmer had previously submitted soil for total SOM testing either through MSU or a commercial laboratory. In addition, all farmers expressed knowledge of the importance of SOM and nearly half mentioned that SOM was the first indicator that they examined when receiving soil test results back. Farmers mentioned that SOM results were used to guide inorganic and manure fertilizer application and other important management decisions. Furthermore, over half the farmers mentioned that managing for SOM had important implications for the future of their farms. This sentiment is not unique to this study, as Kimble (2007) found that farmers across the United States are concerned about the environment and strive to improve soil health for the next generation of farmers. Furthermore, these findings illustrate that farmers are utilizing a wide range of management practices that the total SOM test failed to detect. For example, the total SOM test often did not pick up differences between fields receiving heavy amounts of manure and fields that had not received manure in over twenty years. Given the level of importance that farmers place on SOM, it is problematic that the total SOM test results did not correlate with farmer perceptions of soil health. Meetings with farmers demonstrated that in some cases the lack of correlation between test results and farmer perception hinders appropriate management practices.

Bridging the gap between scientific testing and farmer knowledge

Farmer involvement in this study led to new understandings regarding the relationship between soil quality and soil testing. For example, an important theme that emerged from the meetings is that farmers recognized the discrepancies between their perceptions and experiences of soil health and total SOM test results. Furthermore, farmers voiced dissatisfaction that soil health tests like active C are not commercially available. The disconnect between total SOM test results and farmer perceptions illustrates a consistent problem that occurs when scientific

assessment contrasts with farmer knowledge (Barrera-Bassols and Zinck, 2003). Ethnopedologists, who study and document farmer perceptions of soils and approaches to management, argue that farmer knowledge needs to be reflected in basic soil science research (Lamarque et al., 2008). Farmers have a wealth of knowledge regarding the physical, chemical, and biological aspects of their soils, but this vast knowledge is rarely incorporated in agricultural research (McCallister et al., 1999). Ethnopedologists have made gains in linking farmer soil descriptions with soil surveys and classification, especially in indigenous communities; however, more farmer knowledge needs to be incorporated in soil fertility research worldwide (Barrera-Bassols and Zinck, 2003). The active C test is an attractive example of a scientific tool that can detect short-term changes in management that are undetectable by total SOM and also reflects farmer perceptions of SOM.

Creating a stronger link between farmer perceptions of SOM and soil testing could help farmers make more informed decisions on management that could lead to economic and environmental benefits. For instance, farmers in this study invested a large amount of time and money in a variety of management practices in hopes of increasing SOM. In certain cases, the total SOM test hindered farmers from adopting more economically viable practices. In addition, the active C test can be an important indicator of long-term soil C dynamics as well as agronomic performance (Culman, 2013). From an environmental standpoint, scientists and policymakers continuously encourage farmers to adopt best management practices for C sequestration on-farm to offset CO_2 emissions from agricultural systems (Jarecki and Lal, 2011). Farmers will be more likely to meet target C sequestration goals if active C or other tests that are sensitive to changes in management are more widely available.

Future directions

While the active C test results can better reflect farmer perceptions compared to total SOM, soil scientists need to work with extension educators to make active C more interpretable before it can be useful to farmers. During farmer meetings, farmers mentioned that active C was difficult to follow because of its dynamic nature in comparison to total SOM. This critique is important because other studies have illustrated that active C can change within a given growing season based on crop growth and fertilizer application (Culman et al., 2013). If samples are taken at different points during the growing season, it could be difficult to make informative comparisons from year to year. Thus, farmers should test for active C either in the spring before planting or in the fall after harvest. This recommendation is similar to with the Cornell soil health lab sampling instructions, where farmers are encouraged to sample once in late fall (http://soilhealth.cals.cornell.edu/extension/test.htm#when). Other farmers asked what the average active C rates were for the county, across different soil types, and in different cropping systems. These types of aggregated results are not yet known for active C and will require further research. Overall, this study shows that farmers see value in the active C test along with other soil health indicators and are interested in using soil health testing in the future. Finally, future research should explore how the active C test can be used to inform soil management plans and how to make the active C test more available and understandable to farmers.

CONCLUSIONS

Farmers depend on soil testing to make important management decisions that have consequences at both the local and global scales. Collecting and submitting samples requires time and money and therefore should reflect farmer perceptions of SOM and changes in

management. These findings demonstrate that total SOM, the principle soil C indicator used by farmers in the United States, is ineffective at separating best performing and worst performing fields. In contrast, I found that the active C test reflects significant differences across farmer fields and corroborated with investigator field observations and farmer perceptions. The qualitative analysis in this study revealed that every participant farmer was actively trying to maintain or increase SOM through a variety of different management practices. In addition, farmers voiced frustration with the time required to build SOM. Even worse, some farmers were refraining from incorporating sustainable management practices because total SOM tests did not accurately reflect the health of their soils. Active C can serve as a powerful tool for farmers that use SOM measures to make important management decisions and should therefore be widely offered at both university and commercial soil testing laboratories.

APPENDIX

Table 6.1 Type and scale of participating farms in Michigan.

Farm	Michigan State County	Farm size (hectare)	Crops grown
No. 1	Isabella	364	Corn, Soy, Wheat
No. 2	Isabella	526	Corn, Soybeans, Oats
No. 3	Isabella	324	Corn, Soy, Wheat,
No. 4	Isabella	526	Corn, Soy, Alfalfa
No. 5	Isabella	607	Corn, Soybeans, Wheat, Alfalfa
No. 6	Presque Isle	789	Corn, Soybeans, Wheat
No. 7	Presque Isle	304	Corn, Soybeans, Oats, Alfalfa
No. 8	Presque Isle	809	Corn and Soybeans
No. 9	Presque Isle	32	Strawberry
No. 10	Van Buren	202	Corn and Alfalfa
No. 11	Van Buren	486	Corn, Soybeans, Alfalfa
No. 12	Van Buren	2023	Corn and Soybeans
No. 13	Van Buren	202	Corn, Soybeans, Wheat

Table 6.2 Mean total SOM and active C for all fields and paired t-tests and mean CVs for field comparison across 13 farms in Michigan.

Fields	Total SOM	Active C		Field Comparisons	Total SOM	Active C	Total SOM	Active C
	g SOM kg^{-1}	µg C g^{-1} soil day^{-1}			t-test	t-test	Coefficient of Variation	Coefficient of Variation
Best	38.6 ± 0.1	43.0 ± 0.3		Best vs. Worst	2.8	5.8**	0.05 ± 0.01	0.30 ± 0.03
Worst	37.0 ± 0.1	29.1 ± 0.2		Best vs. Choice	0.8	1.9	0.10 ± 0.02	0.43 ± 0.04
Choice	36.5 ± 0.3	30.8 ± 0.5		Worst vs. Choice	0.4	-0.3	0.11 ± 0.02	0.25 ± 0.03
NRC	47.0 ± 0.3	40.3 ± 0.4		Best vs. NRC	-2.2*	0.8	0.20 ± 0.03	0.24 ± 0.03
				Worst vs. NRC	-2.3*	-2.8**	0.20 ± 0.03	0.31 ± 0.04
P<0.05=*, P<0.01=**				Choice vs. NRC	-2.2*	-1.4	0.21 ± 0.03	0.40 ± 0.04

Table 6.3 Penetrometer resistance (psi) in four fields from each farm (means ± SE). Instances where areas in the field exceeded the maximum resistance, values are preceded by '>', which denotes an underestimated psi value.

	Best Field Depth		Worst Field Depth		Choice Field Depth		Non-Row Crop Field Depth	
Farm	0-15 cm	15-46 cm	0-15 cm	15-46 cm	0-15 cm	15-46 cm	0-15 cm	15-46 cm
	Resistance (psi)		Resistance (psi)		Resistance (psi)		Resistance (psi)	
No. 1	12.2 (0.9)	50.3 (2.1)	19.3 (2.9)	64.2 (4.9)	18.2 (1.2)	58.5 (4.7)	27.0 (2.4)	60.0 (4.1)
No. 2	19.0 (1.6)	43.8 (2.3)	19.0 (2.3)	74.5 (2.4)	19.2 (2.3)	37.1 (1.6)	15.9 (1.5)	40.8 (3.3)
No. 3	18.2 (0.7)	42.0 (2.9)	7.2 (1.9)	37.0 (4.0)	23.0 (2.7)	55.5 (4.1)	8.5 (0.8)	27.5 (1.3)
No. 4	16.9 (0.8)	56.1 (3.4)	22.5 (1.7)	56.5 (5.9)	18.0 (1.9)	59.1 (2.6)	12.7 (1.2)	34.7 (2.6)
No. 5	13.3 (1.3)	53.5 (3.7)	27.5 (2.8)	64.5 (2.5)	53.3 (3.0)	69.0 (2.8)	36.0 (2.3)	74.5 (3.2)
No. 6	26.1 (3.1)	59.8 (5.8)	24.5 (2.7)	55.5 (4.4)	16.6 (2.8)	65.8 (4.7)	< 95.0 (5.0)	< 100.0 (0.0)
No. 7	43.5 (1.5)	82.5 (4.8)	30.1 (1.8)	95.0 (4.0)	79.5 (5.2)	< 99.0 (1.0)	48.3 (2.7)	< 87.0 (5.9)
No. 8	13.3 (2.4)	70.5 (5.5)	52.5 (2.1)	85.8 (3.9)	43.5 (1.9)	< 87.8 (3.9)	40.5 (2.7)	< 83.3 (5.5)
No. 9	68.0 (11.4)	< 100.0 (0)	< 95.6 (4.4)	47.0 (3.7)	< 96.0 (2.2)	43.5 (3.9)	51.0 (9.4)	< 100.0 (0)
No. 10	13.5 (3.2)	43.8 (3.4)	18.2 (2.9)	< 66.0 (5.1)	33.0 (1.5)	59.0 (4.5)	35.0 (2.8)	< 64.5 (6.5)
No. 11	19.3 (1.9)	<62.5 (6.8)	22.4 (2.4)	67.5 (4.4)	29.5 (3.4)	56.5 (2.9)	30.0 (3.3)	84.5 (2.9)
No. 12	6.0 (2.5)	52.9 (5.3)	26.2 (2.3)	47.3 (3.8)	26.0 (3.4)	52.2 (5.6)	38.5 (3.7)	< 54.5 (2.9)
No. 13	15.9 (2.9)	43.5 (2.8)	26.5 (3.7)	47.0 (3.7)	19.0 (1.6)	43.5 (3.9)	12.3 (1.2)	26.4 (1.9)
Average	21.9 (4.8)	58.6 (4.7)	30.1 (6.1)	62.1 (4.6)	36.5 (7.0)	60.5 (4.8)	34.7 (6.3)	64.4 (7.3)

Table 6.4 Investigator field observation descriptions of farmers' Best, Worst, and Choice Fields in addition to C flux trends in Isabella, Presque Isle and Van Buren Counties.

Fields		
Best	Worst	Choice
• Darker in color (7 Farms) • Good soil structure (6 farms) • Earthworm activity (5 farms) • Poor soil structure (2 Farms)	• Poor soil structure (8 Farms) • Sandier ground (5 Farms) • Evidence of drainage issues; oxidation (4 Farms) • Crusted surface (3 Farms • Pale in color (2 Farms)	• Earthworm activity (5 Farms) • Good soil structure (4 Farms) • Evidence of drainage issues; oxidation (3 Farms) • Adequate soil structure (2 Farms) • Poor soil structure (2 Farms)

Table 6.5. Summary and frequency of farmer field descriptions for Best and Worst Fields

Fields	Field descriptions
Best	• High yielding (11 /13 Farmers) • Good soil structure (7/13 Farmers) • Receives Manure (5/13 Farmers) • Higher soil organic matter (3/13 Farmers) • No disease presence (2/13 Farmers) • Good drainage (2/13 Farmers)
Worst	• Low soil organic matter (5 /13 Farmers) • Poor soil structure or health (11/13 Farmers) • Low Yielding (7/13 Farmers) • Badly managed in the past (4/13 Farmers) • Disease (1/13 Farmers)

Table 6.6. Choice field farmer descriptions separated by performance (Good performing field, Intermediate field, Problematic field) as described by farmer across 13 farms.

Good Performing Field	Intermediate Field	Problematic Field
4 Farmers • Higher yields • Good soil structure and friendly to till • Reliable field	**2 Farmers** • High pH that locks up fertilizer • Sandier areas	**7 Farmers** • Lower yields • Compaction problems • Low soil organic matter • Drainage issues

Table 6.7 Management practice thematic categories and selected examples of approaches that farmers use to build soil organic matter.

Crop Rotation	**Cover crop**	**Manure**	**Perennials**	**No-till**	**Residue Management**	**Products (Amendments)**
(13 Farmers) I got a lot more organic matter …a lot more roots there to hold everything. I'm sure there's a lot more earthworms... (No. 11)	(9 Farmers) I want to see what these cover crops are going to do, because we can stop burning up the C, we can start sequestering, and get those numbers up a little bit (13).	(7 Farmers) I get a little manure from a neighbor… typically looking to build on all the organic matter…(No. 2)	(6 Farmers) I planted… alfalfa into it. And I left it almost four years the soil starting to coming around. It's chock full of roots. To me, that's what I want…I want them roots decaying…(No.7)	(6 Farmers) We switched to no-till, [so] we can build up that organic matter (farm 13).	(4 Farmers) You got guys that come along to buy straw. I won't sell the straw, I want to put that straw back into the field. (No.6)	(2 Farmers) I mean organic matter is very important in growing the crop...And that's why we tried this summagrow is it's your biological activity…(farmer No.5)

Table 6.8 Investigator field observation descriptions of farmers' Best, Worst, and Choice Fields in addition to C flux trends in Isabella, Presque Isle and Van Buren Counties.

Isabella County				
Farm	Best Field	Worst Field	Choice Field	Active C (C mineralization) trends
No. 1	Cloddy, crusted, poor soil structure and darker in color.	Structure is poor and lighter in color compared to Best field.	Clear drainage problems (high levels of mottling).	Best>Choice>Worst I noted differences in soil color between Best and Worst fields, which often reflects differences in SOM. Active C rates were substantially higher in the Best field and lowest in the Worst. The active C test results supported my field observations.
No. 2	Earthworm activity, evidence of mycorrhizae, good soil structure and dark in color.	Evidence of earthworm activity, poor soil structure, crusted at surface, pale soil color.	Earthworm activity and better structure than worst field.	Choice>Best>Worst I noted that the Choice and Best field had better soil structure than the Worst. The Choice field had slightly higher fluxes compared to the Best field. The Worst field had poor soil structure and was lighter in color, which is reflected in active C.

Table 6.8 (cont'd)

No. 3	Earthworm activity, dark top soil, and good structure.	Sandy, windswept, and burnt parts of field, rocky surface.	Topography, poor structure, oxidation and potential drainage problems.	Best>Worst>Choice I observed large differences in soil structure, color, compaction, and texture amongst the three fields. I found evidence for poor soil structure in the Choice and Worst fields. The active C test results strongly supported my field observations.
No.4	No-till field, soil structure is excellent and dark in color. A large amount of residue on field, evidence of earthworm activity.	Field varies in quality of soil, compacted, evidence of oxidation and drainage issues, decent structure.	Earthworms, crusty surface, adequate soil structure.	Best>Worst>Choice Differences in soil quality and structure were noted across the three fields. The Best field had better physical structure and darker topsoil compared to the Worst and Choice fields. My observations did not reflect the large differences found in the Worst and Choice field.

Table 6.8 (cont'd)

No.5	Crusty at surface, dark in color, sandier areas where soil doesn't hold structure as well.	Surface crusting, weedy, topography, and drainage issues.	Compacted, poor structure, high in clay content	Choice>Best>Worst My observation of dark topsoil in the Best field was reflected in the active C test, where the Best field had greater C fluxes compared to the Worst field. My observations did not correspond with the large C flux found in the Choice field, which could be caused from the established wheat system or the Sumagrow® that was added.

Table 6.8 (cont'd).

Presque Isle				
Farm	Best Field	Worst Field	Choice Field	Active C (C mineralization) trends
No. 6	No-till field, residue, dark in color but sandier soil	Hilly field, oxidation and drainage problems, sandy soil with several rocks	Sandier than Best field, poor drainage, adequate structure	Best>Worst>Choice I observed that the Best field had soils that were darker in color, which often reflects greater soil organic matter and was supported by the active C test results. There were no note-worthy differences in the Worst and Choice field observations. However, the test found that C fluxes in the Worst were greater than in the Choice.

Table 6.8 (cont'd)

No. 7	Soil is dark and rich in color, has good structure, rocky in some areas, and minimal oxidation.	Evidence of earthworms, compacted, dark soil	Well aggregated, compacted, adequate structure, and soils darker in color.	Best>Worst=Choice I noted that soils were darker in color with good structure in the Best field. I noted similar characteristics in the Worst and Choice fields, but they were both more compacted. My observations aligned with the active C test results.
No. 8	Earthworm activity and good soil structure	Cracked dry surface with a large amount of weeds, rocky. Past 3 cm clay content seems higher and more compacted.	Crusted surface, darker soil and good structure, oxidation evidence for drainage problems.	Best>Worst=Choice My observations noted that the Best field had good soil structure and earthworm activity, while, both the Worst and Choice fields had cracked surfaces that had compaction issues. My observations were supported by the active C test.

Table 6.8 (cont'd)

No. 9	Evidence of earthworms, good soil structure, less compaction than other fields	Extremely compacted, small amounts of oxidation and evidence of drainage problems	Earthworm activity, sandier soil, good soil structure, compaction	Best>Worst>Choice I noted that both the Best and Choice fields had good soil structure and evidence of earthworm activity, which is often associated with greater SOM. I also observed surface crusting in the Worst and Choice fields. Overall, my observations aligned with the active C results, especially between the Best and Worst fields.

Table 6.8 (cont'd)

Van Buren				
Farm	Best Field	Worst Field	Choice Field	Active C (C mineralization) trends
No. 10	Sandy loam, Clear evidence of a rye cover crop, poorer soil structure	Sloped field, poorer soil structure, some corn residue	Wetter soil, soils darker in color, good aggregation	Best>Choice>Worst I observed darker soils with better aggregation in the Choice field compared to the Best field. However, C fluxes were greater in the Best field. My observation of poor soil structure in the Worst field align with active C test results because the Worst field had the lowest C flux.
No. 11	Higher in clay, strong soil structure, high in residue.	Poor soil structure, earthworm activity, sandier soils, some areas were darker with more clay, residue on field.	Sandy but good soil structure, earthworm activity, and corn residue.	Worst=Best=Choice C fluxes were extremely even across the three fields. The only noteworthy differences in my observation amongst the fields, was the poor soil structure in the Worst field. All fields were no-till with plenty of residue left on field. I found no difference in active C across the three fields.

Table 6.8 (cont'd)

No. 12	Sandy loam, good aggregation, darker in color	Extremely sandy, some areas appeared to be nitrogen stressed, earthworm activity	Field extremely variable, some areas dominated by clay others by sand and gravel.	Best>Choice>Worst The Worst and Choice fields were sandier and more variable compared to the Best field. The active C results supported my observations of the large differences in soil quality in the Best field compared to the Worst and Choice fields.
No. 13	No-till, sandy but good soil structure, corn residue	Poor soil structure and extremely sandy	No-till, dark sandy soil, a few grubs, lots of corn residue	Best>Worst>Choice I noted good soil structure in the Best field and poor soil structure in the Worst field, which corresponds with the active C results. However, I only noted positive characteristics in the Choice field, which ended up having the lowest active C rates.

Table 6.9 Farmer descriptions and experiences of Best, Worst, and Choice fields and C flux trends in Isabella County.

Isabella County				
Farmer	Best Field	Worst Field	Choice Field	Active C (C mineralization) result trends
No. 1	"Yield and the way the dirt works… it's much more mallow than say the other ones"	I think it's heavier soil than the other one and it doesn't crumble…it just doesn't work nearly as nice as the other one. SOM not as high as the [best] field"	"Wetter for certain… more loamy and heavier…pretty good in production"	Best>Choice>Worst The Worst field had the lowest C fluxes and clearly detected the lower soil organic matter described by the farmer.
No. 2	"That has been manured pretty regular… and "high yielding"	"Typically lower yielder and poorer soil structure…a never manured field"	"It's fairly friendly to till it's coarse enough textured… but yet it seems to hold water"	Choice>Best>Worst The active C test detected the differences in management practices between the Choice and Worst fields.
No.3	"That's just my best producing field…it's not light soil and it's not heavy soil, it's in-between soil."	"I call it the bad field because there's a sandy ridge that was growing nothing"	"Works up really good… heavy dirt, chunky"	Best>Worst>Choice The Choice field had the lowest active C rate; it could be that I did not sample the sandy ridge described by farmer. However, active C supported farmer perceptions of differences between Best and Worst fields.
No. 4	"Production... the soil structure, we've been applying manure…over the last several years"	"I just don't get the yield… it tends to crust over in certain spots…some of it is sandier"	"damp soil—it's really nice soil, as soon as it dries out, you can play basketball on it".	Best>Worst>Choice The test detected differences between Best and Worst and detected lower C fluxes, possibly due to field compaction described by the farmer.

Table 6.9 (cont'd)

No. 5	"Loamier ground…and that's where the yields are really high and gets manure"	"One of our lowest yielding fields…compaction…there is something in that ground and I don't know what it is"	"Heavier ground…added sumagrow, it's supposed help with the biological"	Choice>Best>Worst The extremely high flux in the Choice field could be due to the Sumagrow, which is designed to make C and N more available. In addition, the test detected differences in soil quality between Best and Worst fields, described by the farmer.

Table 6.9 (cont'd).

Presque Isle County				
Farmer	Best Field	Worst Field	Choice Field	Active C (C mineralization) result trends
No. 6	"That's pretty much where all the manure got hauled for 25 years" and "high yields"	"I just can't get the fertility balanced to where I want it" and "I've had some pretty poor yields…compared to what we have in the neighborhood there"	"That one's got the variations of rolling hills" • Problems include lower yields and water holding capacity due to sandy area	Best>Worst>Choice Both the Worst and Choice fields had problematic characteristics that caused lower active C rates. Thus, the active C test corroborated with farmer perceptions of soil health for each field.
No. 7	"Better organic matter…and higher earthworm population…and…I have less disease pressure, it's always higher in yield"	"soil health [reduced]…due to the farming practices prior to me taking over…it was heavily tilled and over-fertilized"	"low soil organic matter"	Best>Worst=Choice The active C test detected the greater organic matter described by the farmer in the Best field and reflected the reduced soil health and lower SOM in the Worst and Choice fields.

Table 6.9 (cont'd).

No. 8	Higher yields, higher SOM due to years of cattle grazing	"We didn't have the yields that we thought we were going to have…and I am surprised that the soil organic matter is that high [farmer brought tests from previous years]."	"High pH…it's locking up our fertilizers"	Best>Worst=Choice This farmer brought total SOM test results from previous years and was surprised that the total SOM was as high as it was in the Worst field because his yields have been lower then expected. The active C test detected a large difference between the Best and Worst fields and corroborated with farmer perceptions of SOM.
No.9	"I haven't had any incidents of black root rot in the strawberries."	"Black root, compaction, and wet soil"	"Compaction…water laying in between the rows"	Best>Worst>Choice Important characteristics for a good field, as described by the farmer included no incidence of disease, while problematic fields were classified as compacted. While not always correlated, the higher active C in the Best field could be a reflection of an overall healthier system (no disease) compared to the Worst and Choice fields.

Table 6.9 (cont'd)

Van Buren County				
Farmer	Best Field	Worst Field	Choice Field	Soil health (C flux) result trends
No. 10	"It's the field that I've had the longest and it's a consistent producer".	"Yields were down, organic matter is down".	Compaction and drainage issues	Best>Choice>Worst The C mineralization results aligned with farmer perceptions of lower SOM in the Worst field, as active C rates were lowest in the Worst field.
No. 11	"It's well-drained. It's got a variety of soils in it - clay, a little bit of muck on the creek side".	"Well, that's kind of hilly, for one, not flat like the rest of them." and "there's hardly any clay".	"Sandy loam….adds manure to sandiest spots"	Worst=Best=Choice The farmer did not have major problems across the three fields. The differences that he noted were mainly in regards to soil texture. Here our test did not detect any difference across the three fields, which in many ways reflects the farmer's perceptions in terms of differences in soil C.

Table 6.9 (cont'd)

No. 12	"a good yielding field…good water holding capacity, good mellow flow"	"It's just really poor. It wasn't taken care of". Problems include: Sandy texture, soil fertility and difficulties increasing soil organic matter	Due to road construction in the 1950s, the field has lost top soil. "they leveled all the field off, they didn't put the topsoil back on". "I don't know how to fix it…I'm trying to build SOM".	Best>Choice>Worst The farmer mentioned that it was difficult to build soil C in the Worst and Choice fields. Our test detected large differences between the Best and Worst field, which closely aligns with the farmer's experience.
No. 13	"It's the history with this field and the management…banking micronutrients" and "The yields are always the one to watch, it's a field I can always count on".	"Bought [field] not that long ago and it was beaten death" "Corn, soy, and wheat you can count on a 10-20% hit" "the soil organic matter levels are problematic"	"Basically it's decent, it's always been a good field"	Best>Worst>Choice The farmer mentioned lower SOM for the Worst field, which was reflected in the active C test. The Choice field had even lower active C than the Worst field, which didn't align with the farmer perceptions of SOM. The active C test corroborated with farmer perceptions Best field.

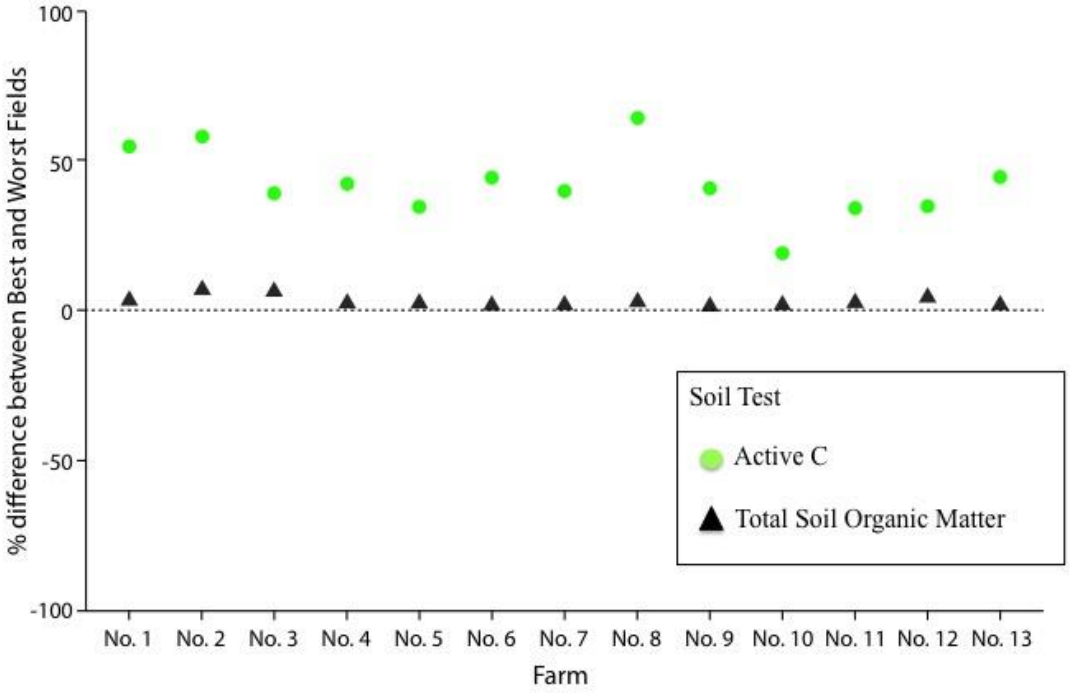

Figure 6.1 Percent difference for Total SOM and C mineralization between Best and Worst fields across 13 farmer field in Michigan.

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