INTERACTIVE EFFECTS OF COVER CROPS AND TOPOGRAPHY ON SOIL ORGANIC CARBON AND MINERAL NITROGEN

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Crop and Soil Sciences - Doctor of Philosophy

2015

ABSTRACT

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Including winter cover crops in agricultural rotations has shown a great potential to enhance soil carbon (C) content and to supply nitrogen (N) for subsequent main crops. However, the benefits of cover crops have been mostly examined in small-scale research plots and the actual performance of cover crops in farmer practiced agricultural fields is not well understood. Large spatial variations in performance of cover crops and soil properties in agricultural fields cause uncertainty about the cover crop-induced benefits. My overall goal in this study was to analyze the variations in cover crop performance across topographically diverse agricultural fields and to assess how those variations control cover crop influences on soil organic C and mineral N.

In chapter 2 I used power analysis to assess how useful the total organic carbon (TOC), particulate organic carbon (POC) and short-term mineralizable carbon (SMC) can be in predicting changes induced by cover crop management. I collected soil samples from ten fields under management with and without cover crop and analyzed them for TOC and for two active C pool characteristics, such as POC and SMC. The results showed that to be able to detect differences between the management systems with an acceptable type II error of 0.20 an 80% difference in TOC and a 50% difference in SMC were needed. The statistical power for POC was never in an acceptable range. I recommend SMC as the preferred C fraction for detecting differences between treatment induced changes in C processes in agricultural field experiments.

In chapter 3, I, first, assessed the spatial distribution of TOC, POC and SMC across 20 fields under conventional, low-input, and organic managements, where the latter two contained cover crops in their rotation. The results showed that POC was significantly lower on topographical slopes of fields under conventional agricultural management compared to managements with cover crops. Then, I monitored the specific contribution of cover crops to POC and SMC across topography during 2011, 2012 and 2013. I established microplots with and without cover cops on three topographical positions of "depression", "slope" and "summit" where I collected soil samples and measured cover crop biomass inputs. Red clover had significant positive effects on SMC in slopes and summits. Rye had significant positive effects on SMC only on summits. The cover cropping practice has a potential to increase soil C, hence soil quality, in depressions and summits and maintain it in slopes.

In chapter 4, I monitored the spatial and temporal distributions of soil NO_3^--N in 20 agricultural fields. The results showed that temporal and spatial distributions of NO_3^--N were mainly controlled by soil organic C content. Then, I assessed the contribution of cover crops to controlling NO_3^--N and potential mineralizable nitrogen (PMN) across topography. Red clover had significant positive effects on NO_3^--N across all topographical positions. Rye had significant negative effects on NO_3^--N only in depressions. The spatial variations in amounts of residue from cover crops were found to be an important factor controlling the effects of cover crops on soil mineral nitrogen across topographically diverse fields.

Dedicated to Farnoosh, for her love and support.

ACKNOWLEDGEMENTS

I am very thankful to my major professor Dr. Sasha Kravchenko for her guidance through my research. What I learned from her geos well beyond the content of this dissertation. Thank you for letting me explore ideas, teaching me statistics and for your magnificent insight and patient support during my research. I would like to express my gratitude to all of my committee members for their willingness to help guide my research and my future career. The insight and expertise of Dr. Philip Robertson, Dr. Alvin Smucker and Dr. David Rothstein enhanced the quality of my research greatly.

I would like to thank my current and past lab members: Dr. David Munoz, Dr. Wei Wang, Dr.Wakene Chewaka, Dr. Ehsan Toosi, Dr. Abdul Basir, Mellisa Erikson, Richard Price and Michelle Quigley for their tremendous help with collecting samples, brainstorming ideas and their help with my presentation practices. I appreciate greatly the hard work of many undergraduate helpers who made this research possible particularly Edward Sczyzgiel, Jon Roney and Mike Klein.

I would like to extend a special thank you to Jim Bronson for his endless support and cooperation in setting up my field plots and sample collection.

I am very appreciative of support and encouragement of my wonderful family and friends. My deepest gratitude goes to Farnoosh, my wife, who made me believe in myself by her love and support. She stood by me during long sample collection days and late night manuscript preparations and listened to several presentation practices.

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Chapter 1: Introduction

Concerns about increasing atmospheric greenhouse gases (Robertson et al., 2000) and soil degradation have promoted the use of conservation agricultural systems. Conservation agricultural practices can reverse soil degradation by limiting erosion, mitigate greenhouse gas emissions by enhancing soil carbon (C) content and promote soil productivity by recycling and supplying nitrogen (N) in soil. One of the promising conservation practices is including winter cover crops in agricultural rotation (Sainju et al., 2006; Hurisso et al., 2014). The cover crops typically are planted after harvest of the main crop and are terminated before planting the next main crop in spring. Application of winter cover crops has been found to increase soil organic C (Grandy and Robertson, 2007; Syswerda et al., 2011; Culman et al., 2013), supply mineral N for the subsequent crop (Snapp et al., 2005), decrease soil erosion (Lal et al., 2003), stabilize the soil structure, increase soil water holding capacity (Raimbault and Vyn, 1991; Fageria, 2007), suppress weeds (Caamal-Maldonado et al., 2001, Snapp et al., 2005), and reduce nitrate leaching (Sainju et al., 1998; Dinnes et al., 2002; Schomberg and Endale, 2004).

The role of cover cropping in increasing soil C and limiting C emissions into the atmosphere has been a subject of intensive research. Soil C is a key indicator of soil quality that controls many soil physical, chemical and biological properties (Carter, 2002; Fageria, 2007). Changes in soil C induced by cover cropping in the short term (2-5 years) are not adequately reflected by total organic C (TOC) because of the slow response of TOC to management changes (Haynes, 2000; Nascente et al., 2013; Plaza-Bonilla et al., 2014). TOC consists of functional pools that can be divided into labile and recalcitrant based on their turnover rates. The labile pools have turnover rate of days to a few years while the recalcitrant pools have turnover rates of decades to centuries (Hungate et al., 1995; Paul et al., 1999; Six et al., 2001; Guimaraes et al. 2013). The assessment of labile pools can reveal the short-term effects of management practices on C processes (Marriott and Wander, 2006; Awale et al., 2013). Various biological, chemical and physical methods are applied to separate TOC labile pools. Physical fractionation based on size can be applied to separate labile pools from the recalcitrant ones. Dispersion and then sieving the soil samples lead to separation of the particulate organic C (POC), that is particles of organic material 0.05- 2 mm in size (Cambardella and Elliott, 1992; Chan, 2001; Collins et al., 2000). The amounts of C respired from soil in a short period of time is another indicator of soil labile pools such as mineralizable C (Paul et al., 1999; Franzluebbers et al., 2000; Culman et al., 2013).

While high sensitivity of a measure of labile C to changes in management is an important criterion in determining how useful labile C can be as a tool in comparing different management practices, another criterion is variability. The larger variability in the measurements of a certain soil C measure, the lower are the chances of detecting the differences among studied management practices using that C measure. The main body of research on the use of active C fractions for management comparisons focused primarily on the larger management effects observed for active C fractions as compared to TOC. However, the variability of POC (Gregorich et al., 2006; Hernandez-Ramirez et al., 2009; Mao and Zeng, 2010) and SMC (Zagal et al., 2009) can be substantially higher than that of TOC. To the best of my knowledge, implications of potentially larger variability of POC and SMC for statistical power of comparisons among agricultural management practices have never been addressed.

Another important and extensively studied effect of cover crops is their ability to supply N to subsequent main crops (Cueto-Wong et al., 2001; Kue and Jellum, 2002; Schomberg and

Endale, 2004; Collins et al., 2007; Asagi and Ueno, 2009). N comprises about 2% of crops biomass and is frequently deficient in crop production. In soil, N exists in both organic and mineral forms with the organic forms comprising 95% of overall soil N pools (Havlin et al., 1999). The two major absorbable forms of mineral N available to plants from soil are nitrate (NO_3^--N) and ammonium (NH_4-N) (Bair et al., 2008). In well-aerated soils during the growing season NO_3^--N becomes the main form of plant available N as the microbial activity transforms NH_4^+-N into NO_3^--N (Addiscott, 2005). Leguminous cover crops have the ability to fix the atmospheric N, assimilate it into their biomass and release it into soil in absorbable forms. N fixation by legumes is carried out by reducing N gas to NH_4-N via a symbiotic relationship between legumes root and rhizobium (Belvins et al., 1990). Non-leguminous cover crops assimilate soil NO_3^--N during the winter (Ruffo et al., 2004). After the biomass of cover crops (both leguminous and non-leguminous) is returned to the soil microbial mineralization transforms the organic N into mineral forms (Haynes 1997).

It is crucial to keep NO_3^--N levels optimal during the crop growing season because on one hand low levels of NO_3^--N in soil can limit crop production and on the other hand the high levels of NO_3^--N can be an environmental pollutant (Cambardella et al., 1999; Di and Cameron, 2002). The levels of NO_3^--N in soil vary across space and over time. Proper agricultural management needs to consider site-specific variation and temporal patterns in soil NO_3^--N to supply optimum amounts of NO_3^--N from organic and mineral N fertilizer sources for plant growth. The temporal variation in soil NO_3^--N provided by cover crops is an important factor that controls the benefits of cover crops (Mirsky et al., 2009; Parr et al., 2011; Wortman et al., 2012). The beneficial effects of cover crops on N will only occur if the N supplied by cover crops becomes available during the high uptake period of the main crop (Kramberger et al., 2009).

PROBLEM DEFINITION

The benefits of cover crops in enhancing soil C and supplying N for crops have been much studied, however, most of the previous research on cover crops has become carried out in controlled, and in many cases small, experimental plots. However, the effects of cover crops on soil C and N across large agricultural fields with substantial topographical and soil diversity where the actual farming takes place are also likely to vary and possibly differ, at least in their magnitude, from the observations made in homogeneous experimental plots. The topographical variations typical of large agricultural fields in the US Midwest could have substantial impact on spatial variability of soil properties and performance of cover crops (Guretzky et al., 2004; Munoz et al., 2014). Topography governs many of the process that control soil C accumulation in a landscape, including erosion and deposition (Gregorich et al. 1998; Lal, 2003; Ritchie et al., 2007), variations in soil moisture and temperature (Bennie et al., 2008), and magnitudes of organic C decomposition processes (Jacinthe et al., 2002). Soil can have variable amounts of organic matter based on their topographical position (Chan et al., 2007) and the amount of organic matter was found to be one of the main sources of NO_3^--N in soil by Dharmakeerthi et al., (2005). Corre et al. (2002) suggested that spatial variation in soil organic matter, soil microbial biomass, natural drainage, growth performance of the plants, and water and nutrient redistribution caused by topography are the main factors controlling the dynamics of soil mineral N. Kay et al. (2006) found that along with weather, landscape accounted for most of the variation in plant available N. Therefore, effects of any type of conservation management with cover crops on C and N also vary across diverse topography (VandenBygaart et al. 2002; Jacinthe et al., 2004; Senthilkumar et al 2009). The effects of topography and cover crops on soil N and soil C have been considered separately by many researchers; however, their combined effects have not been adequately investigated.

The spatial variability of cover crop performance and uncertainty about cover crop effects on soil C and N across large agricultural fields may be one of the reasons for low adoption of cover cropping practices by farmers (Snapp et al., 2005; Munoz et al., 2014) despite the documented benefits of cover cropping in small-plot research. Knowledge about unknown effects of topography and its interactions with cover cropping may resolve many of the ambiguities about cover cropping practice. Therefore, a field-scale approach that considers both spatial and temporal cover cropping effects and their interactive influences on soil N and C pools within farmer-practiced agricultural managements will help farmers to include cover crops in their agricultural practices more successfully.

OBJECTIVES

My overall goal is to assess the effects of agricultural management with and without cover crops on soil organic C and mineral N. The specific objectives are:

1) To evaluate the statistical power of detecting effects of agricultural management for POC and SMC given their observed variability; and then, to investigate the ways to enhance the statistical power when testing management effects across large agricultural fields;
2) To assess the field-scale spatial variability of cover crop biomass in relation to topography; and then to investigate the spatial and temporal distributions of soil C

pools and NO_3^--N in agricultural management with and without cover crops;

3) To investigate the particular role of cover crops on active pools of C across topographically diverse fields; and
4) To assess the contribution of cover crops to soil NO_3^--N across topographical positions during the growing season.

OUTLINE OF THESIS

In Chapter 2 I outline a use of power analysis to assess the suitability of different measures of soil organic C for studying management effects in field scale experiments. In investigated the variability of POC and SMC as the two measures of active C and total organic C and the detectability of management-induced effects on POC and SMC. I discussed the ways to decrease the variability and enhance the statistical detectability of the management effects are discussed as well. In Chapter 3 I outline the contribution of legume and non-legume cover corps to soil C pools across large agricultural fields. I discussed the topography driven differences in cover crops residue inputs and the role of topography in controlling cover crop effects on soil C pools. In Chapter 4 I outline the temporal and spatial distribution of soil NO_3^--N under managements with and without cover crops. The particular role of cover crops in controlling temporal and spatial distribution of NO_3^--N is presented. In Chapter 5 I summarize conclusions of the study.

Chapter 2: Which soil carbon fraction is the best for assessing management differences? View from statistical power perspective

ABSTRACT

Active fractions of soil C such as particulate organic C (POC) and short-term mineralizable C (SMC) respond faster than total organic C (TOC) to management induced changes in soil C. However, the active fractions of organic C can possibly have larger variability that decreases the detectability of management effects on soil C. The objectives of this study are to 1) assess the relative usefulness of TOC, POC and SMC as criteria of management induced changes on soil C and 2) investigate if using auxiliary soil and topographical information can aid in increasing the usefulness of these criteria in studies conducted across large spatial scales. The results showed that to be able to detect differences between the management systems with an acceptable type II error of 0.20, an 80% difference in TOC and a 50% difference in SMC were needed. The statistical power for POC was never in an acceptable range. The use of auxiliary soil and topography data decreased the size of the minimal detectable differences (i.e. increased statistical power) for TOC from 80% to 50%. Given the faster reaction to management of SMC as compared to TOC, and its lower variability as compared to POC, I recommend SMC as the preferred C fraction for detecting treatment induced differences in C processes in agricultural field experiments.

INTRODUCTION

The role of conservation agricultural management practices in increasing soil carbon (C) and limiting C emissions into the atmosphere has been a subject of intensive research. However, testing the effects of management on soil C is difficult because changes in C take a long time to occur and the spatial variability of soil organic C is large (Brock et al., 2011). It might take decades until the effects of management on total organic C (TOC) can be observed (Smith, 2004). However, it is well known that active fractions of TOC react more rapidly to management and can provide assessments of management effects in a shorter time (Hungate et al., 1995; Paul et al., 1999; Six et al., 2002; Guimaraes et al., 2013). Thus active fractions of TOC have been proposed as more sensitive measures for assessing the effects of management practices on soil C processes.

An active fraction of soil C commonly used in management practice comparisons is particulate organic carbon (POC), that is, the organic C particles between 0.50 and 2 mm in size (Cambardella and Elliott, 1992). POC is one of the components of TOC that has been reported to rapidly react to the changes in management (Sequeira et al., 2011). POC is typically found to be positively correlated with TOC (Yan et al., 2007), thus is often used when assessing the influence of management on soil C (Christensen, 2001; Six et al., 2002; Hernandez-Ramirez et al., 2009; Mao and Zeng, 2010; Guimaraes et al., 2013). POC constitutes 8-25 percent of TOC (Chan, 2001) and represents a transitional stage in transformation of plant residue to soil C storage (Mao and Zeng, 2010). Therefore, in a long-term an increase in POC translates into an increase in TOC (Cambardella and Elliott, 1992).

Another measure of biologically active C is short-time mineralizable C (SMC). Franzluebbers et al. (2000) observed that C mineralized in a short (three days) period of time was positively correlated with active C, suggesting that SMC could be a good indicator of active soil C fractions. Under controlled temperature and moisture conditions soil microbes consume the labile fractions of TOC. Therefore, measuring the amounts of CO_2 respired in short time periods reflects the labile fractions of TOC (McLauchlan and Hobbie, 2004). SMC was reported to be sensitive to management changes and thus suggested as a good measure to evaluate management effects on soil organic C (Franzluebbers et al., 2000; Culman et al., 2013). Saviozzi et al. (2014) reported significant correlation between C mineralization and TOC (R^2=0.68). Ahn et al. (2009) also found significant positive correlation between C mineralization and TOC (R^2=0.62) and suggested C mineralization as the most suitable measure of short-term C changes in sandy soils. The increase in SMC reflects an increase in labile fractions of TOC and a long-term increase in soil C storage (Alvarez and Alvarez, 2000).

While high sensitivity of a measure to changes in management is an important criterion for determining how useful the measure can be as a tool in comparing different management practices, another criterion is variability. The larger is variability in the measurements of a particular soil property, the lower are the chances of detecting differences between different management practices. In statistical terms the probability of detecting statistically significant differences between the studied treatments is called power of the test. The power depends on the size of the differences among the mean values of the studied measure from the treatments, on the variability of the studied measure, and on the number of replicates.

While different aspects of power analysis, including minimum detectable differences among the management practices, magnitude of variability, and sample size requirements, have been extensively researched for TOC (e.g. Hungate et al., 1995; Garten and Wullschleger, 1999; Conant et al., 2003; Smith, 2004; Poussart and Olsson, 2004; VandenBygaart and Angers, 2005;

Homann et al., 2008; Allen et al., 2010; Schrumpf et al., 2011; Kravchenko and Robertson, 2011; Brock et al., 2011), power analysis for active C fractions has received considerably less attention. The main body of research on the use of active C fractions for management comparisons has focused primarily on the larger management effects observed for active C fractions as compared to TOC. However, the variability of POC (Gregorich et al., 2006; Hernandez-Ramirez et al., 2009; Mao and Zeng, 2010) and SMC (Zagal et al., 2009) can be substantially higher than that of TOC. To the best of my knowledge, implications of potentially larger variability of such fractions for statistical analysis for management comparisons and its power have never been addressed.

In addition, even for TOC, most of the research on power analysis and sampling requirements has been conducted in small field experiments with relatively low variability from one experimental plot to another. The effects of conservation management systems on soil C content at larger scales, e.g., the scale of entire agricultural fields, have received less attention (VandenBygaart, 2006). One of the main reasons is likely that numerous sources of variability in large fields reduce statistical power for assessment of management effects (Conant et al., 2003; VandenBygaart and Angers, 2005). Large variations in soil properties, including soil particle size distribution, bulk density, and initial soil organic C, lead to larger variability across an entire agricultural field thus making assessment of changes in soil organic C difficult (Chan et al., 2007; Schrumpf et al., 2011).

In Midwest agricultural systems topography is one of the main drivers in spatial patterns of soil properties. Topographical variations in agricultural fields increase soil C spatial variability via varied plant inputs (Jiang and Thelen, 2004; Munoz et al., 2014), decomposition (Chan et al., 2007; Allen et al., 2010), soil texture (Hook and Burke, 2000), and soil C erosion and deposition (Lal, 2003; Ritchie et al., 2007). A potential solution to capture some of the variability across large fields is collecting additional data about soil properties and topography and including them in the data analysis as covariates (Carter et al., 2003). Additional soil and topography data can help decrease experimental error and increase statistical power when assessing management effects on TOC (VandenBygaart, 2006). However, until now there is no literature on how useful such information could be for analyzing changes in POC and SMC. The main objectives of this study are to 1) assess the variability in POC and SMC as the two most commonly measured active fractions of soil C, and compare it with variability in TOC across large agricultural fields at multiple soil depths; 2) to evaluate the statistical power of detecting effects of agricultural management for POC and SMC, given the observed variability; and 3) to investigate the benefits of collecting additional soil and topography data for enhancing the statistical power when testing management effects across large agricultural fields.

MATERIALS AND METHODS

Fields and Treatments set-up

The study was conducted in ten undulated agricultural fields of the Scale-up experiment at the W.K. Kellogg Biological Station (KBS) Long-term Ecological Research (LTER) (www.lter.kbs.msu.edu) site located in southwest Michigan (42° 24' N, 85° 24' W). The dominant soil series are Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs). All fields were in a corn-soybean-wheat rotation. Three of the studied fields were under conventional management without cover crops and seven fields were under management with cover crops. The agricultural managements were established in 2006. The rotations of cover crop management included two cover crops: a leguminous cover crop, red clover (*Trifolium pratense* L.) and a non-leguminous rye (*Secale cereale*) cover crop. Red clover was frost-seeded in winter wheat (i.e. in March) and then plowed into the soil prior to corn planting in the spring of the following year, i.e. in May. Rye was planted after corn harvest, i.e. October, and then plowed into the soil prior to soybean planting in spring of the following year, i.e. in May. Details of the management related activities (i.e. fertilizer, tillage, planting, harvesting, etc.) can be found at http://lter.kbs.msu.edu/protocols/104 (verified 6 Feb. 2015).

Topographical attributes of the fields were derived from a 1 m resolution digital elevation model of the area. They included terrain slope, relative elevation, flow length, flow accumulation, and solar radiation. Linear discriminant analysis was applied to the topographical attributes to classify the land within the fields into three topographical positions: summit, slope and depression as described by Munoz and Kravchenko (2011). For this study, in each field I identified one 10x10 m observational plot within each slope and depression topographical position (Figure 2.1) for subsequent soil sampling (described below).

Sample collection and analysis

Deep soil cores were collected from the observational plots in spring of 2010, four years after establishment of agricultural managements. One soil core was collected from each of the observational plots, for a total of 20 cores. The cores were taken using a hydrolic sampler (Geoprobe, Salina, KS) that uses a direct push and vibratory driving method to avoid compaction. The cores were 7.62 cm in diameter and were taken from a 1-m depth. The cores were cut into five depth intervals: 0-20, 20-35, 35-50, 50-70 and 70-90 cm. Three of the depth intervals (0-20, 35-50 and 70-90 cm) were used for further analysis in this study. For each interval I measured TOC, POC, SMC, bulk density, and particle size distribution.

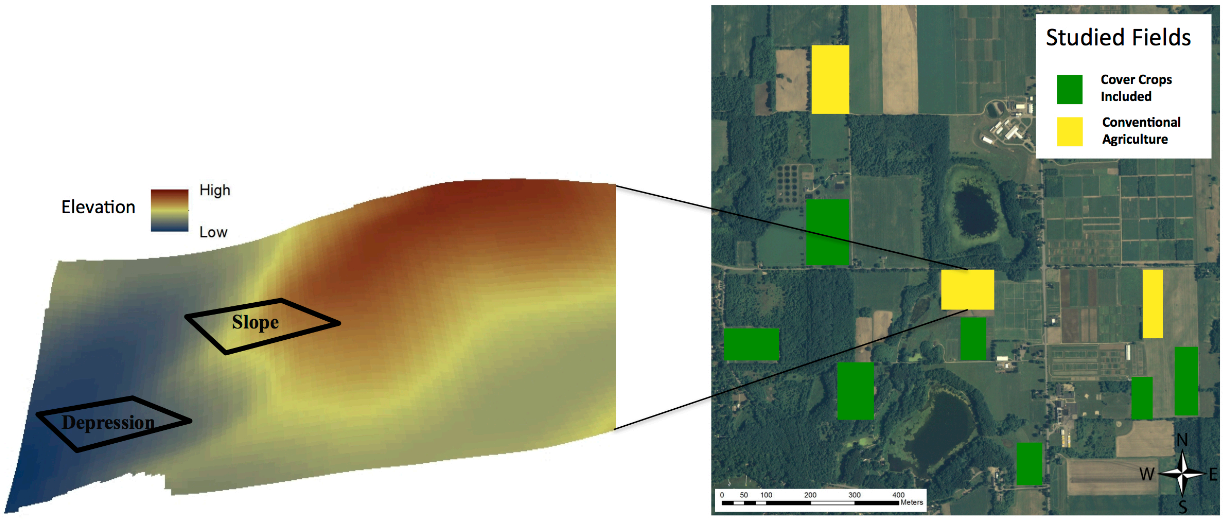


Figure 2.1 Fields used in the study (Scale-up experiment at W.K. Kellogg Biological Station Long-term Ecological Research site) and a schematic representation of the observational plots in contrasting topographical positions in one of the studied fields used for soil sampling.

TOC was measured via dry combustion method using the Costech ECS 4010 CHNSO analyzer.

POC was measured as per Cambardella and Elliott (1992) with slight modifications. Specifically, 60 ml of 5 g L^{-1} sodium meta-phosphate was added to 20 grams of soil in a 250 ml glass beaker. The beaker was shaken for 16 hours on a rotary shaker at 180 round per minute. Then, the contents of the jar were sieved through a 0.053 mm sieve. The remaining material on the sieve was dried and analyzed for C content using a Costech ECS 4010 CHNSO analyzer as above for TOC.

The minimal amount of C that Costech ECS analyzer was able to detect in the standard samples was 0.01 mg. The actual soil samples used in the analysis weighed around 25 mg. Therefore, the minimal C concentration that could be detected by the Costech ECS Analyzer in the studied samples was approximately 0.04% C.

SMC was measured following procedures of Franzluebbers et al. (2000) and Culman et al. (2013). Specifically, 10 grams of air-dried soil was placed in a 230 ml mason jar. The water holding capacity was determined for each soil sample as instructed by Haney and Haney (2010) and water in an amount of approximately 50% of water holding capacity was added to each jar (around 2 ml). The jars were then capped and incubated at 25^{o}C. Once after 24 hours and then after 72 hours the concentration of CO_2 was measured by taking a 5 ml gas sample from each jar using a syringe and injecting that into LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE).

Soil bulk density was calculated by dividing the weight of the undisturbed soil core by its volume. Particle size distribution was analyzed using the pipet method (Kroetsch and Wang, 2008).

In addition to assessing variability of the conducted measurements due to variability in the experimental plots, I also assessed the variability in measurements due to laboratory measurements. For SMC, four samples were randomly selected at each depth interval and SMC was measured at each of them three times. Then the coefficient of variation (CV) was computed for each sample based on the three lab replicates and the average CV was calculated from all four samples. For TOC and POC, 10 randomly selected samples were analyzed with four lab replications each. The CVs were calculated for each sample and the average CV was calculated for TOC and POC.

Statistical analysis and power calculations

Detailed descriptions of power analysis and its applications in soil C studies can be found in a number of statistical texts (Littell et al., 2006; Stroup, 2013) and papers (Garten and Wullschleger 1999; Stroup, 2002; Poussart and Olsson, 2004; Homann et al., 2008; Brock et al., 2011). Thus, here I only briefly mention the inputs needed for conducting power analysis and the considerations for selecting the values for these inputs that I used in the current study.

In a prospective power analysis the required inputs for calculating the probability of detecting statistically significant differences, i.e., power, are: 1) the hypothesized differences between the studied treatments; 2) the estimates of the variances; 3) the desired probability of type I error, α; and 4) the proposed number of replicates. For determining the hypothesized differences between the studied treatments, I used the settings that I expect would constitute the most contrasting comparison scenario, specifically, comparing conventional and cover crop based managements at topographical slope positions. On slopes I expect the conventional management to result in the greatest C losses (Yoo et al., 2006; Ritchie et al., 2007); while I expect the cover crop based management to result in gains in C due to reduction in erosion, extra biomass inputs, and other benefits attributed to cover crop presence (Chan et al., 2007; Senthilkumar et al., 2009). I assume that all three studied C fractions, i.e., TOC, POC, and SMC, would decrease in the slope areas of conventional management and that they would increase in the slopes of the cover crop management fields.

For the power calculations, I hypothesized the decreases on the slopes of conventional management treatment to range from 0 to 50% of the initial soil organic C and the increases on the slopes of cover crop management to range from 0 to 50% of the initial soil organic C, in 10% increments. The hypothesized differences were applied to the grand means of TOC, POC and

SMC yielding a range of hypothesized means for the slope areas of each management. Then the difference between hypothesized means of C measures on slope of conventional management and hypothesized means of C fractions on slope of cover crop management was computed. Finally, I calculated the percent difference ranging from 0 to 100 % between the two studied treatments. Note that the range of the hypothesized differences used in my study is sufficiently large to encompass the difference that is likely to be achieved in typical agricultural field experiments.

Two sets of variances were obtained. The first set was gained from the analysis of variance (ANOVA) of TOC, POC and SMC data from the collected soil samples. The ANOVA was conducted using the MIXED procedure of SAS 9.2 (Cary, NC). The design was a two factor split-plot in a completely randomized set-up. The agricultural management and topographical position were the two studied fixed factors with agricultural management being the whole-plot factor and topographical position being the split-plot factor. Field nested within the agricultural management was treated as the random factor in the statistical model and was used as an error term for testing the effect of agricultural management. Normality of the residuals and homogeneity of variances were checked for all studied variables.

The second set of variances was gained from the analysis of covariance (ANCOVA) of TOC, POC and SMC data with the use of soil bulk density, percent of sand content, and the studied topographical attributes as covariates. ANCOVA uses the linear relationships between the covariates and the response variable to explain the variability in the response variable. If the correlation between the covariates and the response is strong, then ANCOVA results in a lower experimental error and smaller variances. Thus, the use of covariates can increase the statistical power of the analysis. A detailed description of ANCOVA can be found in Milliken and Johnson

(2002). In conducting ANCOVA I started with including all of the covariates in the statistical model and then deleted them from the model in a step-wise manner until only those significant at the $p<0.1$ level remained. ANCOVA was conducted using the MIXED procedure of SAS.

The type I error for the power analysis was set to 0.05. The number of desired replications was set to 5, that is, in the considered scenarios it was assumed that five replicated fields were assigned to each management practice, for a total of 10 fields. The five replicates for each management that I used in my power calculation scenario is comparable to the number of replicates typically used in field studies.

The statistical power was calculated using the above inputs, i.e. the hypothesized differences, the estimated variances, the potential number of replicates, and the desired α. The power calculations were conducted using the MIXED procedure of SAS (Stroup, 2002). A statistical power of 80%, i.e. probability of type II error of 20%, was considered to be acceptable.

RESULTS

Variability in C fractions across topography and depth

In both management systems the depression areas had significantly higher TOC, POC and SMC as compared to the slopes ($p<0.05$) (Table 2.1). POC in depressions was almost five times greater than that in the slopes in 0-20 cm depth, while TOC and SMC in depression areas were about two times greater than in the slopes. Lower sand content and lower bulk density values were observed in depressions compared to the slopes (Table 2.2).

Coefficient of variation was used as a unit-less characteristic to compare variabilities of TOC, POC and SMC (Table 2.1). Across all positions and depths SMC had the smallest CV followed by TOC and POC. For POC the CVs were substantially larger in depression areas compared to slopes. For TOC the CVs were slightly larger in depressions compared to slopes and for SMC both positions were similar in terms of their CV values. In general, the CVs of the studied C fractions were larger at lower depths as compared to the surface soil.

Table 2.1. Descriptive statistics of total organic C (TOC), particulate organic C (POC) and short-term mineralizable C (SMC) at the studied topographical positions and soil depths. Data are the means with coefficients of variation (%) shown in parenthesis.

	TOC %			POC%			SMC (mg CO_2 $kg^{-1}day^{-1}$)		
Soil depth (cm)	0-20	35-50	70-90	0-20	35-50	70-90	0-20	35-50	70-90
Depression	1.6	0.7	0.7	0.6	0.2	0.1	60	16	7
	(51)*	(43)*	(112)*	(131)*	(179)	(167)	(28)*	(30)*	(35)
Slope	0.9	0.2	0.1	0.1	0.02	0.02	39	6	3
	(22)	(60)	(74)	(46)	(96)	(72)	(23)	(49)	(59)

* mark the cases when the means in the same column were significantly different from each other ($p<0.05$).

The CVs from lab replicates followed the same patterns as the CVs from entire data set (Table 2.3). They were smallest for SMC and the largest for POC and substantially increased with depth. The lab measurement CVs for SMC were slightly larger in measurements taken after 24 hours compared to those taken after 72 hours. Thus I used only SMC measured after 72 hours in the rest of the data analysis.

Table 2.2. Sand content and soil bulk density at the studied topographical positions and soil depths of 7 fields.

	Sand (%)			Bulk density (Mg m^{-3})		
Soil Depth (cm)	0-20	35-50	70-90	0-20	35-50	70-90
Depression	45 *	46 *	54 *	1.4 *	1.7 *	1.8
Slope	64	70	86	1.6	1.8	1.9

* mark the cases when the means in the same column were significantly different from each other ($p<0.05$).

Table 2.3 Coefficient of variation (CV) for lab replicates of total organic C (TOC), particulate organic C (POC) and short-term mineralizable C (SMC) at different soil depths. TOC and POC were measured in four lab replicates of 10 samples at each depth. SMC was measured in three lab replicates of four samples at each depth.

Soil depth (cm)	Coefficient of variation %			
	TOC	POC	SMC	
			24 Hours	72 Hours
0-20	7	11	7	5
35-50	22	100	12	7
70-90	40	110	38	20

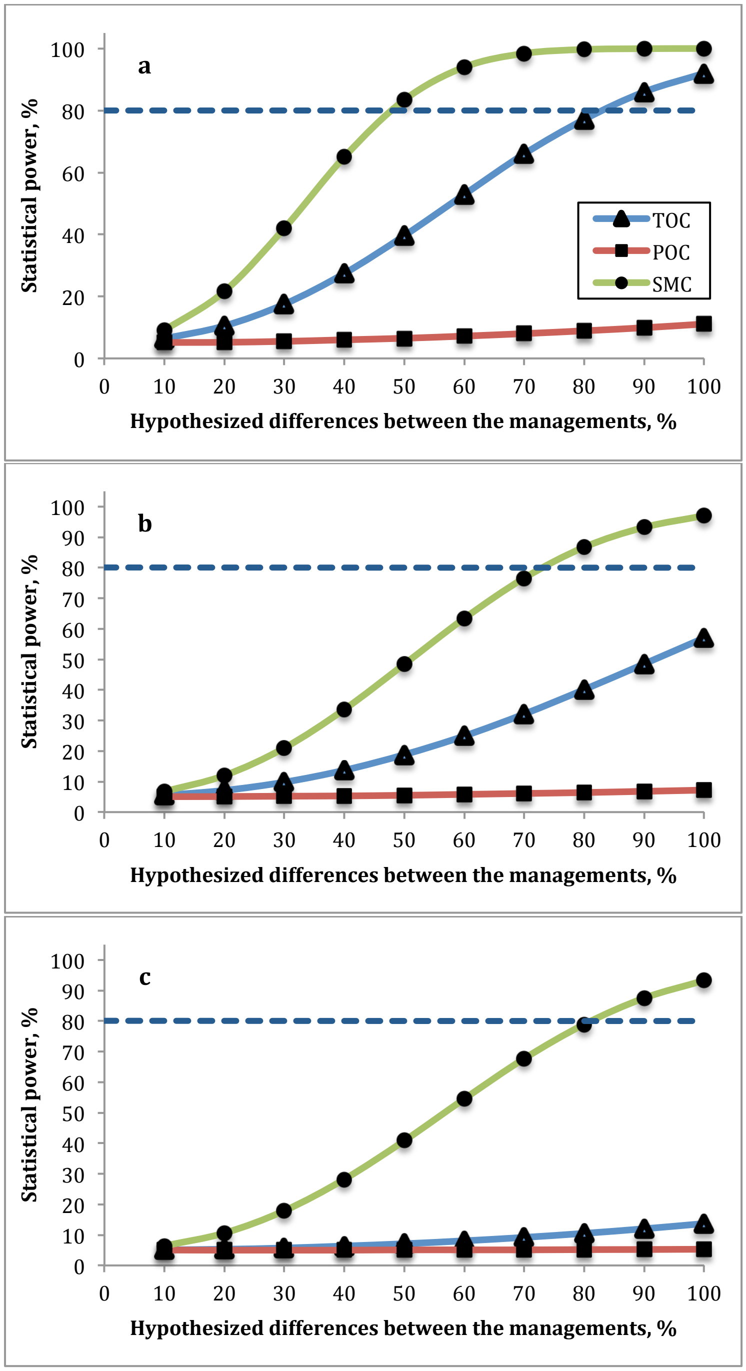


Figure 2.2 Statistical power for testing the hypothesized differences between the conventional and cover crop based managements for a) 0-20, b) 35-50, and c) 70-90 cm depths. The dashed lines mark the acceptable power (80%) (corresponds to the probability of type II error of 20%)

Statistical power for testing the differences between managements

As expected from the variability of the studied soil C fractions (Table 2.1) the largest statistical power was observed for SMC data followed by TOC across all three studied depths (Fig. 2.2). For the 0-20 cm depth the power for SMC reached the acceptable level when the difference between the two management systems was at approximately 50% of the grand mean of C. For the same depth, an 80% difference between the management systems in TOC would be needed to reach the acceptable statistical power (Fig. 2.2). Statistical power was higher in the surface soil compared to the deeper layers for both TOC and SMC. The statistical power for POC was less than 20% across all studied depths (Fig. 2.2).

The benefits of using soil and topography data as covariates

TOC was significantly negatively correlated with soil bulk density and sand content at all studied depths (Table 2.4); while SMC was negatively correlated with soil bulk density across all three studied depths. Correlations between SMC and topographical covariates were present at 35-50 and 70-90 cm depths, but not at the soil surface.

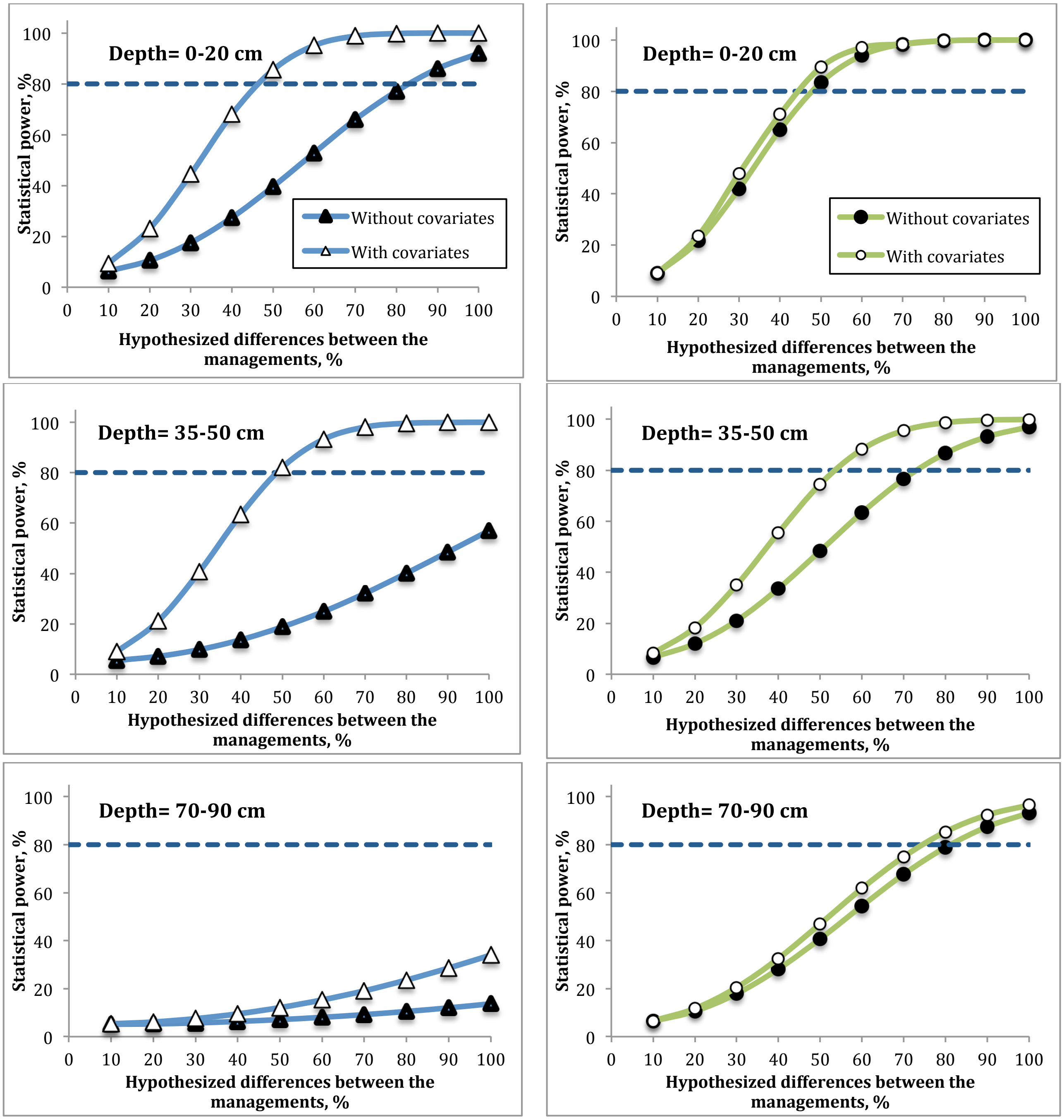
At 0-20 cm depth sand content was used as a covariate only for TOC analysis. The other soil and topography data did not explain a significant portion of variance when added to the ANCOVA ($p<0.1$). At the 35-50 cm depth sand content and slope degree were used as covariates in TOC analysis and bulk density and flow accumulation were used as covariates in SMC analysis (Table 2.3).

Table 2.4. Pearson correlation coefficients between the studied C fractions, i.e, total organic C (TOC), particulate organic C (POC), and short-term mineralization C (SMC), and soil bulk density (BD), sand content, topographical slope and flow accumulation (Flow Acc). Only correlation coefficients significant at 0.05 level are shown.

Depth (cm)	Fraction	Soil BD	Sand	Slope	Flow Acc
0-20	TOC	-0.68	-0.81 *	-0.48	
	POC				
	SMC	-0.62			
35-50	TOC	-0.87	-0.77 *	-0.82 *	0.81
	POC				
	SMC	-0.69 *		-0.74	0.74 *
70-90	TOC	-0.85 *	-0.79 *		0.64 *
	POC	-0.67	-0.66 *		
	SMC	-0.65 *	-0.62 *	-0.56	0.77 *

* marks the cases where the covariate was found to be contributing significantly ($p<0.1$) to the ANCOVA.

Including covariates in the analysis for both TOC and SMC decreased the variances, hence smaller hypothesized differences in both TOC and SMC were detectable with the acceptable level of statistical power (Fig. 2.3). However, the improvement in the power for TOC was larger compared to that for SMC. Including additional soil and topography information did not substantially increase the power for POC (results not shown).



a. Total Organic Carbon b. Short-term Mineralizable Carbon

Figure 2.3. Statistical power for testing the hypothesized differences between the conventional and cover crop based managements with and without covariates for a) TOC and b) SMC at the three studied depths. The dashed lines mark the acceptable power (80%) (corresponds to probability of type II error of 20%)

Including covariates produced a greater increase in statistical power in deeper soil as compared to the surface. At the 0-20 cm depth adding the covariates decreased the detectable differences for TOC from 80% to 45%. At the depth of 35-50 cm detecting the difference between the treatments as small as 50% was possible for TOC with covariates; while without the covariates even a 100% difference was not detectable with acceptable power.

At the 0-20 cm and 70-90 cm depths the use of covariates resulted in only minor improvements in power for SMC. However, for the 35-50 cm depth the detectable differences for SMC decreased from 70% to 55% when the covariates were added to the analysis.

DISCUSSION

Variability of TOC, POC and SMC across topography and depth

As expected, topography had a major influence on the amounts of TOC, SMC and POC. Five times larger POC and two times larger TOC and SMC in depressions compared to slopes probably resulted from a combination of factors, including larger crop residue returns (Jiang and Thelen, 2004), lower decomposition rates (Allen et al., 2010), and deposition of plant material brought from higher areas (Gregorich et al. 1998; Lal, 2003) in the depression areas.

Of the three studied C variables SMC had the lowest variability, followed by TOC and by POC. My observed CVs for SMC for the surface soil (25%) were similar to those reported by Zagal et al. (2009), e.g., CV of 21% for SMC, while lower than the 52% CV reported by Ahn et al., (2009). However, my average CVs for TOC and POC in the 0-20 cm depth (36% and 87%, respectively) were larger than those of Mao and Zeng (2010) who observed CV of 22% (on average) for TOC and around 50% for POC in the 0-15 cm depth, and than those of Hernandez-

Ramirez et al. (2009) who reported CV of 6% for TOC and 29% for POC in the 0-15 cm depth. The larger CVs are likely due to the much larger spatial scale of my experiment with samples collected across multiple agricultural fields; while most of the previous studies were carried out in small experimental plots.

The larger CVs for POC as compared to TOC observed in this study are consistent with earlier reports (Gregorich et al., 2006; Hernandez-Ramirez et al., 2009; Mao and Zeng, 2010). The greater variability of all studied C variables at deeper soil layers is also consistent with previously reported results (e.g., VandenBygaart et al.,2011; Schrumpf et al., 2011).

Assessing the CVs from lab replications (Table 2.3) indicated that high variability of TOC and POC data in deep soil layers was caused not only by high variability among the soil samples from different experimental plots, but also by low lab repeatability of these measurements. While my CVs for lab measurements of both TOC and POC were at acceptably low 7% and 11% levels in samples from 0-20 cm depth, in 70-90 cm depth the CVs increased >5 fold and 10 fold for TOC and POC, respectively.

One cause for higher variability in POC, and in part in TOC at deeper layers that was encountered in this study were very low levels of C; that is C levels approaching the minimum C level detectable by a CN analyzer. The measurement accuracy of the Costech CN analyzer machine used for sample analysis depends on the amounts of C present in the sample. For soil samples with C contents lower than 0.04% the ability of the machine to detect it decreases. The detection limit of the historic Walkley-Black method was reported to be 0.1% (De Vos et al., 2007). The detection limit of Costech CN analyzer that I used is smaller than the detection limit of Walkley-Black method. Many of the samples from the deeper soil had POC and even TOC contents lower than 0.04%, which caused less accurate results and larger CVs.

Another reason for high POC variability is that POC determination heavily depends on the presence of organic particles, typically the remains of plant/animal residues, within a relatively small soil sample. The presence or absence of such particles can vary substantially from one sample to another and thus can cause large variations in POC data even in lab replicates of the same soil sample. This effect is probably exacerbated in my study because of the presence of a clay layer with larger percentage of stones and textural variations, formed on parent material different from the parent material of the surface soil (2Bt pedogenic layer).

Statistical power for testing the differences between the managements

The results of power analysis suggested that assessing management effects using TOC requires substantial differences between the managements to have taken place in order to be statistically detectable. More than an 80% difference between the managements would be needed to detect differences with an acceptable power in these sites. The statistical power values that I observed are comparable to those of Homann et al. (2008). However, larger statistical power was reported by Conant et al. (2003), Schrumpf et al. (2011) and Brock et al. (2011). The latter two studies were replicated in small experimental plots with minimum heterogeneity in soil organic C and other soil properties, while my study was conducted in large fields with several factors contributing to variability in TOC.

Data from a smaller scale long-term experiment adjacent to my studied fields indicated that after 12 years of cover cropping practice the difference between the conventional and the cover crop based managements in terms of TOC in the soil Ap horizon was merely 20% (Syswerda et. al., 2011). Results of my power analysis indicate that in a large scale field study such as mine, a larger difference in TOC will be needed in order to be detectable with an acceptable power. Gaining such a difference will require a much longer time than what is typically employed in most field experiments.

Several researchers have suggested that POC could reflect the management effects in a shorter time due to its rapid response to management changes (Six et al., 2002; Mao and Zeng, 2010; Guimaraes et al., 2013). However, my results suggest that caution is needed in using POC to assess management effects. The statistical power for POC never reached the acceptable level in these fields, primarily due to very high variability of POC data.

In contrast to TOC and POC, detecting the differences between managements would be more easily attained by using SMC. My results are in contrast with those of Culman et al. (2013) who observed larger long-term as well as short- term management effects on POC compared to SMC. The main reason for larger detectability of changes in SMC in my study is its lower CV as compared to those of POC and TOC. In addition, SMC is an active fraction of C with faster reaction to management effects (Franzluebbers et al., 2000). Therefore, the benefit of using SMC is two-fold: 1) it has a lower variability and therefore smaller differences between managements can be detected with an acceptable statistical power; and 2) it changes more rapidly as compared to TOC.

A promising feature of SMC data observed here is that the decrease in statistical power with depth for SMC was less than that for TOC or POC. The assessments of management effects on soil organic C in lower depths are of great interest and importance, however they have often produced controversial results because of difficulties in measurements and detectability of the changes, largely due to greater variability in deeper soil (Syswerda et al., 2011). The sharp decreases in power for TOC with depth consistent with my results have been reported by Yang et al. (2008) and Kravchenko and Robertson (2011).

SMC's ability to reflect differences due to presence of dissolved organic C contributes to its potential advantage as an indicator of C changes in deeper soil. Agricultural management can cause varied amounts of dissolved organic C to be leached into deeper soil (Toosi et al., 2012). Differences between managements in terms of dissolved organic C moving to deeper soil cannot be detected by POC measurements, and might not be detectable by measuring TOC, since dissolved organic C typically constitutes a very small portion of TOC (Kalbitz and Kasier, 2008). However, assessing SMC could reveal the changes caused by dissolved organic C transport and thus could help with detecting the differences between management practices (Buscot, 2005).

Statistical power for management differences with additional soil and topography data

Soil and topography covariates explained some of the variability in the studied C fractions and increased the statistical power for assessing the differences between management practices. Hence, a shorter span of time would be needed to detect differences between the managements when including covariates in the statistical model. The TOC detectable differences obtained in the analysis with covariates of my large field study data were similar to those observed in relatively small experiments by Yang et al. (2008), Conant et al. (2003), Schrumpf et al. (2011), and Brock et al. (2011) who reported changes less than 50% in TOC to be detectable. Therefore, using covariates would facilitate detection of changes in TOC across larger scales with diverse topography. My observations agreed with Schrumpf et al. (2011) and VandenBygaart (2006) who suggested that information about soil heterogeneity at landscape scales could help to detect changes in soil organic C stocks.

Including additional data about soil and topography in the analysis did not help to explain the variability and hence the statistical power for either POC (not reported) or SMC in the surface soil. This suggested that inherent soil properties and landscape features control variations in TOC more than variations in SMC and POC. SMC is reflective of accessible C pools from recent plant inputs (Ahn et al., 2009) and thus it can be expected that the role of soil properties in its variability is less influential. However, it is the soil properties, which govern physico-chemical processes of soil C cycling, control TOC changes (Six et al., 2002).

At the 35-50 cm depth the use of covariates increased statistical power for both TOC and SMC. The increased power of SMC at 35-50 cm depth suggested that effects of soil properties on SMC varied across soil depth. Similarly, Fierer et al., (2003) showed a varied response of C mineralization to soil properties over depths. Frequent changes in soil moisture and temperature, along with large additions of fresh plant residues likely masked the role of inherent soil properties in controlling SMC in the surface soil. In contrast, the relative role of soil properties and topography in controlling the inputs of C in deeper soil layers becomes more evident (Buscot, 2005; Gregorich et al., 2006; Salome et al., 2010;Toosi et al., 2012). This explains why the content of SMC in deeper soil was more correlated to soil properties and why using those soil properties in ANCOVA increased the statistical power.

Because of very high variability in all studied C variables at 70-90 cm depth, even the analysis with covariates failed to produce results with acceptable statistical power.

CONCLUSIONS

By assessing variability of TOC, POC and SMC at 0-90 cm depth in 10 agricultural fields I found that:

- SMC is advantageous for assessing changes in soil C because of its relatively rapid response to management and environmental changes and because of its relatively low variability. POC could be more reflective of management changes, but its large variability leads to substantially lower statistical power in analyses of POC data.

- Collecting additional information about soil and topography data and using them as covariates in the data analysis increases the statistical power and the chances for detecting statistically significant differences in C variables. It is especially important for studies conducted across large agricultural fields where heterogeneities in soil properties and landscape variations are large.
- The changes in C in deeper soil are unlikely to be detectable by measuring TOC because of its slow response to management and environmental changes. Sharp increases in TOC and POC measurement variability with depth further reduce usefulness of these variables. Low variability and relatively quick response of SMC suggests it to be a particularly useful variable to assess the management effects on deep C.

Chapter 3: Interactive effects of cover cropping and topography on soil organic carbon

ABSTRACT

The use of cover crops is a management technique that can potentially increase the amount of carbon (C) sequestered in soil. Information on cover crop's role in increasing soil C comes mostly from small uniform experimental plots. However, the magnitude of C gains in large agricultural fields may vary spatially in response to large scale topographical and soil variability. Here I assess cover crop effects on soil organic C in large agricultural fields across a diverse topographical landscape. Studied agricultural practices are conventional, low-input, and organic managements in corn-soybean-wheat rotation. The low-input and organic managements included rye and red clover cover crops as part of their rotations. The managements were implemented in twenty large undulated fields starting from 2006 and the sampling for this study took place during 2011, 2012 and 2013. Micro-plots with and without cover crops were laid out within each field at three contrasting topographical positions of "depression", "slope" and "summit". Soil samples were collected and analyzed for total organic carbon (TOC), particulate organic carbon (POC) and short-term mineralizable carbon (SMC). Cover crops had positive effects on soil POC and SMC but the mechanisms of those effects varied across topography. Five years of conventional management resulted in a loss of POC on slopes as compared to managements with cover crops. On slopes, cover crops maintained soil C by limiting soil erosion. In depressions, cover crops increase soil C in long-term by their greater C inputs. The beneficial effects of cover crops enhanced soil quality particularly in slope areas where soil organic C and crop yield is the lowest.

INTRODUCTION

Conventional agricultural management practices have increased the release of C from soil to the atmosphere (Lal, 2002) thus contributing to raised levels of atmospheric CO_2 and changing climate. During the last decades several conservation practices that can potentially slow or even revert soil C depletion have been proposed. One of such conservational practices consists of inclusion of winter cover crops in agricultural rotations (e.g., Sainju et al., 2006; Hurisso et al., 2014). Cover crops are planted after harvest of the main crop and are terminated before planting the next main crop in spring. Cover cropping practice increases the amount of residue returned to soil and thereby has a potential to increase soil organic C pools (Thomsen and Christensen, 2004; Motta et al., 2007; Weil and Kremen, 2007; Mutegi et al., 2013; Abdollahi and Munkholm, 2014).

The magnitude of cover crop effects on soil C depends on the amounts of aboveground and belowground biomass produced by cover crops (Gardner and Sarrantonio, 2012; McDanniel et al., 2014). However, biomass production varies spatially, and in the US Midwest the spatial variability of biomass produced by cover crops is mostly influenced by topographic variations (Guretzi et al., 2004; Munoz et al., 2014). The lower areas in an undulated agricultural field usually have higher soil C contents (Chan et al., 2007; Corre et al., 2002) and greater availability of soil moisture (Kang et al., 2003; Bennie et al., 2008), which promote the cover crop growth compared to other areas (Munoz et al., 2014). Therefore, a larger contribution of cover crops to C accumulation is likely to take place in topographical depressions as opposed to topographical summits and slopes. Moreover, topography driven variations in soil moisture and temperature can also influence rates of decomposition of cover crop residue (Jacinthe et al., 2002), e.g., enhancing decomposition in soil of slopes and summits as compared to colder and wetter depression areas. These combined influences could possibly result in increased spatial variability of soil C across topographically diverse fields. On the other hand, cover crop presence is known to limit soil erosion, which can decrease C redistribution across topographical gradients, and thus produce the opposite effect of reducing the spatial variability of soil C across topographically diverse fields (Jacinthe et al., 2004; Ritchie et al., 2007). Because of variety of these counter-acting influences, it can be expected that the effects of conservational management with cover crops on soil C may vary across agricultural fields with diverse topography (VandenBygaart et al., 2002; Jacinthe et al., 2004; Senthilkumar et al., 2009).

Despite the general understanding that topography can moderate cover crop management effects on soil C, the interactions between topography and cover crops have not been adequately addressed in field experiments. Most of the cover crop research is performed in small experimental plots where conditions are far more controlled and homogeneous and topographical variations are minimal as compared to actual agricultural fields (VandenBygaart, 2006). The lack of knowledge about the performance of cover crops in undulated agricultural landscapes and their effects on soil C limits accuracy in assessing the potential contribution that cover crops can make in terms of increasing soil organic C and improving soil health. This uncertainty about the performance of cover crops makes management decision-making difficult and may inhibit farmers' willingness to include cover crops in cropping rotations.

The other issue associated with studying cover crop effects on soil C is the slow response of soil organic C to management changes (Nascente et al., 2013). Changes in soil C induced by cover cropping in the short term (2-5 years) studies may not be detected by assessing soil total organic carbon (TOC) levels because of TOC's slow response to management changes (Haynes, 2000; Plaza-Bonilla et al., 2014). TOC consists of functional pools that can be divided into labile and recalcitrant pools based on their turnover rates. The labile pools have a turnover rate of days to a few years while the recalcitrant pools have a turnover rate of years to centuries (Hungate et al., 1995; Paul et al., 1999; Six et al., 2001; Guimaraes et al., 2013). The assessment of labile pools can reveal the short-term effects of management practices on C processes (Marriott and Wander, 2006; Awale et al., 2013). Various biological, chemical and physical methods have been developed to separate TOC into different fractions based on their biological availability and decomposition rate. Physical fractionation of soil organic C leads to separation of the particulate organic carbon (POC) (Cambardella and Elliott 1992). POC has been suggested as a labile fraction of soil C that reflects management effects in a short span of time (Cambardella and Elliott 1992; Collins et al., 2000; Chan, 2001; Haynes, 2005). Incubation of soil samples produce information on short term mineralizable C (SMC), which is related to other labile pools of organic C and can indicate the effects of management on soil C in short-term (Franzluebbers et al., 2000; Culman et al., 2013). Franzlubers et al. (2000) observed that C that mineralized in a short (three days) period of time was positively correlated with active C, suggesting that SMC could be a good indicator of active soil C fractions. SMC was reported to be sensitive to management changes and thus suggested as a good measure to evaluate management effects on soil organic C (Franzlubers et al., 2000; Culman et al., 2013).

The goal of the study is to assess how presence of cover crops influences soil C across topographically diverse terrain. I hypothesized that, while positive effect from cover crops will be present everywhere, its magnitude will differ at different topographical elements. The variations in magnitudes of cover crop effect will reflect topographically driven differences in cover crop growth patterns and residue decomposition rates as well as cover crop influences on processes of soil erosion. The first objective in this study is to assess the changes in soil C, as represented by TOC, POC and SMC, induced by cover cropping across topographically diverse landscape. The second objective is to assess the specific contributions that the presence of cover crops makes to changes in active soil C fractions, POC and SMC, at contrasting topographical position locations within large agricultural fields.

MATERIALS AND METHODS

Fields and Treatments set-up

The study was conducted in twenty one topographically diverse agricultural fields of the Scale-up experiment established in 2006 at Long Term Ecological Research (LTER) site of the Kellogg Biological Station, southwest Michigan (42° 24' N, 85° 24' W). The size of the fields ranges from 3.1 to 7.9 ha. The dominant soil series are Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs).

Three agricultural management practices were established in 2006: conventional, low-input and organic management. The studied fields were in corn-soybean-wheat rotation, and every year all three phases of rotation were present in all three studied management practices. The rotations of low-input and organic management treatments included two cover crops: a leguminous cover crop, red clover (Trifolium pratense L.), and a non-leguminous rye (Secale cereale) cover crop. Red clover was frost-seeded in winter wheat (i.e. in March) and then plowed into the soil prior to corn planting in the spring of the following year, i.e. in May. Rye was planted after the corn harvest, i.e. October, and then plowed into the soil prior to soybean planting in spring of the following year, i.e. in May.

The conventional management received N and K fertilizers and necessary pesticides at the rates recommended by Michigan State University Extension and soil-test. All of the pesticide, K fertilizer and a third of the N fertilizer were applied by broadcasting in spring before corn planting. The remaining of N fertilizer was applied banded within rows approximately one month after planting. The low-input management received 1/3 of the N fertilizer and herbicide amounts applied to the conventional systems. N fertilizer and herbicide were broadcast in spring before planting corn. The organic system did not receive any chemical inputs.

The primary tillage in all three management practices was spring chisel plowing. The secondary tillage consisted of disking before wheat planting, field conditioning with a soil finisher before soybean and corn planting, and inter-row cultivation for soybean and corn. The low-input and organic systems received additional inter-row cultivation and rotary hoeing as needed for weed control. Details of the management related activities (i.e. tillage, planting, harvesting, etc.) can be found at http://lter.kbs.msu.edu/protocols/104 (verified 9 Nov. 2014).

Data for this study were collected in 2011, 2012 and 2013. In 2011, I sampled 4 fields from conventional management, 5 fields from low-input management, and 5 fields from organic management. In 2012, 4 fields from conventional management, 4 fields from low-input management, and 4 fields from organic management were sampled. In 2013, I sampled 4, 5, and 3 fields from conventional, low-input, and organic managements, respectively. Only those of the Scale-up fields that had marked variations in field topography were used in this study.

Topographical attributes of the fields were derived from the digital elevation model of the area at 2 m resolution. The attributes included relative elevation, terrain slope, flow length, and flow accumulation. Linear discriminant analysis was applied to the topographical attributes to classify the studied fields into three topographical positions: summit, slope and depression as described by Munoz et al. (2012).

Within each field I identified topographical transects across summit, slope, and depression positions. The number of transects depended on the size of the field and the

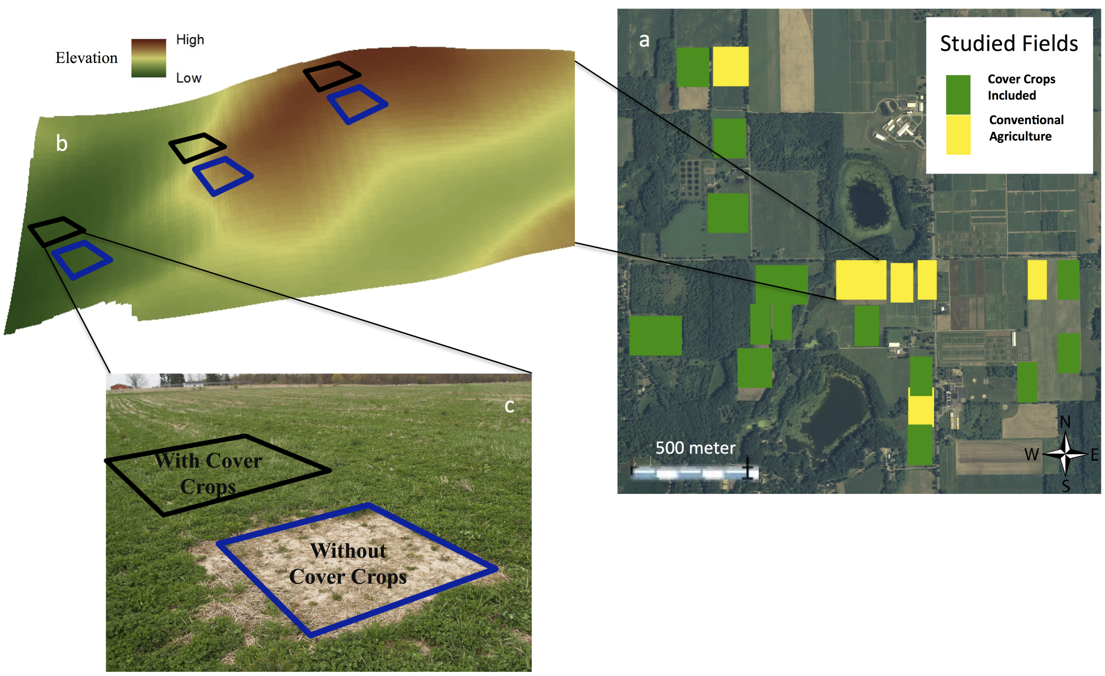


Figure 3.1. a) The study area showing locations of the monitored fields from the three studied treatments; b) a schematic presentation of a topographical transect in one of the studied fields; and c) a 4×4 m cover crop and bare micro-plot within a topographical position.

topographical variability within the field. In larger, more undulated fields up to four transects were identified whereas small and relatively flat fields typically had only one transect. For the fields under cover crop management along each transect in each of the three topographical positions I laid out two adjacent 4 x 4 m micro-plots. One of the micro-plots had the cover crops similar to the entire field ("cover"), while the other was kept bare of weeds and cover crop plants by herbicide applied in early spring after plant emergence ("bare") (Fig. 2.1). In conventional management fields, which did not have cover crop in the rotation, I established only bare micro-plots. Soil and cover crop biomass was collected from micro-plots as described below.

Sample collection and analysis

I sampled biomass of rye and red clover from all cover crop micro-plots in spring 2-3 days before their termination. For biomass sampling a 0.5 by 0.5 m quadrant was randomly thrown at a corner of a cover micro-plot and the total aboveground cover crop biomass within the quadrant was collected. The biomass sample was dried and weighed after drying. Each year I randomly selected 10 samples of cover crop biomass for C content measurements via dry combustion method using a Costech ECS 4010 CHNSO analyzer. Estimates of the C inputs from above-ground cover crop biomass were then obtained by multiplying the amount of biomass by the average C% from the 10 measured samples.

I sampled biomass of corn and soybean from the areas adjacent to the cover micro-plots in low-input and organic fields and to the bare micro-plots in conventional fields at the harvest time in falls of 2011 and 2012. The total biomass was measured along the two crop rows of 1 m length. I dried the biomass sample in a 70^{o}C oven for one week and weighed it after drying. I also used the corn and soybean biomass data available from samples collected form the cover micro-plots in 2010 (unpublished data). To estimate the biomass of corn and soybean during 2006-2009 period and to estimate the biomass of wheat during 2006-2012 I used corn, soybean, and wheat yield data collected from the studied fields using yield-monitors from the start of the experiment in 2006 (the data are available at KBS LTER website at http://lter.kbs.msu.edu/datatables).

Soil samples at micro-plots were collected from 0-15 cm depth three times during each growing season of 2011, 2012, 2013. The first soil sampling was conducted in spring 2-3 days before cover crop termination. The second sampling was conducted two months after plowing, and the final sampling was conducted after the harvest of the main crop. After each sampling the soil samples were transferred to the lab and air-dried.

TOC was measured in soil samples collected from all topographical positions of 15 fields in spring of 2011. TOC was measured via the dry combustion method with Costech ECS 4010 CHNSO analyzer following procedures described in Robertson et al. (1999).

POC was measured in all of the collected samples per Cambardella and Elliott (1992) with slight modifications. Specifically, 60 ml of 5 g/l sodium meta-phosphate solution was added to 20 g of soil in a 200 ml glass jar. The jar was sealed and shaken for 16 hours on a rotary shaker at 180 rounds per minute. Then, the contents of the jar were sieved through a 0.053 mm sieve. The material remaining on the sieve consisted of sand and particulate organic matter. POC was obtained by analyzing the remaining material for C content using a Costech ECS 4010 CHNSO analyzer.

SMC was measured in all of the collected samples following the procedures of Franzluebbers et al. (2000) and Culman et al. (2013). Specifically, 10 g of air-dried soil was placed in a mason jar. The water holding capacity was determined for each soil sample as instructed by Haney and Haney (2010) and water in an amount of approximately 50% of water holding capacity was added to each jar (around 2 ml). The jars were then capped and placed in 25^{o}C incubator. After 72 hours the concentration of CO_2 was measured in 5 ml gas samples taken from each jar using LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE).

The decomposition rate of cover crop residue was measured using litter bags (Robertson et al., 1999). The biomass of red clover and rye was sampled form areas close to the micro-plots. Approximately 7 grams of air-dried plant residue cut into 5-10 cm pieces was placed in 15 x 15 cm nylon mesh litter bags (1.5 mm mesh size). The litter bags were buried for two months (from June 18 to August 18) in 8 fields with corn in 2013. At each micro-plot I buried one bag of red clover and one bag of rye litter at 0-15 cm depth for a total of 90 bags. The material remaining in the bags was burned in a muffle furnace at 400^{o}C and for each bag the amount of ash free material remaining was calculated.

Sand content was measured for all of the samples as a part of POC measurement. Soil bulk density and particle size distributions for 10 of the studied fields were available from a previous study (Ladoni et al., 2015, in review). Soil bulk density of the undisturbed soil was measured as instructed by Hao et al. (2008). Particle size distribution was analyzed using the pipet method (Kroetsch and C. Wang, 2008).

Statistical analysis

Two sets of data analyses were conducted using the MIXED procedure of SAS 9.2 (Cary, NC). The purpose of the first set of analyses was to address the study's objective 1, that is, to compare the conventional and cover crop based managements at different topographical positions. For this analysis I used data from micro-plots in conventional management fields and from cover micro-plots in the cover crop based management fields. The statistical model included agricultural management, topographical position and the interaction between them as the fixed factors. Fields nested in agricultural management were included in the model as the random factor and were used as an error term for testing the agricultural management effect. Post-hoc power analysis was conducted following procedures outlined by Stroup (2002). As part of the power analysis I calculated minimal detectable differences between the managements that could be declared as statistically significant at $\alpha = 0.05$, given the observed variability, with a power of 80%.

The purpose of the second set of data analyses was to address the study's objective 2, that is, to directly assess the effects of cover crop presence on C measures at different topographical positions. For this analysis I used the data from bare and cover micro-plots from the fields of low-input and organic management treatments. The statistical model for this analysis included topographical position, cover crop presence, and the interaction between them as the fixed factors. Fields and interaction between fields and topographical positions were included in the model as random factors. Fields essentially served as a blocking factor, while the field by topography interaction was used as an error term for testing the effect of topography.

For both sets of data analysis I added percentage of sand and terrain slope as covariates to the statistical models and kept covariates in the model if their contribution was statistically significant at $p < 0.1$ level. The details of the analysis of covariance are described in chapter two.

Normality of the residuals and homogeneity of variance assumptions were checked for all studied variables in both sets of data analyses. Means of agricultural management treatments, topographical positions, and their combinations were compared when the respective main and or interaction effects were statistically significant at $p < 0.05$.

Linear regression analyses were conducted to examine influences of cover crop biomass on SMC and POC. I used the difference in SMC between each pair of cover and bare micro-plots as the dependent variable in the linear regression analysis. The cover crop biomass from the respective cover micro-plot was used as the independent variable. I also ran linear regression analysis between the difference in POC between cover and bare micro-plots and the cover crop biomass from the respective cover microplot. The regression analyses were conducted using the MIXED procedure of SAS, which enabled us to include fields nested within year in the random statement of the regression analyses.

Table 3.1. Means and standard errors (in parentheses) of topographical features for 20 studied fields and soil properties in 10 studied fields.

	Agricultural management		
	Conventional	Low-input	Organic
Slope percent	5.6 (0.9)	5 (1)	5.1 (1)
Relative elevation (m)	3.8 (1.4)	5.9 (1.8)	4.5 (1.3)
	Topographical position		
	Depression	Slope	Summit
Sand %	45 (4) a	65 (4) b	62 (4) b
Clay %	12 (1)	10 (1)	10 (1)
Bulk density Mg m^{-3}	1.44 (0.04) a	1.56 (0.04) b	1.56 (0.04) b

Different lowercase letters mark significant differences among the topographical positions ($p<0.05$).

RESULTS

No significant differences in slope percent and relative elevation existed between the fields from the three studied managements (Table 3.1). Topography had a significant influence on sand content and soil bulk density ($p<0.05$). Depressions had the smallest soil bulk density and sand content (Table 3.1).

Table 3.2. Means and standard errors (in parentheses) of total organic carbon (TOC), particulate organic carbon (POC) and short-term mineralizable carbon (SMC) after five years of practicing the three studied managements in 20 studied fields across all three studied topographical positions.

Soil C fraction	Agricultural management			Minimal detectable difference at type II error of 0.20
	Conventional	Low-input	Organic	
TOC, %	1.1 (0.06)	1.0 (0.05)	1.0 (0.06)	0.48
POC, %	0.26 (0.04)	0.18 (0.03)	0.23 (0.03)	0.59
SMC, $mgCO_2\ kg^{-1}day^{-1}$	44 (2.1)	42 (1.9)	45 (2)	22

Effects of management on TOC, POC and SMC

By 2011 the studied management practices have been in place for 5 years (since 2006). However, I observed no statistically significant differences among the managements for either TOC, POC, or SMC (Table 3.2) across all topographical positions of all 14 sampled fields. To further examine the lack of significant differences I conducted post-hoc power analysis and assessed the minimal detectable differences (MMD) between the managements. Power analysis revealed that 50%, 200% and 50% changes from the initial TOC, POC and SMC levels, respectively, would have to have occurred in order to be detected as statistically significant in my study.

Topography modifications of management effects on soil C

As expected, topography had a substantial effect on TOC, POC and SMC in both conventional and cover crop-based managements, with highest levels observed at depressions, followed by summits and slopes (Fig. 2.2). Note that since the results for all three studied C fractions were very similar in the organic and low-input fields, for the analyses of topographical influences I combined the data from organic and low-input management treatments, referred to from now on as management with cover crops.

An interesting numeric trend was observed for conventional management having higher levels of all three studied C fractions as compared to the management with cover crops in topographical depressions. At topographical slopes the cover crop management tended to have higher values of POC and SMC than conventional management (the difference was statistically significant for POC). In conventional management the differences between topographical depressions and slopes in terms of POC and SMC were numerically higher as compared to the cover crop management (Fig. 3.2).

Cover crop contribution to POC and SMC across topography

The aboveground inputs of C from both red clover and rye residue in depressions were significantly larger than that in slopes (Fig. 3.2; $p<0.05$).

The results of micro-plot data analyses indicated that soil covered with red clover had higher SMC levels in all topographical positions ($p < 0.1$ and $p<0.05$) (Fig. 3.4a). Soil in rye cover micro-plots had significantly higher SMC only on summits (Fig. 3.5a) ($p<0.05$). POC in cover microplots was numerically higher than that in bare plots, but the differences were not statistically significant (Figs. 3.4b and 3.5b).

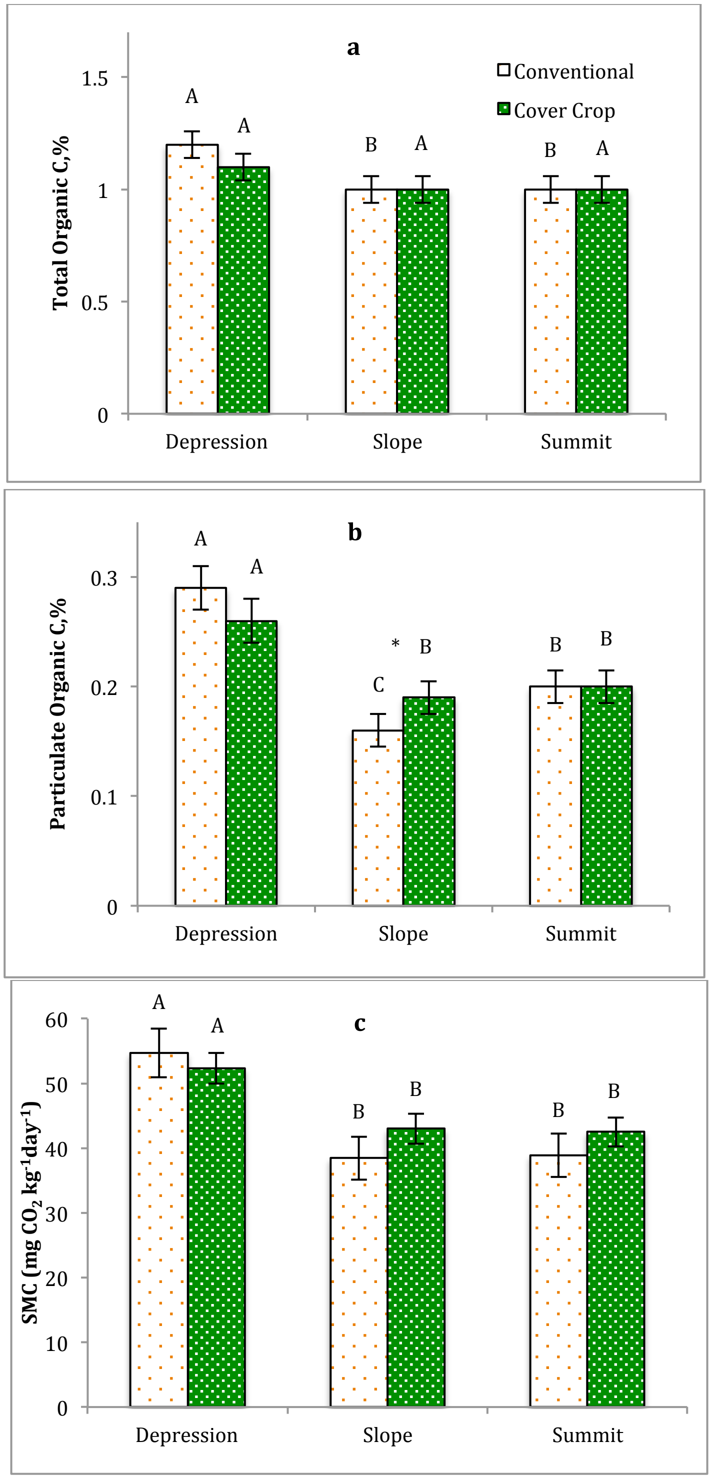


Figure 3.2. Total organic carbon (TOC), particulate organic carbon (POC) and short-term mineralizable carbon (SMC) in the studied fields after five years of practicing the three studied managements. Error bars represent standard errors. Bars with different letters indicate significant differences between topographical positions within the same management type ($p < 0.05$). The * shows significant difference between conventional and cover crop ($p<0.05$).

The regression analysis between SMC differences in cover-bare microplots and cover crop biomass showed that the aboveground biomass of red clover was positively related to increases in SMC in cover micro-plots on summits ($p<0.1$), while rye biomass was positively related to SMC increases on slopes and summits ($p<0.1$). The relationship between cover crop biomass and increase in SMC was not statistically significant in depressions. The regression

Table 3.3. Relationships between cover crop biomass and short-term mineralizable carbon (SMC).

Dependent variable	**Independent Variable (Mg ha^{-1})**	**Topographical position**	**Regression Slope**	**p-value**
SMC difference between cover and bare (mgCO$_2$ kg^{-1}day^{-1})	Red clover biomass	Depression	0.2	0.70
		Slope	1.0	0.10
		Summit	0.9	0.09
	Rye biomass	Depression	4.5	0.14
		Slope	10.0	0.06
		Summit	18.3	0.001
POC difference between cover and bare (%)	Red clover biomass	Depression	0.02	0.03
		Slope	0.01	0.10
		Summit	0.00	0.20
	Rye biomass	Depression	0.08	0.01
		Slope	0.10	0.03
		Summit	0.14	0.01

analysis showed significant relationship between difference in POC between bare and cover microplots and red clover biomass only in depressions while the difference in POC between bare and cover was significantly related to rye biomass across all topographical positions (Table 3.3). Unfortunately, the litter bag analyses did not allow identification of differences in residue decomposition conditions at different topographies. In more than 95% of both red clover and rye residue litter bags the residue completely decomposed after two months in soil (data not shown).

Significantly larger SMC was observed in the soil under red clover compared to bare soil at the beginning of the growing season before termination of red clover, as well as two months after red clover termination and after the main crop harvest ($p<0.1$) (Fig. 3.6a). Numerically higher SMC was observed in the soil under rye compared to bare soil before cover crop termination and two months after cover crop termination (Fig. 3.6b); however, the differences

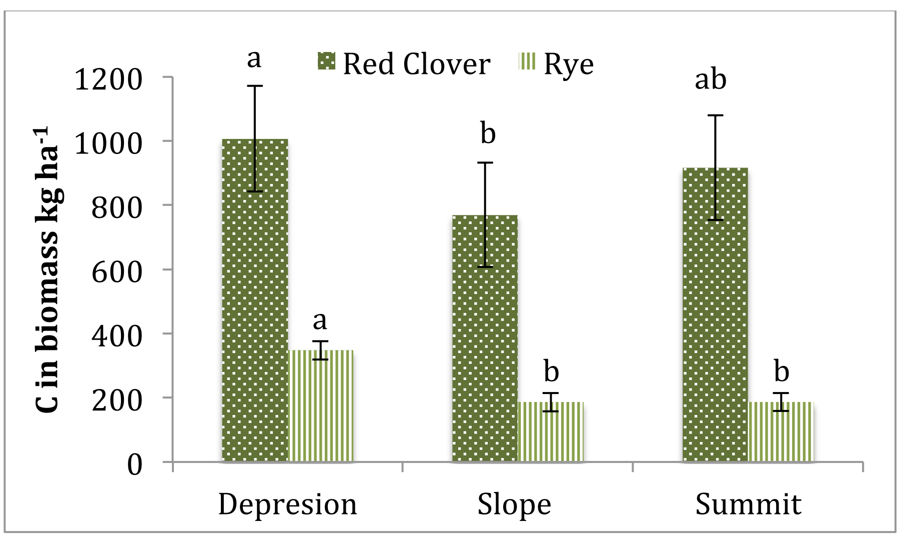


Figure 3.3. C returned to soil from aboveground biomass of cover crops sampled in spring before termination. The data shown are averages from 2011, 2012 and 2013 results. Error bars represent standard errors. Bars with different letters show significant differences between the topographical positions for each of the cover crops ($p<0.05$).

were not statistically significant ($p<0.1$). The effects of cover crops on POC during the growing season were not statistically significant (data not shown).

DISCUSSION

Effects of management on TOC, POC and SMC

The three studied management practices were substantially different from each other in terms of several components relevant to soil C, including differences in C inputs due to cover crop presence, differences in C inputs due to differences in main crop growth, and differences in the fertilizer inputs. Below I assess the relative contributions of these components in the three studied management systems during the five-year period from the start of the experiment in 2006 to the 2011 sampling.

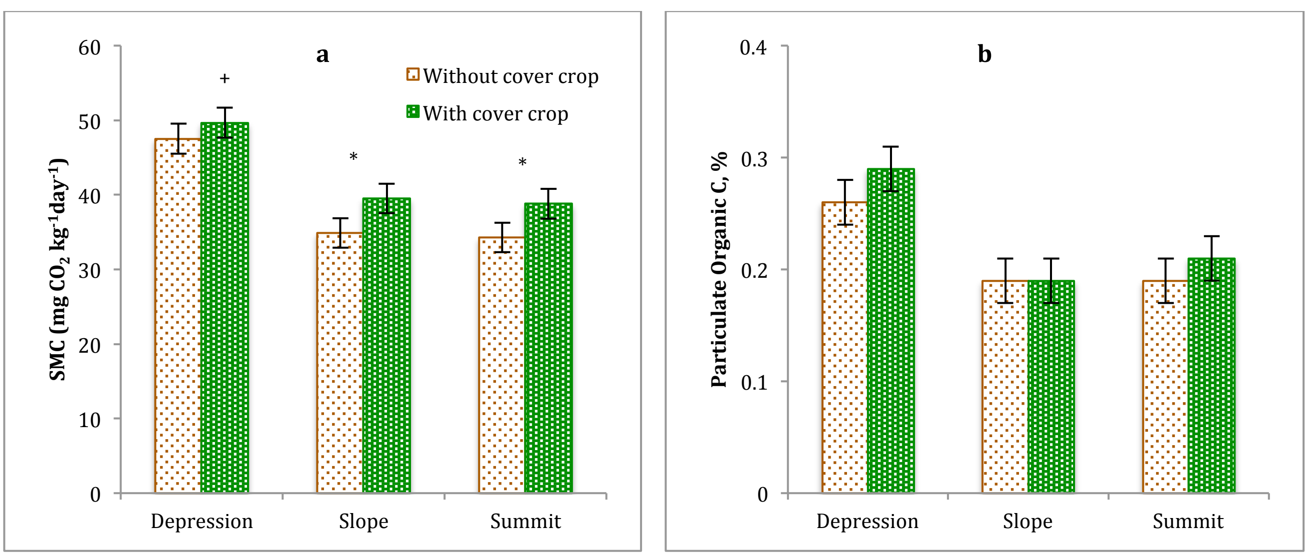


Figure 3.4. Short-term mineralizable carbon (SMC) and particulate organic carbon (POC) in bare and cover micro-plots of the studied topographical positions following red clover cover crop. The data shown are averages from 2011, 2012 and 2013 results. Error bars represent standard errors. The * and $^{+}$ mark the cases when the differences between cover and bare micro-plots were statistically significant at $p<0.05$ and $p<0.1$, respectively.

The five years of practicing the managements consisted of one season of red clover, one season of rye and another season of either red clover or rye (depending on the rotation). Note that no data on cover crop biomass was collected from the experiment prior to 2011. Thus I used the historical data for cover crop biomass from the adjacent LTER research plots of the same rotation and management practices collected from 2006-2011 (data are available at KBS LTER website at http://lter.kbs.msu.edu/datatables). The estimates of the amounts of C inputs to soil from the aboveground cover crop biomass constituted 2022 and 2582 kg ha^{-1} in the low-input and organic managements, respectively. If I use a root-to-shoot ratios of 55% (Snapp et al., 2007) and 30% (Hùgh-Jensenand Schjoerring, 2001; Puget and Drinkwater, 2001) for rye and red clover respectively, the total amount of C inputs from cover crops at the time of incorporation can be estimated as 3725 kg ha^{-1} in low-input and 4479 kg ha^{-1} in organic

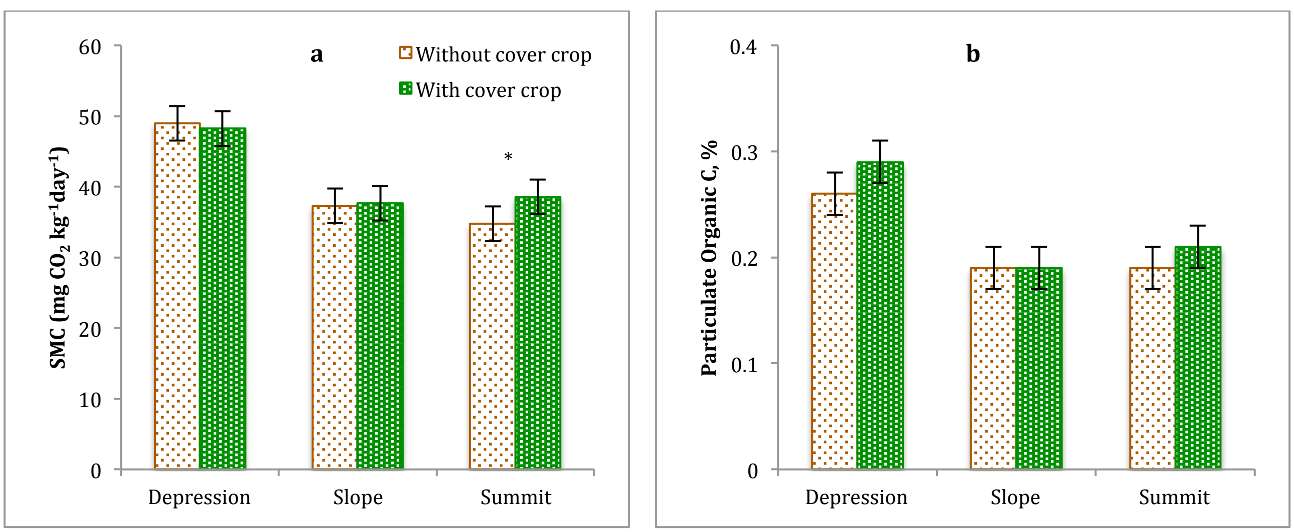


Figure 3.5. Short-term mineralizable carbon (SMC) and particulate organic carbon (POC) in bare and cover micro-plots of the studied topographical positions following rye cover crop. The data shown are averages from 2011, 2012 and 2013 results. Error bars represent standard errors. The * indicates the cases where the differences between cover and bare was statistically significant at $p<0.05$.

managements, respectively. This is a conservative estimate, as it does not account for C inputs into soil via root exudates during cover crop growth.

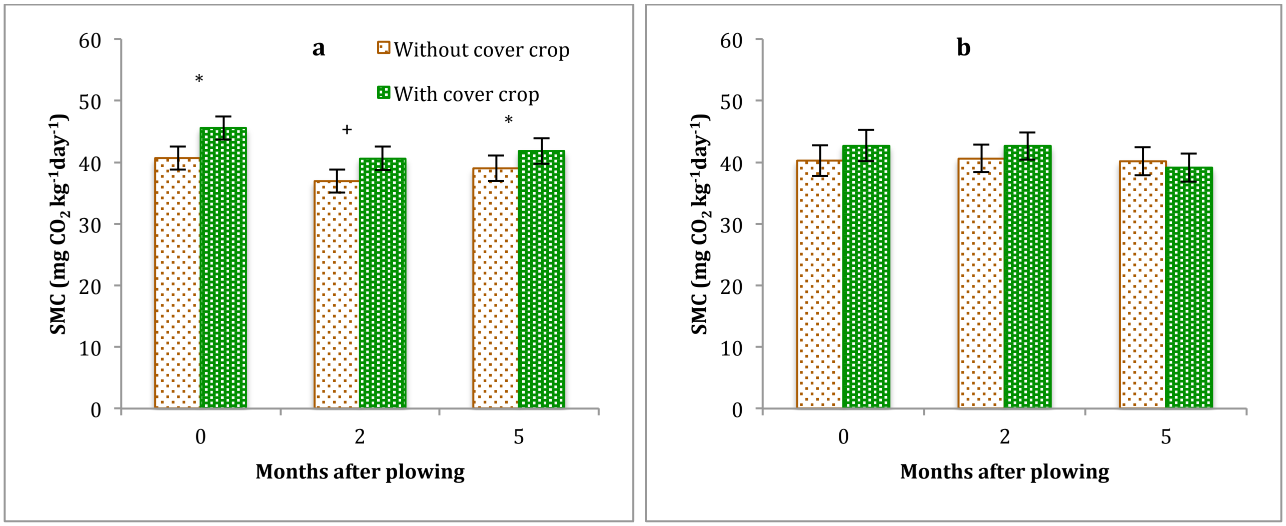


Figure 3.6. Short-term mineralizable carbon (SMC) in bare and cover micro-plots of the studied sampling dates following red clover (a) and rye (b) cover crop. The data shown are averages from 2011, 2012 and 2013 results. Error bars represent standard errors. The * and $^+$ mark the cases when the differences between cover and bare micro-plots were statistically significant at $p<0.05$ and $p<0.1$, respectively.

Assessing the net primary production of the studied fields indicated that the total amount of C in the biomass of main crops during the period from 2006 and 2011 was 13460, 10480, and 8190 kg ha^{-1} for conventional, low-input and organic management, respectively. Therefore, a larger residue input into soil from the main crops was expected under conventional management in these fields compared to organic and low-input fields.

The other main difference between managements was application of synthetic N fertilizers. The application of N fertilizer in conventional and low-input managements increased the biomass production of corn. However, the application of N fertilizer may also inhibit the growth of roots of main crops (Lu et al., 2011) and accelerate the decomposition of residues (Mulvaney et al., 2009). However, Grandy et al. (2013) reported no effects from N fertilizer on residue decomposition in the same study area.

The differences in C inputs of cover crops, residue inputs of main crops and fertilizer application may lead to differences in soil C. Reports from the adjacent LTER convincingly demonstrated that, after >10 years of implementation, the organic management resulted in higher soil TOC levels as compared to those of the conventional management (Robertson et al., 2000; Grandy and Robertson, 2007; Hao and Kravchenko, 2007; Senthilkumar et al., 2009; Syswerda et al., 2011); while TOC in low-input management was numerically but not statistically significantly higher than that of conventional management (Syswerda et al., 2011). Syswerda et al. (2011) reported that only a 20% change in TOC occurred after 12 years of continuous cover cropping. Thus I realized that only five year duration of this study probably would not be sufficient to generate detectable differences in TOC. However, I did expect to observe differences in POC and SMC, since these C variables are known for their more rapid reaction to management changes (Haynes, 2005; Culman et al., 2013).

However, the sizes of changes that could be detected, as suggested by power analysis, for TOC and POC were much greater than the effects of cover crop presence that could be expected to take place in 5 years in the studied area (Table 3.2). The most likely possible cause for the large MMD values is extremely large variability of the studied C fractions due to high soil and topographical diversity of the large fields used in my study. Large variability translates into smaller statistical power and larger MMD values. VandenBygaart (2006) also reported that one of the difficulties of studying soil C at the landscape scale is multiple sources of variation contributing to greater variability, and thus to low power of statistical comparisons. Large topographical variations typical to agricultural fields of the Great Lakes region is one of the main sources of variations in soil properties (Kravchenko and Bullock, 2000; Yoo et al., 2006), crop biomass (Jinag and Thelen, 2004; Munoz et al., 2014), including soil C (VandenBygaart and Kay, 2004; Senthilkumar et al., 2009) across agricultural fields. Therefore, the next step of my study in examining the effect of management on soil C in these large fields was to explore and account for the contribution of topographical variability.

Topography modifications of management effects on soil C

The observed spatial trends under managements (Fig. 3.2) probably resulted from a combination of three main influences: spatial redistribution of soil C in the fields due to erosion (Ritchie et al., 2007), spatial patterns in C inputs from cover crops and main crops, and differences in decomposition rates across topographical gradients.

While data collected in this study do not allow us to explicitly evaluate the role of erosion, it is reasonable to assume that in topographically diverse fields of this study it plays an important role in C redistribution. Jacinthe et al. (2004) reported that as much as 41.3 C kg ha^{-1} per year was a subject of erosion in agricultural fields under tillage system similar to mine in northern Ohio with terrain slopes comparable in steepness with those of my study. My visual observations indicated that soil on the slopes under conventional management was particularly prone to erosion during heavy precipitation events in falls and early springs. Erosion was reported to have a greater effect on redistribution of POC, due to movement of smaller pieces of plant residue, as compared to other C variables (Jacinthe et al., 2002). Greater difference between POC in slopes and depressions under conventional management observed in my study thus can be an indirect indication of erosion effects. While somewhat smaller differences between depressions and slopes in management with cover crops could be seen as an indicator of lower erosion under cover crops (Fig. 3.2b). These observations are consistent with results of Jacinthe et al., (2004) who reported that presence of cover crops during fall and early spring decreased the chance of erosion from slopes.

Cover crop contribution to POC and SMC across topography

I assessed the specific contribution of cover crop presence to soil C at different topographical positions by using the data from the cover-bare micro-plot pairs on each of the topographical positions of the cover crop fields. The first advantage of having the micro-plot pairs located in close proximity to each other was to be able to assess the cover crop effects on POC and SMC across large fields while minimizing the influences of soil and topographical variability on the cover crop contributions. The second advantage was that, unlike the soil C trends from the entire fields under managements with or without cover crops that were affected both by the cover crop growth patterns and by erosion, the micro-plots allowed us to specifically focus on the influence of the cover crop presence. Indeed, in depressions it is safe to assume that both the bare and the cover micro-plots are equally influenced by the influx, if any, of eroded material from the higher located areas. While for the micro-plot pairs in summits and slopes it can be assumed that the magnitude of losses of soil from the bare plots due to erosion, if any, is much smaller than what can be expected from similar topographical positions in fields entirely under conventional management without cover crops. During the study period I observed no visual indications of soil erosion occurring at the bare plots of summits and slopes.

As expected, cover crop presence, red clover in particular, and the extra C inputs that it provided, resulted in a tendency for a gradual increase of active C pools (Fig. 3.4a). My results are in agreement with those of Steenwerth and Belina (2008) who observed positive effects from cover crops on mineralizable pools of soil C. The results are consistent with results of Franzlubers (2002) and Culman et al. (2013) who found SMC to be reflective of management effects. However, my observations are in contrast with those of Haynes (2005) and Plaza-Bonilla et al. (2014) who reported POC to be the sensitive fraction of soil C that can reflect management changes. High variability of POC data experienced in this study is likely the reason for lack of statistical significance (chapter 2). As I mentioned earlier, an increase as large as 200% was needed for POC to be detected as statistically significant. Presence or absence of large pieces of plant residue increases the variations in POC values, hence increases the measurement errors. This, in turn, results in low statistical power in detecting differences between the management.

The larger cover crop biomass returned to soil in depression areas (Fig. 3.3) is most likely related to better growing conditions there, driven by higher soil organic C (Table 3.2), lower sand content (Table 3.1), and better soil structure in depressions as compared to slopes and summits. Thus I hypothesized a presence of a feed-forward mechanism of better cover crop growth because of, in part, higher soil C, followed by still a further increase in soil C due to greater C inputs from the well growing cover crops in depression areas. However, the results did not fully support this hypothesis. The effect of cover crop presence on SMC actually tended to be smaller in depressions as compared to slopes and summits in red clover (Fig 4a) and smaller than in summits in rye (Fig. 3.5a). Moreover the regressions of the differences in the SMC levels between bare and cover micro-plot pairs versus the cover crop biomass suggested larger effects of red clover and rye on SMC in slopes and summits compared to depressions (Table 3.3). In a near-by experimental site, depressions under cover crops management had higher soil CO_2 emissions than depressions under conventional management, while no differences in CO_2 fluxes between cover and conventional managements were observed in summits (Negassa et al., 2015, in review). This observation points to possibly faster decomposition rates of cover crop C inputs in depression areas, and explains why greater cover crop biomass inputs in depressions did not turn into greater SMC differences in this study. My results are similar to those reported by Steenwerth and Belina (2008). They observed that cover crops increased C mineralization, however, the increased mineralization was not related to the amounts of aboveground cover crop biomass. They suggested that greater root biomass and root exudation to be the likely reasons for larger SMC under cover crops. My litter bags results demonstrated that added cover crop material can completely decompose in the studied soil in just two months. Unfortunately the litter bag analyses did not allow identification of differences in residue decomposition among the studied topographical positions.

Tendencies for positive response of soil SMC and POC to cover crop inputs on slopes and summits are especially important (Table 3.3). These are the areas within the fields with the lowest soil fertility and the lowest crop yields (Munoz et al., 2014), therefore increasing organic C there would be of interest to farmers.

The temporal patterns of cover crops on SMC and POC observed here are similar to those reported by Steenwerth and Belina (2008) who observed an increased in potential microbial respiration during the growing season starting in spring before cover crop termination. The smaller role of the aboveground cover crops biomass on SMC (Table 3.3), fast decomposition of cover crop residue, and the increased SMC even before the aboveground biomass was returned to soil indicates that the roots of cover crops play an important role in increasing active pools of soil C. The study of cover crop roots are rare due to difficulties associated with measuring root activity, however, a few studies showed that cover crops produced larger belowground biomass than aboveground (Gardner and Sarrantonio 2012). Using C isotopes Steinbeiss et al. (2008) reported that soil C storage was derived more from root biomass than from the aboveground biomass. Gelixner (2013) suggested that the aboveground plant biomass may decompose rapidly by soil microorganisms while the activity of roots and its association with soil particles is what governs the soil C storage. The roots of cover crops exude labile C that enhances microbial activity and hence the SMC (Kong et al., 2011; McDaniel et al., 2014).

Another interesting observation was that larger SMC values in red clover microplots as compared to bare micrplots were observed as far as five months after the red clover termination. This suggested that even though red clover residue may decompose rapidly, its positive effects on soil C could last for a long time. My results are consistent with those of Steenwerth and Belina (2008) who reported that positive effects of cover crops on C mineralization lasted till the end of growing season. However, my results are not in agreement with those of by Culman et al. (2013) who reported significant differences in SMC between managements during growing season but no significant differences at the end of growing season. My observations suggested that red clover presence could gradually increase active pools of soil C and increase the soil quality in the long-term.

CONCLUSIONS

- The effects of five years agricultural management on TOC, POC and SMC were not detected across large fields. However, when the managements were compared in three distinct topographical positions, i.e. depressions, slopes and summits, the conventional management tended to have lower POC on the slopes compared to managements with cover crops. This indicates a gradual loss of C pools on topographical slopes of conventional management compared to management with cover crops, possibly as a result of erosion.

- The magnitude of cover crop effects on SMC and POC varied across topography. Numerically higher POC was observed in depressions and slopes due to presence of rye and red clover. Red clover had significantly positive effects on SMC in slope and summit. The positive effects of cover crops in slopes could be due to their effects in limiting C depletion by soil erosion.
- The aboveground biomass of cover crops was found to be responsible for a small portion of positive effects of cover crops on SMC and POC. Most of aboveground biomass of cover corps decomposed within two months after they were returned to soil. In addition, positive effects of red clover on SMC started before its aboveground biomass was returned to soil. These evidences suggested that root activity and growth of cover crops could play an important role in controlling the effects of cover crops on soil C pools.

Chapter 4: Interactive effects of cover crops and topography on potential mineralizable nitrogen and soil nitrate levels

ABSTRACT

Supplying optimum levels of soil nitrate (NO_3^--N) for plant growth during the growing season and across large agricultural fields is a challenge for conservational agricultural systems with cover crops. Knowledge about cover crop effects on NO_3^--N comes from small mostly flat research plots and performance of cover crops across topographically diverse agricultural fields is not well understood. Our objective was to assess the cover crop effects on NO_3^--N levels across diverse topographical landscape. The studied agricultural practices are conventional, low-input, and organic managements in corn-soybean-wheat rotation. The low-input and organic managements include rye and red clover cover crops as part of their rotation. The managements were implemented in twenty large undulated fields in Southwest Michigan starting from 2006 and the sampling took place during 2011, 2012 and 2013. Observational micro-plots with and without cover crops were laid within each field on three contrasting topographical positions of “depression”, “slope” and “summit”. Soil samples were collected multiple times during the growing season and analyzed for NO_3^--N and potential mineralizable nitrogen (PMN). The results showed that temporal and spatial distribution of NO_3^--N was similar under all three managements. Red clover had a significant positive effect on NO_3^--N across all topographical positions. Rye had a significant negative effect on NO_3^--N in topographical depressions but not in slope and summit positions. Both cover crops had positive effects on PMN, however, those

effects were not detected as statistically significant. The spatial variations in the amounts of residue from cover crops were found to be an important factor controlling the effects of cover crops on soil mineral nitrogen across topographically diverse fields.

INTRODUCTION

Keeping plant available soil nitrogen (N) at an optimum level evenly across large agricultural fields during the growing season to support plant growth and production can be a challenge for agricultural management systems. Nitrogen comprises about 2% of crops biomass and is frequently deficient in crop production. Soil N exists in organic and mineral forms with organic forms comprising 95% of soil N pools (Havlin et al., 1999). The two forms of soil mineral N majorly absorbable by plants are nitrate (NO_3^--N) and ammonium (NH_4^+-N) (Bair et al., 2008). In well-aerated soils during the growing season NO_3^--N becomes the main form of available N for crops as the microbial activity transforms NH_4^+-N into NO_3^--N (Addiscott, 2005). It is crucial to keep the NO_3^--N levels at optimum because in one hand low levels of soil NO_3^--N can limit crop production and on the other hand the high amounts of NO_3^--N can lead to environmental pollution (Cambardella et al., 1999; Di and Cameron, 2002). The levels of soil NO_3^--N vary across space and over time. A proper agricultural management needs to consider site-specific variations and temporal patterns in soil NO_3^--N to supply optimum amount of NO_3^--N from both organic and mineral N fertilizer sources for the plant growth.

In the US Midwest, including cover crops in the rotation grew in popularity after increasing environmental concerns about air and water pollution and soil degradation (Sainju and Singh, 2008; Fageria, 2007). The leguminous cover crops have the ability to fix the atmospheric

N, assimilate it into their biomass and release it into soil in plant absorbable forms. Non-leguminous cover crops assimilate the excess NO_3^--N amounts in soil during winter (Ruffo et al., 2004). After cover crop biomass (both leguminous and non-leguminous) is returned to the soil, microbial mineralization transforms the organic N into mineral forms (Haynes 1997). The benefits of cover crops in supplying N for main crops were extensively studied (Cueto-Wong et al., 2001; Kue and Jellum, 2002; Schomberg and Endale, 2004; Collins et al., 2005; Jewett and Thelen, 2007; Asagi and Ueno, 2009). However, most of the previous research on cover crops was carried out in controlled, and in many cases small, experimental plots. Subsequently, the ability of cover crops to supply N for the subsequent main crop across large real agricultural fields remains not well understood.

The topographical variations typical to large agricultural fields could have a substantial impact on dynamics of soil mineral N and performance of cover crops (Munoz et al., 2014). Soil organic matter levels vary in response to variations in topography and the amount of organic matter is regarded as one of the main sources of NO_3^--N in soil (Chan et al., 2007; Dharmakeerthi et al., 2005). Corre et al. (2002) suggested that spatial variations in soil organic matter, soil microbial biomass, natural drainage, plant growth, and water and nutrient redistribution caused by topography are the main factors controlling the dynamics of soil mineral N. Kay et al. (2006) found that along with weather, landscape topographical patterns accounted for most of the variations in plant available N. Priyashantha et al. (2007) also observed an increasing trend in the NO_3^--N levels from higher areas to valleys in the landscape.

Interactions between cover crop presence and topography driven factors, such as soil organic matter levels and erosion, make study of their combined effects on soil N difficult. For example, variations in soil organic matter across topography lead to variations in cover crop growth, thus varied effects of cover crop on soil mineral N. On the other hand, cover crops influence surface runoff in topographically diverse fields, thus controlling redistribution of soil mineral N through the landscape.

The temporal variation in soil NO_3^--N provided by cover crops is another factor that controls the magnitude of the benefits that cover crops can supply (Mirsky et al., 2009; Parr et al., 2011; Wortman et al., 2012). The beneficial effects of cover crops on N will only occur if the N supplied by cover crops becomes available during the period of high N uptake by the main crop (Kramberger et al., 2009). The N in the residue of cover crops becomes available by microbial mineralization. The rate of the mineralization depends on the C/N ratio of cover crop residues (Jensen et al., 2005), and on environmental factors, such as soil moisture and temperature (Fageria, 2007; Wortrman et al., 2012). Topographical variations in soil organic matter, soil moisture and soil temperature may cause differences in mineralization rates across large agricultural fields (Dharmakeerthi et al., 2005). The management practices such as timing of cover crop termination and planting of the succeeding main crop also control the synchrony between the release of mineral N from cover crop residue and the main crop N demands (Dabney et al., 2001).

Uncertainty about the performance of cover crops across large agricultural fields is among the reasons for low adoption of cover cropping practices by farmers despite its documented benefit in a research context. Knowledge about unknown effects of topography and its interactions with cover cropping will resolve many of the ambiguities about cover crop performance. Therefore, a field-scale approach that considers both spatial and temporal cover cropping effects on soil NO_3^--N levels within farmer-practiced agricultural managements will help farmers to include cover crops as a source of fertilizers in their agricultural practices more successfully.

In this study we assessed the field scale spatial and temporal variability of NO_3^--N in large agricultural fields under contrasting, i.e., conventional and cover crop based, management and contrasting topography. We specifically focused on NO_3^--N as the form of N of primary use to plants. We hypothesized that the magnitude of the effects of cover crops on soil NO_3^--N depends on the amount of biomass produced by cover crops, therefore the extent of cover crop effect on NO_3^--N varies across a topographical transect. Our first objective was to assess temporal and topography driven variations of soil NO_3^--N in different agricultural managements and to identify the factors that control these variations. Our second objective was to assess the particular contribution of cover crops to soil NO_3^--N levels across topographical transects and during the growing season.

MATERIALS AND METHODS

Fields and Treatments set-up

The study was conducted in twenty topographically diverse agricultural fields of the Scale-up experiment established in 2006 at Long Term Ecological Research (LTER) site of the Kellogg Biological Station, southwest Michigan (42° 24' N, 85° 24' W). The size of the fields ranges from 3.1 to 7.9 ha. The dominant soil series are Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs).

Three agricultural management practices were established on different fields in 2006: conventional, low-input and organic management. The studied fields were in corn-soybean-wheat rotation, and every year all three phases of rotation were present in all three studied management practices. The rotations of low-input and organic management treatments included two cover crops: a leguminous cover crop, red clover (*Trifolium pratense* L.) and a non-leguminous rye (*Secale cereale*) cover crop. Red clover was frost-seeded in winter wheat (i.e. in March) and then plowed into the soil prior to corn planting in the spring of the following year, i.e. in May. Rye was planted after the corn harvest, i.e. October, and then plowed into the soil prior to soybean planting in spring of the following year, i.e. in May. The conventional management received N and K fertilizers and necessary pesticides at the rates recommended by Michigan State University Extension and soil-test (Table 1). All of the pesticide, K fertilizer and a third of the N fertilizer were applied by broadcasting in spring before corn planting. The remaining of N fertilizer was applied banded within rows approximately one month after planting. The low-input management received 1/3 of the N fertilizer and herbicide amounts applied to the conventional systems. N fertilizer and herbicide were broadcast in spring before planting corn. The organic system did not receive any chemical inputs.

The primary tillage in all three management practices was spring chisel plowing. The secondary tillage consisted of disking before wheat planting, field conditioning with a soil finisher before soybean and corn planting, and inter-row cultivation for soybean and corn. The low-input and organic systems received additional inter-row cultivation and rotary hoeing as needed for weed control. Details of the management related activities (i.e. tillage, planting, harvesting, etc.) can be found at http://lter.kbs.msu.edu/protocols/104 (verified 9 Nov. 2014).

Data for this study were collected in 2011, 2012 and 2013. In 2011, we sampled 4 fields from conventional management, 5 fields from low-input management, and 5 fields from organic management. In 2012, 4 fields from conventional management, 4 fields from low-input management, and 4 fields from organic management were sampled. In 2013, we sampled 4, 5, and 3 fields from conventional, low-input, and organic managements, respectively.

Topographical attributes of the fields were derived from the digital elevation model of the area at 2 m resolution. The attributes include relative elevation, terrain slope, flow length, and flow accumulation. Linear discriminant analysis was applied to the topographical attributes to classify the studied fields into three topographical positions: summit, slope and depression as described by Munoz et al. (2012).

Within each field we identified topographical transects across summit, slope, and depression positions. The number of transects depended on the size of the field and the topographical variability within field. In larger, more undulated fields up to four transects were identified whereas small and relatively flat fields typically had only one transect. For fields under cover crop management along each transect in each of the three topographical positions we laid out two adjacent 4 x 4 m micro-plots. One of the micro-plots had the cover crops similar to the entire field ("cover"), while the other was kept bare of weeds and cover crop by herbicide applied in early spring after emergence ("bare")(Fig. 1). In conventional management fields, which did not have cover crop in the rotation, I established only bare micro-plots. Soil and cover crop biomass was collected from micro-plots as described below.

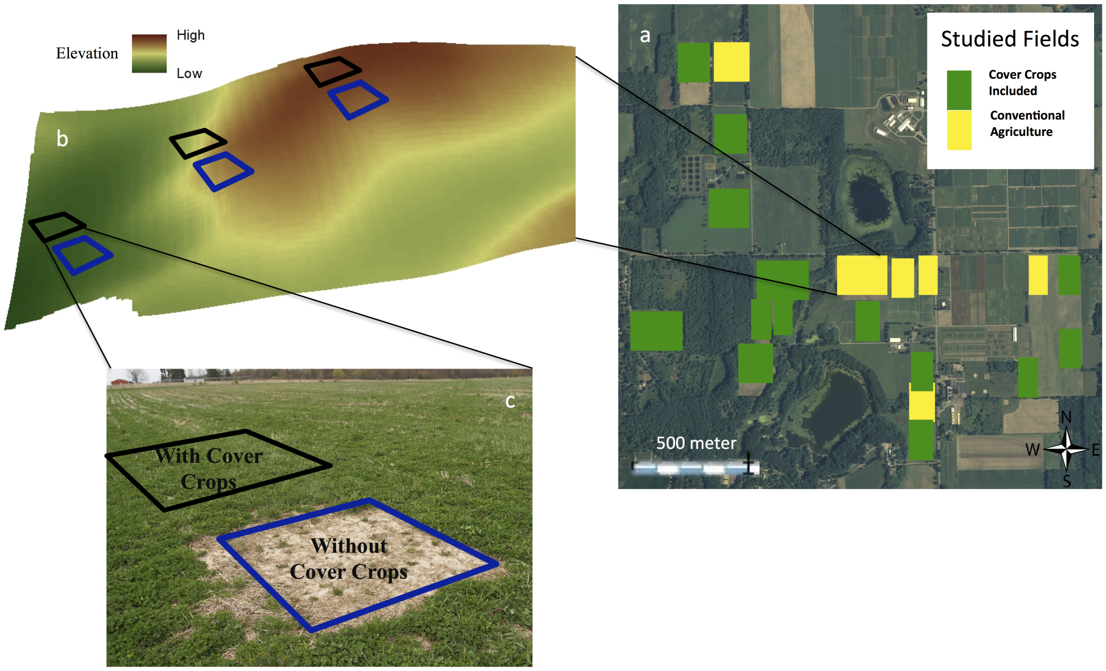


Figure 4.1. a) The study area showing locations of the monitored fields from the three studied treatments; b) a schematic presentation of a topographical transect in one of the studied fields; and c) a 4×4 m cover crop and bare micro-plot within a topographical position.

Sample collection and analysis

We sampled biomass of rye and red clover from all cover crop micro-plots in spring 2-3 days before their termination. For biomass sampling a 0.5 by 0.5 m quadrant was randomly thrown at a corner of a cover micro-plot and the total aboveground cover crop biomass was collected. The biomass sample was dried and weighed after drying.

Biomass of corn and soybean was sampled from cover micro-plots in low-input and organic fields and bare micro-plot in conventional fields at the harvest time in fall of 2010, 2011 and 2012. The total biomass was measured along two rows of 1 m length. The biomass sample was dried in drier and weighed after drying.

Soil samples were collected from 0-15 cm depth from micro-plots four times during each growing season using a push probe (2 cm diameter). The first soil sampling was conducted in spring 2-3 days before cover crop termination. The second sampling was conducted two weeks after plowing, the third sampling was conducted 8 weeks after plowing and the last sampling was conducted after harvesting the main crop. After each sampling the soil samples were air-dried and then sieved to 2 mm.

We measured total organic nitrogen (TON) only once at the beginning of the study in all of the fields. The measurements were made for cover micro-plots in low-input and organic fields and bare micro-plot in conventional fields. TON was measured via dry combustion method using the Costech ECS 4010 CHNSO analyzer.

Soil NO_3^--N was measured in all of the collected samples by shaking 10 g of air-dried soil with 50 ml of 1M KCl for 30 minutes. The mixture was then extracted through paper filter. NO_3^--N content of filtered extracts was determined using the LAChat rapid flow injection unit (Mulvaney, 1996).

Potential mineralizable N (PMN) was measured as instructed by Robertson et al. (1999) at three time points during the growing season, i.e., before plowing, eight weeks after plowing, and after harvest. 10 g of soil was extracted as described previously and amounts of NO_3^--N and NH_4^+-N were measured in the extract. Another 10 g of air-dried soil was brought to 50% water holding capacity moisture (Haney and Haney, 2010) and was incubated in 25^{o}C incubator for 14 days. The sample was then extracted in a similar way as described above and soil NO_3^--N and NH_4^+-N was measured. PMN was calculated as the difference between the total amounts of mineral N (NO_3^--N and NH_4^+-N) in the initial and after-incubation measurements.

Statistical analysis

Two sets of data analyses were conducted using the MIXED procedure of SAS 9.2 (Cary, NC). The purpose of the first set of analyses was to compare the conventional and cover crop based managements at different topographical positions. For this analysis we used data from micro-plots in conventional management fields and from cover micro-plots in the cover crop based management fields. The statistical model included agricultural management, topographical position, date and the interactions between them as the fixed factors with date treated as a repeated measure. Fields nested in agricultural management were included in the model as the random factor and were used as an error term for testing the agricultural management effect.

The purpose of the second set of data analyses was to directly address the effects of cover crop presence at different topographical positions. For this analysis we used the data from bare and cover micro-plots from the low-input and organic fields. The statistical model included topographical position, cover crop presence, date and the interaction between them as the fixed factors with date treated as a repeated measure. Fields and interaction between fields and topographical positions were included in the model as random factors. Field essentially was serving as a blocking factor while field by topography interaction was used as an error term for testing the effect of topography. Normality of the residuals and homogeneity of variance assumptions were checked for all studied variables in both sets of data analyses. Means were compared when the respective main and or interaction effects were statistically significant at 0.05 level.

Three simple linear regression analyses were conducted to examine influences of TON and cover crop biomass on soil NO_3^--N and PMN. The first analysis was conducted between TON as the independent variable and soil NO_3^--N as the dependent variable. We also ran regression analysis between TON as the independent variable and soil PMN as the dependent variable. In the second analysis we looked at differences in NO_3^--N between cover and bare micro-plots at each topographical position as the dependent variable and cover crop biomass as the independent variable. The third analysis was conducted with the difference between cover and bare micro-plots at each topographical position in terms of PMN as the dependent variable and the cover crop biomass as the independent variable. The regression analyses were conducted using the MIXED procedure of SAS, which enabled us to include field nested within year in the random statement of the regression analyses.

RESULTS

Topographical and management differences in the studied plant and soil characteristics

Expectedly the largest corn and soybean biomass was observed in depression areas under all three managements (Table 4.1). The smallest biomass of corn and soybean was observed in slopes under all managements ($p<0.05$). The differences in corn and soybean biomass between slope and depression were numerically the largest under organic management compared to conventional and low-input managements.

Table 4.1. Means and standard errors (in parentheses) of NO_3^--N , TON (total organic nitrogen), fertilizer inputs, and biomass of red clover, rye, corn and soybean in the studied managements across 20 fields. Data are averages from 2011-2013.

	Conventional			Low-input			Organic		
	Depression	**Slope**	**Summit**	**Depression**	**Slope**	**Summit**	**Depression**	**Slope**	**Summit**
NO_3^--N mg kg^{-1}	7.8 (1.6) a	4.2 (1.5) b	7.5 (1.5) a	6.7 (1.4) a	3.3 (1.4) b	4.1 (1.4) b	9.4 (1.4) a	4.5 (1.4) b	6.4 (1.4) b
TON, mg kg^{-1}	0.53 (0.03) a	0.44 (0.03) b	0.48 (0.03) ab	0.53 (0.03) a	0.40 (0.03) b	0.44 (0.03) b	0.44 (0.03) a	0.44 (0.03) a	0.44 (0.03) a
Fertilizer kg ha^{-1}	120	120	120	40	40	40	0	0	0
Red Clover. biomass kg ha^{-1}	N/A	N/A	N/A	1761 (278) a	1162 (278) b	1521 (278) ab	1653 (284) a	1482 (284) a	1617 (284) a
Rye biomass kg ha^{-1}	N/A	N/A	N/A	633 (87) a	329 (87) b	296 (87) b	769 (77) a	496 (77) b	513 (77) b
Corn biomass kg ha^{-1}	18600 (1100) a	13700 (1100) b	17900 (1100) a	15800 (1100) a	11100 (1100) b	11900 (1100) b	15000 (1300) a	7600 (1300) b	10000 (1300) b
Soybean biomass kg ha^{-1}	11664 (490) a	6426 (490) b	6954 (490) b	10033 (480) a	6265 (480) b	8900 (480) a	9263 (510) a	4650 (510) b	5900 (510) b

Different lowercase letters in each row within each management mark significant differences among the topographical positions ($p<0.05$).

TON was the largest in depressions compared to slopes under conventional and low-input managements. I did not observe a significant difference in TON between topographical positions under organic management ($p<0.05$). The regression analysis showed a significant relationship between TON and NO_3^--N and PMN under all three managements ($p<0.05$). The slope of the relationship between TON and NO_3^--N and PMN was the largest under organic management (Table 4.2).

Cover crop biomass was typically the largest in depressions followed by summits and slopes. Red clover aboveground biomass inputs were larger compared to rye (Table 4.1).

Managements had no significant effect on soil NO_3^--N levels. However, the influence of topography on NO_3^--N was statistically significant ($p<0.05$). Under all three managements the slopes had lower NO_3^--N compared to depression areas (Table 4.1). Under low-input and organic management topographical summits also had significantly lower NO_3^--N compared to depressions but under conventional management there was no significant difference between depressions and summits ($p<0.05$).

The effects of cover crops on soil PMN and NO_3^- - N at different topographical positions

There was a trend of higher PMN in cover crop micro-plots as compared to bare micro-plots in depression areas which was significant at $p<0.1$ under red clover (Fig. 4.2a), but not significant under rye (Fig. 4.2c). No significant differences between cover and bare micro-plots in terms of PMN were observed at other topographical positions.

Table 4.2. The relationship between NO_3^--N , PMN (potential mineralizable N) and TON (total organic nitrogen). Data are regression slopes with standard errors (in parenthesis).

Dependent variable mg kg^{-1}	**Independent variable mg.kg^{-1}**	**Management**		
		Conventional	**Low-input**	**Organic**
NO_3^--N	TON	12.7 (2.9) b	11.1 (2.9) b	15.5 (3.1) a
PMN	TON	25.6 (4.3) b	26.6 (4.1) b	34.8 (4.8) a

All of the regression slopes were statistically significant ($p<0.05$). Different lowercase letters in each row show the cases where the regression slopes were significantly different among the managements ($p<0.05$).

Table 4.3. The relationships of the difference between cover and bare micro-plots in terms of NO_3^--N and PMN (potential mineralizable N) with cover crop biomass.

Dependent variable mg kg^{-1}	**Independent variable**	**Position**	**Regression slope/ Std error**
NO_3^--N difference between cover and bare	Red Clover Biomass, kg ha^{-1}	Depression	$0.0014/0.0007^{*}$
		Slope	$0.0001/0.0008^{+}$
		Summit	$0.0013/0.0007^{*}$
	Rye Biomass, kg ha^{-1}	Depression	$-0.0028/0.001^{*}$
		Slope	$-0.0006/0.002^{NS}$
		Summit	$-0.0014/0.002^{NS}$
PMN difference between cover and bare	Red Clover Biomass, kg ha^{-1}	Depression	$0.0015/0.0009^{+}$
		Slope	$0.0004/0.001^{NS}$
		Summit	$0.0007/0.009^{NS}$
	Rye Biomass, kg ha^{-1}	Depression	$0.0018/0.002^{NS}$
		Slope	$-0.0017/0.004^{NS}$
		Summit	$-0.0002/0.0004^{NS}$

The * and + marks the cases where the relationships between the studied variables were statistically significant at $p<0.05$ and $p<0.1$ levels, respectively. NS: not significant at $p<0.1$.

Soil NO_3^--N increased significantly due to presence of red clover in all topographical positions (Fig. 4.2b). Presence of rye tended to have an opposite effect on soil NO_3^--N . Presence of rye significantly decreased NO_3^--N in depressions, while no significant differences between bare and cover micro-plots were observed in slopes and summits (Fig. 4.2d).

I used regression analysis to assess the relationship between cover crop biomass and its effect on soil NO_3^--N, expressed as the difference in NO_3^--N between cover and bare micro-plots (Table 4.3). Consistent with my expectations the regression analysis showed that the aboveground biomass of red clover was positively related to the NO_3^--N difference between cover and bare in depressions and summits ($p<0.05$) as well as on slopes ($p<0.1$). A negative relationship between the aboveground biomass of rye and soil NO_3^--N was observed in depressions ($p<0.05$). Only weak positive relationship ($p<0.1$) was observed between red clover biomass and the cover-bare difference in terms of PMN in depressions, but not in other topographical positions.

The effects of cover crops on temporal patterns of soil PMN and NO_3^- - N

The largest NO_3^--N was observed in soil two to eight weeks after plowing in all three managements ($p<0.05$) and then it decreased in after-harvest measurements (Fig. 4.3). Across all studied topographical positions the presence of red clover increased PMN at the sampling conducted shortly before cover crop termination ($p<0.1$) (Fig. 4.4a). The positive effects of red clover on PMN decreased later in the growing season and eight weeks after plowing no significant difference between bare and cover micro-plots in PMN was observed (Fig. 4.4a). However, positive effect of red clover presence on NO_3^--N appeared two weeks after red clover termination ($p<0.1$) and persisted at the eight weeks after plowing sampling event and even after the main crop harvest ($p<0.05$).

Expectedly, the effects of rye presence on NO_3^--N was opposite of that of red clover. Rye did not have a significant effect on PMN before its termination (Fig. 4.4c); however, soil NO_3^--N decreased under the soil covered with rye compared to bare soil (Fig. 4.4d). The negative effects did not last, as two weeks after termination of rye the NO_3^--N was similar in both cover and bare micro-plots (Fig. 4.4d).

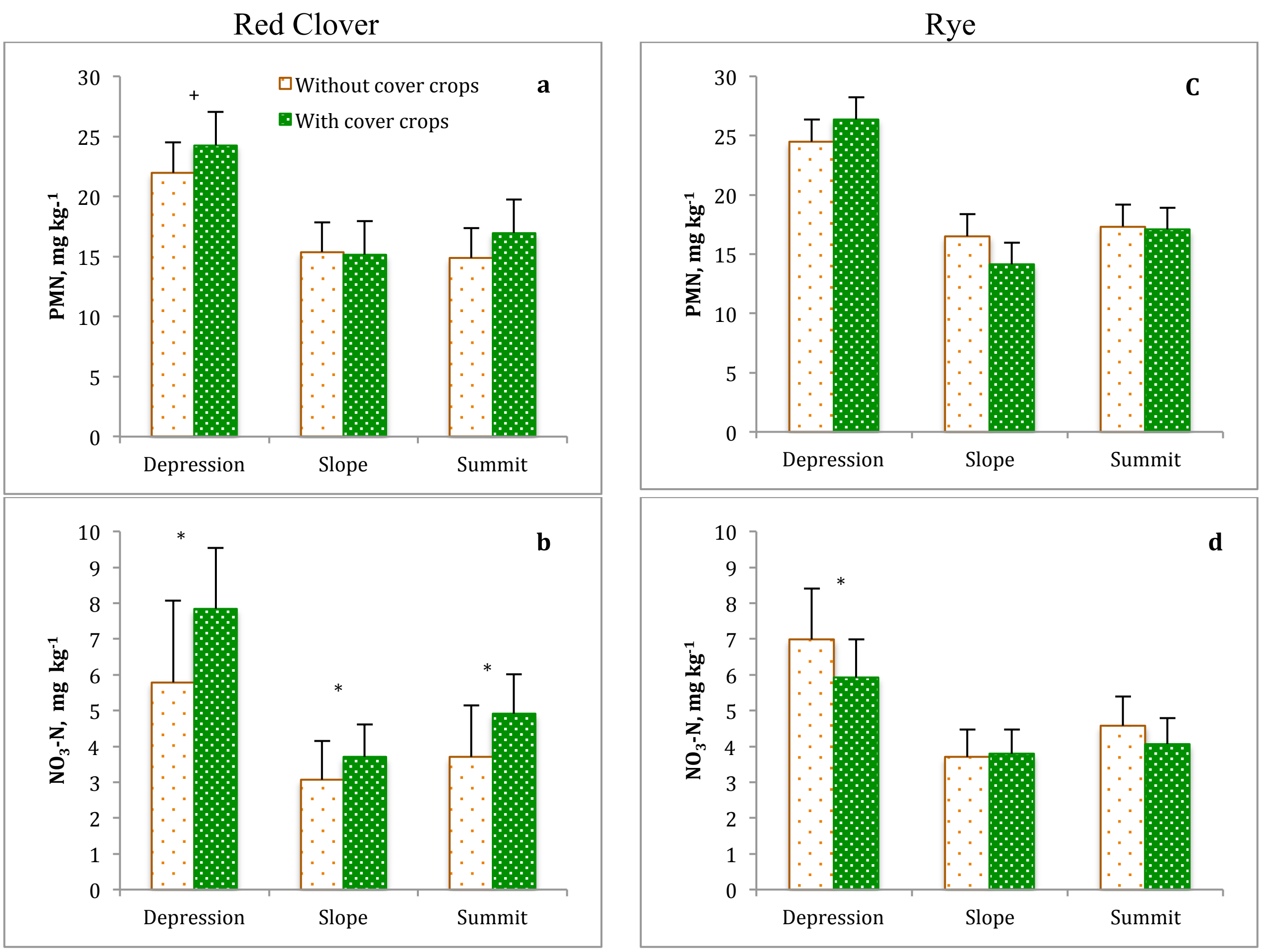


Figure 4.2. Soil potential mineralizable N (PMN) and NO_3^--N in bare and cover micro-plots of the studied topographical positions following red clover and rye cover crops. The data shown are the averages from 2011, 2012 and 2013 results. Error bars represent standard errors. The * and $^+$ mark the cases when the differences between cover and bare micro-plots were statistically significant at $p<0.05$ and $p<0.1$, respectively.

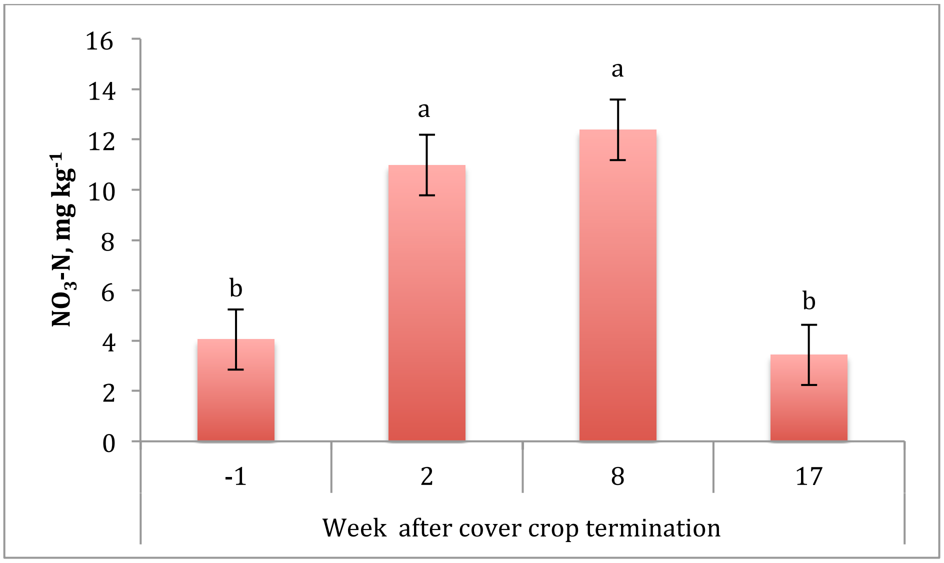


Figure 4.3. NO_3^--N in soil at different sampling dates during growing season. The data shown are the averages from 2011, 2012 and 2013 results of all three studied treatments. Error bars represent standard errors. Bars with different letter indicate significant differences among sampling dates ($p < 0.05$).

DISCUSSION

Spatial distribution of soil NO_3^- - N under different managements

The spatial distribution of NO_3^--N observed under the three studied managements (Table 4.1) was the outcome of a balance between inputs and outputs of mineral N controlled mainly by: spatial distribution patterns in uptake by the main crop, TON mineralization, N fertilizer application, and uptake/input from cover crops. While my results showed that the combined effect of all contributors to soil NO_3^--N resulted in its similar spatial and temporal patterns in all three studied managements, it is important to isolate the specific contribution of each of source of NO_3^--N to the spatial and temporal distribution patterns of NO_3^--N .

Soil organic N as one of the main sources of soil NO_3^--N may have been the main cause for the observed differences in NO_3^--N across topographical positions. The regression analysis showed that across all years and managements for every 1 mg kg^{-1} increase in TON, soil NO_3^--N increased as much as 12.6, 11.1 and 15.5 mg kg^{-1} in conventional, low-input and organic managements, respectively (Table 4.2). These results are consistent with previous studies by Garten and Ashwood (2002), Soon and Malhi (2005), Dharmakeerthi et al. (2005) and Corre et al. (2002) who reported soil organic matter as the main factor controlling the spatial distribution of NO_3^--N . In the organic management, where no N fertilizer is applied, the contribution of TON to NO_3^--N was significantly larger compared to low-input and conventional managements (Table 4.2).

The largest biomass of corn and soybean in depressions indicated a larger plant uptake of NO_3^--N there as compared to slopes, which probably contributed to a reduction in the spatial patterns of NO_3^--N . The effects of topography on the growth and thus biomass of main crops are well-known and my results are consistent with a multitude of previous reports indicating that topographical depressions tend to provide optimal growth conditions for the Midwest corn and soybean crops (e.g., Jinag and Thelen, 2004; Munoz et al., 2014). A larger magnitude of differences between depressions and slopes in terms of corn biomass was observed in the organic fields as compared to conventional and low-input managements. In both low-input and conventional managements corn produced 30% less biomass in slopes as compared to depressions, however, under organic management slopes produced 50% less corn biomass. My results are in agreement with those of Kravchennko et al. (2005) who observed the largest spatial variability in terms of crop grain yield under organic management compared to low-input and conventional managements.

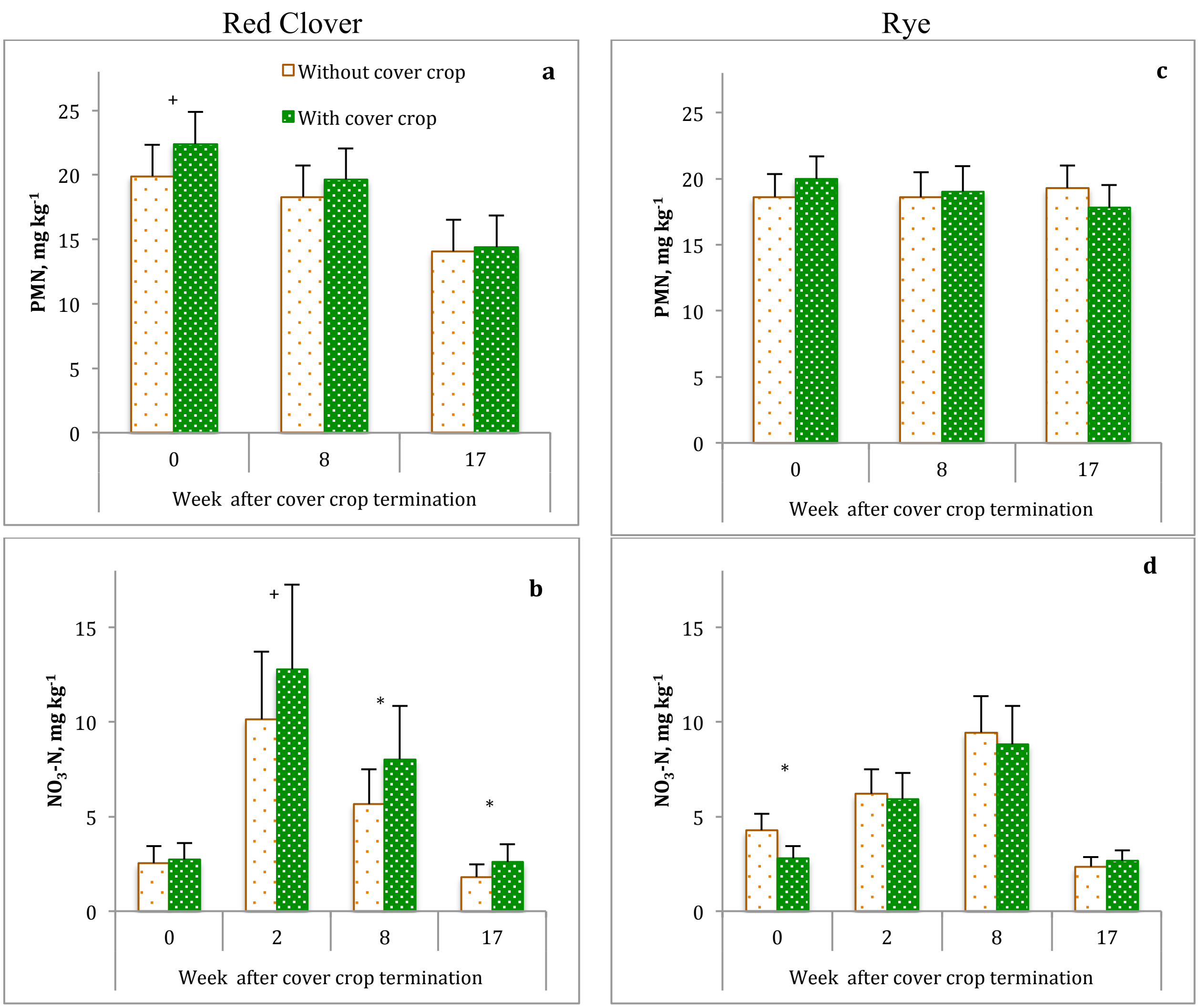


Figure 4.4. NO_3^--N and potential mineralizable N (PMN) in bare and cover micro-plots at the studied sampling times following red clover (a and b) and rye (c and d) cover crops. The data shown are the averages from 2011, 2012 and 2013 results. Error bars represent standard errors. The * and + mark the cases when the differences between cover and bare micro-plots were statistically significant at $p<0.05$ and $p<0.1$, respectively.

Fertilizer was another main source of input in the conventional and low-input fields. However, its application should have had only a relatively minor influence on the variations in NO_3^--N across topography because fertilizers were uniformly applied through entire fields. Nevertheless, spatial redistribution along the topographical gradients via surface run-off could have depleted some of the applied mineral N from slopes and accumulated it in depressions.

The effects of cover crops on spatial distribution of soil PMN and NO_3^- - N

Studying the effect of cover crops across large agricultural field can provide critical information for farmers on the benefits and complexity of cover cropping practice. However, multiple and often counter-acting sources of variation present across large fields have hindered the ability of researchers to assess field-scale effects of cover crops on soil N. In this study my design enabled us to assess the cover crop effects on soil NO_3^--N across large fields with diverse topography with minimum interference from other sources of variability. Better understanding of cover crop's contribution could then help with changing the temporal and spatial patterns in systems with cover crops in favor of crop production. Having a set of two micro-plots with and without cover crops that were laid in close proximity enabled us to tease out the particular effects of cover crops under similar soil properties and plant growth conditions. Note that since the results for cover crop biomass, NO_3^--N and PMN in organic and low input fields were very similar, for the analyses of cover crop influences I combined the data from these two managements.

The larger cover crops biomass in depression areas (Table 4.1) most likely resulted from better growing conditions there, driven by higher soil organic matter and better soil structure as compared to slopes and summits (Munoz et al., 2014).

Large cover crop residue inputs from both cover crops in depressions are the reason for observed PMN differences between cover and bare micro-plots (Fig. 4.2a and 4.2c). While cover crop residue inputs on slopes apparently were not sufficient to notably increase PMN in the cover micro-plots. A larger effect of cover crop presence on PMN for a legume cover crop (Fig.

4.2a) as compared to a non-legume cover crop (Fig. 4.2c) reflects both extra N inputs and longer duration of red clover cover. This result is consistent with reports of Shcomberg and Lindale (2004), who observed larger contribution from crimson clover to soil mineralizable N compared to rye under a cotton production system. The microbial decomposition of cover crops residue releases mineral into soil and thus enhances soil PMN especially under red clover due to its N-rich residue.

Our results are in agreement with the frequently reported positive effects from leguminous cover crop as opposed to negative effects from non-leguminous cover crop on soil NO_3^--N (e.g. Kue and Sainju, 1998; Kramberger et al., 2009). Significant positive effects from red clover on soil NO_3^--N on slopes are of specific importance. Low soil productivity on slopes of these undulated fields results in overall lower main crop yields. The observed increase in soil NO_3^--N on slopes due to legume cover cropping suggests that it can be a solution for improving soil productivity where an improvement is particularly needed. My results are in agreement with findings of Starovoytov et al. (2010) and Tonitto et al. (2006), Zotarelli et al. (2009) who reported positive effects of legume cover crops on soil N and thus the growth of subsequent crop.

Negative effects of rye cover cropping in depressions may act in favor of decreasing the leaching of NO_3^--N . On the contrary, rye did not have a negative effect on NO_3^--N on slopes, indicating that presence of rye on slopes may not limit low levels of soil NO_3^--N there any further. One possible reason for lack of rye cover effect on slopes and summits of this study could be the low biomass of rye in those topographical positions as compared to more fertile depression soils. Tonitto et al. (2006) also reported that low quantities of cover crops may not have an effect on soil N. The presence of rye cover crop was also found to increase soil organic matter on slopes by increasing residue inputs and limiting soil erosion (chapter 3). Therefore, planting rye leads to a win-win situation on slopes due to no negative effect of rye on soil NO_3^--N and positive effects of rye on soil organic matter in long-term.

Our data confirms the hypothesis that magnitude of the effects from cover crops on soil PMN and NO_3^--N varies across a topographical transect, and the amounts of biomass produced by cover crops are driving the size of the effects. However, the overall magnitude of increase in NO_3^--N due to cover presence was relatively low. In order to isolate the effects on NO_3^--N from a combination of topography-driven variations in soil organic matter and in soil surface runoff from the effects of cover crops I calculated the differences in the NO_3^--N levels between the bare and cover micro-plot pairs at each topographical position and regressed that versus the cover crop biomass from respective cover micro-plots (Table 4.3). The regression analysis showed that per 1000 kg ha^{-1} increase in red clover biomass an approximately 1.4 mg kg^{-1} increase in NO_3^--N occurred in depression areas and 1.3 mg kg^{-1} NO_3^--N in summit areas. However, the actual differences that was observed between micro-plots with and without red clover was slightly larger (Fig. 4.3b). This may indicate that the aboveground biomass of red clover was responsible only for a part of the differences in NO_3^--N between cover and bare micro-plots. Shcomber and Endale (2004) showed that red clover enriches soil by N via root activities while its N-rich residue contributes to soil N after termination. Andraski and Bundy (2005) observed that presence of cover crops enriched soil N even when the aboveground biomass of cover crop was harvested. They concluded that roots of cover crops increased soil mineral N.

The result of regression analysis for rye effects were consistent with significantly lower NO_3^--N observed in bare micro-plots compared the cover ones in depressions (Fig. 4.2). The regression analysis showed that per every1000 kg ha^{-1} of aboveground rye biomass a 2.8 mg kg^{-1}

decrease in NO_3^--N occurred in depressions. The well-known ability of rye to scavenge soil mineral N could be one reason for its negative effects observed here. Another reason could be assimilation of soil N due to the low N content of rye residue.

The effects of cover crops on temporal distribution of soil PMN and NO_3^- - N

The temporal patterns observed in my study are consistent with previous reports by Starovoytov et al. (2010), Sarrantonio and Gallandt (2003) and Sainju and Singh (2001) (Fig. 4.3). The increased NO_3^--N two weeks after plowing reflects the release of mineral N form mineralization of soil organic matter in all three studied treatments, decomposition of cover crop residue in low-input and organic managements, and nitrification of the applied fertilizer in conventional and low-input managements. Higher NO_3^--N values persisted until eight weeks after first plowing when plant uptake of NO_3^--N increased and soil NO_3^--N declined (Fig. 4.3). I surprisingly observed no significant differences between NO_3^--N levels among the three studied managements during the growing seasons of the three studied years (Table 4.1).

Greater PMN prior to red clover termination suggested that it increased the pools of soil N even before its aboveground biomass was returned to soil, most likely via root activity of exuding labile C and N-rich compounds (Gardner and Sarrantonio, 2012) (Fig. 4.4a). However, increased PMN did not materialize into higher NO_3^--N at the before cover crop termination sampling event (Fig. 4.4b). The low temperatures and resulting low microbial mineralization of early springs could be one reason that the organic N released by red clover roots was not immediately transformed into NO_3^--N .

Fast decomposition of N-rich residue of red clover in soil is likely the reason for smaller effects of red clover on PMN later in the growing season (Fig. 4.4a). Rapid decomposition of legume cover crop residue was also reported by Schomberg and Endale (2004) and Starovoytov et al. (2010). My assessment of residue decomposition using litter bags showed that more than 95% of red clover residue decomposed during two months after termination (chapter 3) The lasting positive effects of red clover suggested that timing of N from cover crops could be synchronized well with the uptake by main crop plants. The synchrony in release of N from cover crop residue has been a concern in cover cropping practice. Schomberg and Endale (2004) assessed the mineralization of cover crop residue and concluded that only a small portion of N from cover crops becomes available for the subsequent crop. However, my results indicated that red clover had positive effects on NO_3^--N and those effects started early in the season.

The decrease in NO_3^--N early in the season revealed the ability of rye to scavenge the soil NO_3^--N as frequently reported in the literature (Sainju and Singh, 2008; Fageria, 2007). Wortman et al. (2012) reported that negative effects from a mixture of cover crops on soil NO_3^--N lasted until 81 days after termination. The shorter negative effects in my study could be related to a leguminous main crop, i.e., soybean, shortly following after rye termination. Gabriel et al. (2012) suggested that cover crops enhance retention of mineral N in soil profile by controlling leaching. Schomberg and Endale (2004) and Lawson et al. (2012) suggested that rye can assimilate NO_3^--N early in the growing season and then release it to soil later that results in close synchrony with the plant need. My results consistently showed an assimilation of NO_3^--N early in the season, however, I do not have enough evidence that mineralization of rye residue increased the NO_3^--N levels in soil later in the growing season.

CONCLUSIONS

Despite differences in cover cropping and fertilizer application practices, similar spatial and temporal patterns in soil NO_3^--N were observed in all three managements, including conventional management that received synthetic N fertilization and organic management with no fertilizer inputs. Soil organic N was similarly distributed under all managements and was perhaps the main factor controlling spatial distribution of soil NO_3^--N in these large topographically and edaphically diverse fields.

The presence of red clover had a positive effect on soil NO_3^--N across all topographical positions. Including red clover in rotation would have benefits in enhancing NO_3^--N availability, particularly on the eroded topographical slopes where low soil quality results in poor performance of main crops. The residue of cover crops was positively correlated with NO_3^--N levels on slopes, however, growth and residue of red clover on slopes was relatively low. The effects of rye were opposite of those of red clover. Rye significantly decreased soil NO_3^--N in depressions, but not on summits and slopes.

Positive influence of red clover presence on soil NO_3^--N continued through the entire growing season. Apparently, the lack of the synchrony of release of N from red clover residue and the plant needs early in the season was not a concern for the fields in my study. Rye had the ability to scavenge soil NO_3^--N before its termination, however, soil NO_3^--N gradually increased after termination of rye.

Chapter 5: Conclusions

Including cover corps in row crop systems of the US Midwest has a great potential to increase soil organic C and supply mineral N for the subsequent crop. I found that the magnitude of benefits from cover crops varies across topography in large undulated fields. The challenges associated with uncontrolled sources of variations in field-scale experiment set-ups has hindered the ability of researchers to assess the effects of cover crops on soil organic C and mineral N in larger scales. My study showed that studying the effects of cover crops on soil organic C and mineral N across large agricultural fields is crucially needed and is possible, however, special care is required in to tease out the particular role of cover crops across topography.

The larger heterogeneities in soil properties and landscape could be used in favor of increasing the ability to detect management effect on fractions of organic carbon. I demonstrated that the special importance of collecting additional information such as soil particle size distribution, soil bulk density and applying them as covariates in statistical analysis. I found that SMC has the potential to be used for assessment of management effects since it was reflective of management effects and at the same time has lower variability. POC could be more reflective of management changes, however, its larger variability compared to TOC leads to substantially lower statistical power for assessment of its changes due to management. My results suggested that SMC could reveal some of the management effects on fractions of carbon in both surface and deeper layers of soil in a shorter time. Changes in carbon in deeper soil are far smaller to be detectable by measuring TOC. Very low contents of TOC and POC in deeper soil caused large measurement errors. However, the measurements of SMC did not have large error in deeper soil and could be potentially useful to assess the management effects on deep carbon.

The effects of five years agricultural management on TOC, POC and SMC were not detected across large fields. However, when the managements were compared in three distinct topographical positions as depression, slopes and summit the conventional management had less POC on the slopes compared to managements with cover crops. The lower POC on slopes of conventional managements may indicate the gradual loss of C pools on slopes of conventional management compared to management with cover crops. Red clover had positive effects on SMC across all topographical positions despite its varied growth across topography. I suggest that inputs of C to soil from of red clover residue along with its effect in limiting soil erosion are the main reasons for its positive effects on soil C across all topographical positions. Rye has positive effects on SMC only in depressions. My data showed that growth of rye in slopes and summits was perhaps smaller to cause changes in soil C. My assessment of cover crop biomass and its association with pools of C suggested that belowground root activity and growth of cover crops may play an important role in controlling soil C changes. The effects of cover crops were less detectable when POC was assessed. The cover cropping practice has the ability to gradually increase soil C across large field and promote soil quality in long-term.

The spatial distribution of soil NO_3^--N was similar under conventional, low-input and organic management. Depressions had the largest NO_3^--N and slopes had the lowest NO_3^--N . The presence of red clover had positive effects on soil NO_3^--N across all topographical positions. I demonstrated that including red clover in rotation would have benefits in enhancing NO_3^--N availability, particularly on the slopes where the soil quality is low. I also found that the residue of cover crops was positively correlated with NO_3^--N levels in slope, however, growth and residue of red clover was low on slopes and may cause smaller increase in soil NO_3^--N . The effects of rye were opposite of those of red clover. Rye significantly decreased soil NO_3^--N in depressions but not summits and slopes. The growth of rye was not enough to cause changes in soil NO_3^--N in slopes and summits. The positive effects of red clover started early in the growing season and lasted till the end of growing season. I demonstrated that the concern about the synchrony of release of N from red clover residue and the plant need early in the season did not apply to the fields in my study. Rye had the ability to scavenge soil NO_3^--N before its termination, however, soil NO_3^--N gradually increased after termination of rye. The aboveground biomass of cover crops was associated with soil NO_3^--N , however, my results suggested that belowground growth of cover crops also controls the effects of cover corps on soil N.

The findings of this study enhance the understanding of complexities of cover cropping practice across topographically diverse agricultural fields. I showed that cover crops contribute to the soil quality, however, the mechanism of those contributions vary across space. In depression and summits the residue of cover crops increases pools of soil organic C. In slopes, where the residue of cover crops is smaller, cover crops maintain soil quality through controlled erosion. I also showed that a legume cover crop contributes the soil productivity by increasing soil mineral N across all topographical positions. A non-legume cover crop has negative effects on soil mineral N only in depressions. My results suggest that cover cropping practice can be adjusted to meet the specific needs of a farmer. A differential planting of cover crops across topography can be practiced depending on the desired benefit. If the goal is to increase soil quality via controlling erosion planting cover crops only on slopes will be sufficient. In contrast, if the goal is to limit soil NO_3^--N leaching, then planting a non-legume on depressions is needed. The effects of cover crops on soil organic C and mineral N is controlled by biomass production of cover crops. However, aboveground biomass of cover corps may have a smaller role compared to their belowground growth and activity of cover crops. This suggests that farmers can still benefit from cover crops even if the aboveground biomass is harvested or grazed. Understanding the spatial variations of cover corps biomass and their effects on soil C and N enables farmers to obtain the most benefit from cover crops.

APPENDIX

Table A.1. Bulk Density (BD), clay, silt, sand, total organic carbon (TOC) and particulate organic carbon (POC) in deep cores collected from 10 agricultural fields.

Conventional Management								
Position	**No of cores**	**Depth**	**BD Mg M^{-3} Std err =0.06**	**Clay % Std err =0.02**	**Silt % Std err =0.05**	**Sand% Std err =0.07**	**TOC% Std err =0.1**	**POC% Std err =0.07**
Depression	8	0-20	1.45	0.12	0.44	0.44	1.23	0.4
	8	20-35	1.6	0.11	0.47	0.42	0.75	0.18
	8	35-50	1.7	0.12	0.45	0.44	0.58	0.15
	8	50-70	1.7	0.11	0.39	0.5	0.46	0.15
	8	70-90	1.81	0.11	0.38	0.51	0.49	0.09
Slope	8	0-20	1.53	0.09	0.23	0.69	0.84	0.14
	8	20-35	1.76	0.1	0.22	0.68	0.35	0.03
	8	35-50	1.84	0.12	0.16	0.73	0.23	0.01
	8	50-70	1.83	0.07	0.07	0.86	0.14	0.05
	8	70-90	1.87	0.04	0.06	0.9	0.21	0.01
Summit	8	0-20	1.52	0.09	0.2	0.71	0.81	0.19
	8	20-35	1.82	0.09	0.13	0.78	0.34	0.04
	8	35-50	1.86	0.07	0.06	0.87	0.44	0.01
	8	50-70	1.87	0.05	0.04	0.9	0.17	0.02
	8	70-90	1.89	0.04	0.03	0.93	0.39	0.01

Low-input Management								
Position	**No of cores**	**Depth**	**BD Mg M^{-3} Std err =0.06**	**Clay % Std err =0.02**	**Silt % Std err =0.06**	**Sand% Std err =0.07**	**TOC% Std err =0.1**	**POC% Std err =0.08**
Depression	7	0-20	1.35	0.12	0.37	0.5	2.28	0.89
	7	20-35	1.62	0.14	0.34	0.52	0.82	0.2
	7	35-50	1.71	0.1	0.27	0.62	0.45	0.13
	7	50-70	1.73	0.09	0.2	0.71	0.35	0.11
	7	70-90	1.83	0.09	0.19	0.72	0.14	0.04
Slope	7	0-20	1.47	0.11	0.35	0.54	0.87	0.14
	7	20-35	1.78	0.17	0.31	0.51	0.36	0.04
	7	35-50	1.77	0.18	0.17	0.65	0.21	0.02
	7	50-70	1.81	0.11	0.09	0.8	0.19	0.02
	7	70-90	1.82	0.09	0.1	0.81	0.1	0.04
Summit	7	0-20	1.32	0.1	0.37	0.53	0.9	0.16
	7	20-35	1.68	0.14	0.34	0.52	0.43	0.04
	7	35-50	1.78	0.17	0.19	0.64	0.19	0.02
	7	50-70	1.82	0.11	0.11	0.78	0.18	0.04
	7	70-90	1.84	0.08	0.07	0.85	0.1	0.03

Table A.1. (cont'd)

Organic Management								
Position	**No of cores**	**Depth**	**BD Mg M^{-3} Std err =0.06**	**Clay % Std err =0.02**	**Silt % Std err =0.06**	**Sand% Std err =0.07**	**TOC% Std err =0.1**	**POC% Std err =0.08**
Depression	7	0-20	1.47	0.15	0.47	0.39	1.16	0.51
	7	20-35	1.6	0.15	0.49	0.35	0.91	0.52
	7	35-50	1.64	0.15	0.51	0.34	0.71	0.33
	7	50-70	1.61	0.14	0.46	0.4	0.62	0.34
	7	70-90	1.67	0.12	0.48	0.4	0.58	0.33
Slope	7	0-20	1.65	0.13	0.18	0.69	0.73	0.09
	7	20-35	1.78	0.12	0.15	0.72	0.48	0.04
	7	35-50	1.77	0.15	0.13	0.72	0.24	0.02
	7	50-70	1.84	0.11	0.08	0.81	0.14	0.02
	7	70-90	1.86	0.08	0.04	0.88	0.09	0.01
Summit	7	0-20	1.7	0.12	0.3	0.58	0.85	0.21
	7	20-35	1.76	0.19	0.22	0.59	0.42	0.07
	7	35-50	1.8	0.16	0.12	0.72	0.19	0.02
	7	50-70	1.81	0.1	0.05	0.84	0.09	0.03
	7	70-90	1.8	0.05	0.04	0.9	0.07	0.02

Table A.2. Typical soil horizons in the studied fields.

Topographical position	**Soil horizon**	**Depth (cm)**
Depression	Ap	0-25
	A	25-50
	Bt	50-70
	2Bt	70-90
	C	90-100
Slope	Ap	0-20
	E	20-30
	Bt	30-50
	2Bt	50-70
	C	70-100
Summit	Ap	0-20
	E	20-35
	Bt	35-55
	2Bt	55-85
	C	85-100

Table A.3. Means and standard errors (in parentheses) of particulate organic carbon (POC), short-term mineralizable carbon (SMC), NO3, NH4 and potentially mineralizable N (PMN) the three studied managements in during 2011, 2012 and 2013.

Management: **Conventional** and Year: **2011**								
CC	**Pos**	**Cover**	**Date**	**POC**	**SMC**	NO_3^-	NH_4^+	**PMN**
RedC	DE	No	May					
RedC	DE	No	June					
RedC	DE	No	Aug	0.27 (0.07)	57.15 (7.9)	17.28 (3.5)	26.34 (5.2)	18.8 (6.2)
RedC	DE	No	Oct	0.3 (0.06)	58.05 (6.5)	1.47 (2.9)	7.99 (4.4)	31.34 (5.2)
RedC	SL	No	May					
RedC	SL	No	June					
RedC	SL	No	Aug	0.19 (0.07)	41.83 (7.9)	21.41 (3.5)	12.37 (5.3)	8.72 (8)
RedC	SL	No	Oct	0.13 (0.06)	40.92 (5.8)	2.04 (2.5)	4.12 (4.1)	23.73 (4.8)
RedC	SU	No	May					
RedC	SU	No	June					
RedC	SU	No	Aug	0.24 (0.07)	48.41 (7.9)	15.75 (3.5)	3.94 (5.3)	28.7 (5)
RedC	SU	No	Oct	0.16 (0.06)	43.67 (5.8)	1.28 (2.5)	3.35 (4.1)	28.82 (5)
Rye	DE	No	May					
Rye	DE	No	June			3.07 (2.5)	2.6 (6.6)	14.56 (4.2)
Rye	DE	No	Aug	0.25 (0.1)	52.42 (5.3)	10.25 (2.5)	6.75 (4)	21 (4.4)
Rye	DE	No	Oct	0.68 (0.05)	53.58 (5.9)	9.04 (2.5)	18.45 (3.9)	22.64 (7.8)
Rye	SL	No	May					
Rye	SL	No	June			2.05 (2.3)	3 (2.1)	20.3 (4.2)
Rye	SL	No	Aug	0.11 (0.1)	37.71 (4.8)	7.65 (2.1)	5.35 (1.7)	6.26 (4.4)
Rye	SL	No	Oct	0.13 (0.05)	37.25 (5.3)	2.4 (2.3)	8.1 (1.7)	18 (4.6)
Rye	SU	No	May					
Rye	SU	No	June			2.23 (2.3)	5.22 (1.6)	18.05 (5)
Rye	SU	No	Aug	0.18 (0.1)	41.77 (4.8)	6.83 (2.3)	3.42 (1.6)	9.3 (4.3)
Rye	SU	No	Oct	0.15 (0.05)	35.84 (5.3)	3.23 (2.3)	0.09 (1.6)	17.55 (4.6)

Table A.3. (cont'd)

Management: **Conventional** and Year: **2012**								
CC	**Pos**	**Cover**	**Date**	**POC**	**SMC**	**NO_3^-**	**NH_4^+**	**PMN**
RedC	DE	No	May	0.15 (0.08)	50.97 (8)	2.49 (4.9)	4.52 (2.5)	25.92 (8)
RedC	DE	No	June			12.25 (3.5)	7.15 (2.5)	
RedC	DE	No	Aug	0.25 (0.08)	56.68 (8)	14.88 (3.5)	5.38 (2.5)	37.89 (6.2)
RedC	DE	No	Oct	0.26 (0.08)	39.6 (8)	13.52 (3.5)	3.15 (2.5)	31.66 (6.2)
RedC	SL	No	May	0.15 (0.08)	36.41 (7)	2.34 (4.8)	4.26 (2.3)	24.93 (8)
RedC	SL	No	June			13.74 (4.8)	16.52 (2.3)	
RedC	SL	No	Aug	0.24 (0.06)	51.6 (7)	6.2 (2.)	2.65 (2.3)	35.9 (5.5)
RedC	SL	No	Oct	0.18 (0.06)	27.83 (7)	8.79 (2.9)	2.68 (2.3)	14.97 (5.5)
RedC	SU	No	May	0.12 (0.06)	32.62 (7)	4.6 (4.8)	4.07 (2.3)	17.71 (8)
RedC	SU	No	June			15.54 (2.9)	28.09 (2.3)	
RedC	SU	No	Aug	0.21 (0.06)	45.4 (7)	17.06 (2.9)	10.41 (2.3)	24.77 (5.5)
RedC	SU	No	Oct	0.18 (0.06)	25.58 (7)	8.48 (2.9)	2.64 (2.3)	18.83 (5.5)
Rye	DE	No	May	0.16 (0.06)	32.39 (7)	2.9 (4.8)	1.99 (3.6)	26.7 (6.2)
Rye	DE	No	June			12.56 (2.9)	4.26 (3.6)	
Rye	DE	No	Aug	0.21 (0.06)	45.56 (7)	13.69 (2.9)	5.12 (3.6)	29.53 (5.5)
Rye	DE	No	Oct	0.28 (0.06)	39.34 (7)	4.21 (2.9)	2.5 (2.9)	28.83 (5.5)
Rye	SL	No	May	0.16 (0.06)	26.38 (7)	6.73 (3.5)	3.57 (2.9)	23.8 (8)
Rye	SL	No	June			8.08 (2.5)	3.44 (2.9)	
Rye	SL	No	Aug	0.2 (0.05)	37.95 (6)	14.03 (2.5)	1.71 (2.9)	22.74 (5.5)
Rye	SL	No	Oct	0.24 (0.05)	35.17 (6)	2.78 (2.5)	3.7 (2.9)	22.42 (5.5)
Rye	SU	No	May	0.14 (0.05)	25.64 (6)	1.27 (4.9)	4.24 (2.9)	23.25 (5.5)
Rye	SU	No	June			7.85 (2.5)	4.52 (2.5)	
Rye	SU	No	Aug	0.21 (0.05)	37.47 (6)	15.4 (2.5)	7.15 (2.5)	23.35 (5.5)
Rye	SU	No	Oct	0.21 (0.05)	37.3 (6)	3.06 (2.5)	5.38 (2.5)	25.82 (8)

Table A.3. (cont'd)

Management: **Conventional** and Year: **2013**								
CC	**Pos**	**Cover**	**Date**	**POC**	**SMC**	**NO_3^-**	**NH_4^+**	**PMN**
RedC	DE	No	May	0.21 (0.05)	33.12 (6.1)	3.73 (2.6)	4.52 (2.5)	13.87 (5.3)
RedC	DE	No	June			13.11 (2.6)	7.15 (2.5)	
RedC	DE	No	Aug	0.34 (0.05)	74.99 (6.1)	17.83 (2.6)	5.38 (2.5)	29.36 (5.3)
RedC	DE	No	Oct	0.27 (0.05)	33.47 (6.1)	3.06 (2.6)	3.15 (2.5)	4.25 (5.3)
RedC	SL	No	May	0.12 (0.05)	24.05 (5.6)	1.54 (2.3)	4.26 (2.3)	5.77 (4.9)
RedC	SL	No	June			18.39 (2.3)	16.5 (2.3)	
RedC	SL	No	Aug	0.25 (0.05)	32.66 (5.6)	2.06 (2.3)	2.65 (2.3)	16.53 (4.9)
RedC	SL	No	Oct	0.18 (0.05)	31.93 (5.6)	1.1 (2.3)	2.68 (2.3)	3.56 (4.9)
RedC	SU	No	May	0.18 (0.05)	28.81 (5.6)	2.76 (2.3)	4.07 (2.3)	11.27 (4.9)
RedC	SU	No	June			26.79 (2.3)	28.1 (2.3)	
RedC	SU	No	Aug	0.22 (0.05)	27.63 7.9)	21.06 (2.3)	10.4 (2.3)	25.92 (6.5)
RedC	SU	No	Oct	0.23 (0.05)	32.56 (7.9)	1.74 (2.3)	2.64 (2.3)	1.31 (6.5)
Rye	DE	No	May	0.21 (0.05)	33.91 (7.9)	12.13 (3.5)	1.99 (3.6)	7.81 (5.5)
Rye	DE	No	June			2.22 (3.5)	4.26 (3.6)	
Rye	DE	No	Aug	0.37 (0.08)	39.07 (6.7)	6.79 (3.5)	5.12 (3.6)	30.78 (5.5)
Rye	DE	No	Oct					
Rye	SL	No	May	0.16 (0.1)	29.02 (6.7)	4.82 (2.9)	2.5 (2.9)	7.34 (5.5)
Rye	SL	No	June			6.75 (2.9)	3.57 (2.9)	
Rye	SL	No	Aug	0.23 (0.06)	27.88 (6.7)	3.28 (2.9)	3.44 (2.9)	20.92 (5)
Rye	SL	No	Oct					
Rye	SU	No	May	0.17 (0.06)	27.71 (5.2)	8.43 (2.9)	1.71 (2.9)	4.61 (6)
Rye	SU	No	June			4.74 (2.9)	3.7 (2.9)	
Rye	SU	No	Aug	0.22 (0.06)	28.96 (5.2)	2.52 (2.9)	4.24 (2.9)	20.58 (5)
Rye	SU	No	Oct					

Table A.3. (cont'd)

Management: **Low-input**, Cover crop: **Red Clover** and Year: **2011**							
Pos	**Cover**	**Date**	**POC**	**SMC**	**NO_3^-**	**NH_4^+**	**PMN**
DE	No	May	0.19	58.38 (5)			16.71 (4.2)
DE	Yes	May	0.17 (0.05)	60.71 (5.5)			26.69 (4.4)
DE	No	June					
DE	Yes	June					
DE	No	Aug	0.32 (0.05)	54.01 (5)			19.05 (4.3)
DE	Yes	Aug	0.26 (0.05)	52.22 (5)			17.05 (4.6)
DE	No	Oct	0.2 (0.05)	44.31 (5.5)			14.04 (5)
DE	Yes	Oct	0.25 (0.05)	53.55 (5.5)			25 (4.6)
SL	No	May	0.13 (0.05)	39.75 (5)			8.84 (4.3)
SL	Yes	May	0.16 (0.05)	49.65 (5)			11.94 (4.3)
SL	No	June					
SL	Yes	June					
SL	No	Aug	0.16 (0.05)	33.33 (5)			13.25 (4.3)
SL	Yes	Aug	0.21 (0.05)	39.34 (5)			11.08 (4.3)
SL	No	Oct	0.21 (0.05)	40.34 (5.2)			13.12 (4.3)
SL	Yes	Oct	0.13 (0.05)	40.88 (5.2)			10.39 (4.3)
SU	No	May	0.14 (0.05)	37.13 (5.2)			12.6 (4.6)
SU	Yes	May	0.21 (0.05)	49.07 (5.2)			23.7 (4.6)
SU	No	June					
SU	Yes	June					
SU	No	Aug	0.21 (0.05)	33.25 (5.2)			11.18 (4.3)
SU	Yes	Aug	0.21 (0.05)	36.48 (5.2)			9.78 (4.3)
SU	No	Oct	0.12 (0.05)	32.55 (5.2)			6.72 (4.3)
SU	Yes	Oct	0.21 (0.05)	41.63 (5.2)			18.88 (4.3)

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