Scaling-up: cover crops differentially influence soil carbon in agricultural fields with
diverse topography

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ABSTRACT

The use of cover crops is a management technique that can potentially increase the
amount of carbon (C) sequestered in soil. However, information on cover crop's role in
increasing soil C comes mostly from small experimental plots, while the magnitude of C
gains in large agricultural fields may vary spatially in response to topographic and soil

variability. Here we assess cover crop effects on soil organic C in 20 large agricultural fields across a topographically diverse landscape under conventional, low-input, and organic managements in corn-soybean-wheat rotation. The low-input and organic managements included rye and red clover cover crops in their rotations. Micro-plots with and without cover crops were laid out within each studied 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). The magnitude of cover crop effects on SMC and POC varied across topography. The contributions of cover crop's presence to soil C variables tended to be the highest on topographical slopes and summits. Positive correlations between effects of cover crop presence on SMC with cover crop biomass also were primarily observed on slopes and summits. The results indicate that in the studied agricultural environments preferential placement of cover crops on eroded low fertility elements of the relief can be a particularly effective strategy from both environmental and management standpoints.

INTRODUCTION

Intensive agriculture increases the release of C from soil to the atmosphere (Lal, 2002) thus contributing to increasing levels of atmospheric CO₂ and changing climate. A conservation practice that can potentially slow or even reverse soil C depletion is the inclusion of winter cover crops in agricultural rotations (e.g., Thomsen and Christensen, 2004; Sainju et al., 2006; Motta et al., 2007; Weil and Kremen, 2007; Mutegi et al., 2013; Abdollahi and Munkholm, 2014; Hurisso et al., 2014). Cover crops are grown in between consecutive main crops and are typically planted in fall and terminated in spring, thus providing continuous live vegetative coverage.

Row crop farming is most often implemented in large agricultural fields, which in the US Midwest are commonly characterized by undulating topography. In such settings cover crop contribution to soil C cycle can be expected to be a subject of substantial spatial variations. The numerous sources of these variations are driven by interwoven soil/hydrological/plant interactions. Differences in the amounts of above- and below-ground biomass produced by cover crops is arguably the leading source of variation in cover crop effects on soil C (Gardner and Sarrantonio, 2012; McDaniel et al., 2014). Cover crop production can be significantly influenced by topography (Muñoz et al., 2014). For example, cover crop growth in topographical depressions of undulating agricultural fields is often enhanced (Muñoz et al., 2014) due to more favorable edaphic and hydrological settings, as compared to other topographical positions (Chan et al., 2007; Corre et al., 2002; Bennie et al., 2008). The other source of variation is topography-driven differences in soil moisture and temperature and their effects on decomposition rates of cover crop residue (Jacinthe et al., 2002), e.g., enhancing

decomposition in soil of slopes and summits as compared to colder and wetter topographical depressions. Combined, these influences can lead to increased spatial variability of soil C across topographically diverse fields, enhancing soil C gains in topographical depressions as opposed to higher terrain.

On the other hand, cover crop presence is known to limit soil erosion. Reduction in erosion can decrease C redistribution across topographic gradients, and thus produce the opposite effect of reducing the spatial variability of soil C (Jacinthe et al., 2004; Ritchie et al., 2007). Moreover, it is possible that in historically C-enriched topographical depressions soil can be closer to the state of C saturation, which can result in lower C sequestration capabilities due to cover crop inputs. Because of the variety of these counter-acting influences, the effects of conservation management practices 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 a 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 experimentation. Most cover crop research is performed in small experimental plots where conditions are far more controlled and homogeneous and topographic variations are minimal as compared to large agricultural fields (VandenBygaart, 2006). Substantial resource demands for large scale experimentation and logistic difficulties in designing the experiments where unequivocal isolation of topographical and management effects could be possible are the understandable reasons. However, the lack of knowledge on (i) cover crop performance in undulating agricultural landscapes and (ii) on their effects on soil C limits accurate

assessment of the potential contribution that cover crops can make to increase soil organic C and improve soil health. The uncertainty about the performance of cover crops in real size agricultural fields complicates management decisions and may inhibit farmers' willingness to include cover crops in cropping rotations.

Our study takes advantage of a unique replicated large scale experiment where three contrasting management systems, i.e., conventional row crop management and two cover crop based practices, were tested each at 4-5 large undulating agricultural fields. Topographical diversity of the studied fields enabled us to address the effects of management on the spatial patterns in soil C at the field scale. Multiple sets of paired micro-plots established at contrasting topographical positions within cover crop fields enabled us to perform quantitative assessment of the cover crop contribution to soil C, while avoiding confounding effects of other contributing factors.

We hypothesize that, while positive effect of cover crops will be present everywhere, its magnitude will vary at different topographical positions. The variations result from several competing processes, including topographically driven differences in cover crop growth patterns, differences in residue decomposition rates as well as cover crop influences on processes of soil erosion. The topographical depressions in the studied landscape typically support substantially greater plant growth and, due to their tendency for higher soil moisture and lower soil temperature levels, might be less conducive to decomposition of freshly added plant inputs. Thus, we expect greater benefits of cover crop presence to be observed in topographical depressions as opposed to summits and slopes.

Since soil total organic carbon (TOC) relatively slowly responds to land

management and land use changes (Nascente et al., 2013; Haynes, 2000; Plaza-Bonilla et al., 2014), in our study we focused on the C characteristics related to labile C pools, specifically, particulate organic C (POC) and short term mineralizable C (SMC). The labile pools have a turnover rate of days to a few years (Hungate et al., 1995; Paul et al., 1999; Six et al., 2001). Thus, their assessment can reveal the short-term effects of management practices on C processes (Marriott and Wander, 2006; Awale et al., 2013). Both POM and SMC were demonstrated to be closely related to several labile pools of organic C and to be sensitive to management changes (Cambardella and Elliott 1992; Collins et al., 2000; Chan, 2001; Haynes, 2005; Franzluebbers et al., 2000; Culman et al., 2013; Ladoni et al., 2015).

The overall goal of this study is to assess how the presence of cover crops influences soil C on a topographically diverse landscape. The first objective is to compare three management practices in their effects on soil C, as represented by TOC, POC and SMC, across diverse terrain. The three practices include a conventional practice that completely relays on the use of synthetic fertilizers, and two conservational practices that use cover crops with reduced fertilizer inputs (low input) and with no chemical inputs (organic). The second objective is to assess the specific contributions that the presence of cover crops makes to changes in labile soil C fractions, POC and SMC, at contrasting topographical positions within large agricultural fields.

METHODS

Study site

The study was conducted at the Scale-up experiment established in 2006 at the Kellogg Biological Station Long Term Ecological Research (LTER) site in southwest Michigan (42° 24' N, 85° 24' W) (Robertson and Hamilton 2015). The dominant soil series are Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs).

The Scale-up experiment consisted of a total of 27 agricultural fields, with fields ranging in size from 3.1 to 7.9 ha. Scale-up experiment studied three agricultural management practices, namely, conventional, low-input and organic. Each practice was assigned to 9 randomly selected fields. The studied fields were in corn-soybean-wheat rotation, and every year all three phases of the rotation were present in all three studied management practices. Thus, each year for each management practice there were three replicated fields in corn, three in soybean, and three in wheat present in the Scale-up experiment.

Treatment and experiment design

Our study consisted of three factors, namely, management practice with three levels (conventional, low-input, and organic), crop with two levels (corn and soybean), and topographical position with three levels (depression, slope, and summit). Data for this study were collected in 2011, 2012 and 2013. Each year we worked only with the Scale-up fields that were either in corn or in soybean. Out of the total of 18 of corn and soybean fields available each year from the entire Scale-up experiment we selected only the fields with sufficiently diverse topography. In 2011, we sampled 4 fields from conventional management, 6 fields from low-input management, and 5 fields from organic

management. In 2012, we sampled 4 fields from conventional management, 4 fields from low-input management, and 4 fields from organic management. In 2013, we sampled 4, 4, and 5 fields from conventional, low-input, and organic managements, respectively. Figure 1 shows locations of the fields used in the study along with the years at which each field was sampled.

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 we identified topographical transects across summit, slope, and depression positions (Fig. 1). The number of transects depended on the size of the field and the topographic variability within the field. In larger, more undulating fields up to four transects were identified whereas small fields typically had only one transect. For the fields in organic and low input management practices along each transect in each topographical position 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 plants by herbicide applied in early spring after plant emergence (“bare”) (Fig. 1). In conventional management fields, which did not have cover crop in the rotation, we established only bare micro-plots.

Soil and cover crop biomass samples were collected from micro-plots as described below. Note that for the first objective of the study, that is, to compare the three studied management practices we used the data from the micro-plots that were

representative of the respective management. For example, comparisons between organic, low-input, and conventional systems were conducted using the data from the cover micro-plots of the organic and low-input systems and the bare micro-plots from the conventional system. For the second objective of assessing the effect of cover crop presence at different topographical positions we conducted comparisons between bare and cover micro-plot pairs at each position of each studied field from the organic and low-input systems.

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 cereal rye (*Secale cereale*), a non-leguminous cover crop. Red clover was frost-seeded in winter wheat in March and then plowed into the soil prior to corn planting in May of the following year. Rye was planted after the corn harvest, in October, and then plowed into the soil prior to soybean planting in May of the following year.

The conventional management received N and K fertilizers and necessary pesticides at the rates recommended by Michigan State University Extension and soil test results. All of the recommended pesticides and K fertilizer as well as a third of the recommended N fertilizer were applied by broadcasting in spring before corn planting. The remaining 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 was broadcast in spring before planting corn. The organic system did not receive any synthetic 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).

Sample collection and analysis

We sampled biomass of rye and red clover from all cover crop micro-plots in spring 2-3 days before plowing. For biomass sampling a 0.5 by 0.5 m quadrant was randomly thrown from 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 we randomly selected 10 samples of cover crop biomass for C content measurements via the 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.

We 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 Fall 2011 and 2012. The total biomass was measured along the two crop rows of 1 m length. We dried the biomass sample in a 70°C oven for one week and weighed it after drying. No data on cover crop biomass was collected from the

experiment prior to 2011. Thus we 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>). To estimate the biomass of corn and soybean and wheat during 2006-2011 period in the fields we used yield data collected from the studied fields using yield-monitors from the start of the experiment in 2006 (data available 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, and 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 a 25°C incubator. After 72 hours the concentration of CO₂ was measured in 5 ml gas samples taken from each jar using a LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE).

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). 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 Wang, 2008).

Estimation of C inputs from cover crops prior to start of the study

We assessed the relative contributions of cover crops and main crops to the C inputs in the three studied management systems during the five-year period from the start of the Scale-up experiment in 2006 to the 2011 sampling. The five years of 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). The estimates of the amounts of C inputs to soil from the aboveground cover crop biomass from 2006-2011 constituted 2.0 and 2.6 Mg ha⁻¹ in the low-input and organic managements, respectively. With root-to-shoot ratios of 55% (Snapp et al., 2007) and 30% (Høgh-Jensen and Schjoerring, 2001; Puget and

Drinkwater, 2001) for rye and red clover respectively, we estimated the total amounts of C inputs into the soil from cover crops at the time of incorporation. The estimates should be treated as conservative, as they do not account for C inputs into soil via root exudates during cover crop growth.

Statistical analysis

Two sets of data analyses, one for the objective 1 and the other one for the objective 2, 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 topographic positions. For this analysis we used data from bare micro-plots in conventional management fields and from cover micro-plots in the cover crop based management fields. Note that micro-plots in conventional management and cover micro-plots in the low-input and organic managements were not subject to any manipulations and thus were representative of their respective management practices.

The statistical model for this analysis 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 Kravchenko and Robertson (2011). In the course of power analysis we calculated minimal detectable differences (MDD) between the managements, that is, the smallest differences between the managements that could be detected as statistically significant given the numbers of experimental fields. The considered MDDs were such that could be declared as

statistically significant at probability of Type I error, α , of 0.05, given the observed variability, with a power of 80%.

The purpose of the second set of data analyses was to directly assess the effects of cover crop presence on C measures at different topographical positions. For this analysis we 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 management (low-input and organic, only), topographical position, cover crop presence, and the interaction between them as the fixed factors. Fields nested within managements and interaction between fields and topographical positions were included in the model as random factors. The fields were used as an error term for testing the effect of management, while the field by topography interaction was used as an error term for testing the effect of topography.

For both sets of data analysis we 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 Ladoni et al. (2015).

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. We 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. We 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.

RESULTS

Effects of management and topography on TOC, POC and SMC

The fields in the studied three managements did not differ in terms their overall topographical characteristics. No significant differences in slope percent and relative elevation existed between the fields from the three studied managements (Table 1). Across all fields topography had a significant influence on sand content and soil bulk density ($p < 0.05$). Depressions had the lowest bulk density and sand content levels (Table 1).

By 2011 the studied management practices had been in place for 5 years, i.e., since 2006. However, the overall effect of managements across all topographic positions was not statistically significant for either TOC, POC, or SMC (Table 2). To further examine the lack of significant differences among the managements we conducted post-hoc power analysis. 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 our study.

341 As expected, topography had a substantial effect on TOC, POC and SMC in both
342 conventional and cover crop-based managements, with highest levels of all three C
343 variables observed at depressions, followed by summits and slopes (Fig. 2). Since the
344 results for all three studied C fractions were very similar in the organic and low-input
345 fields, for the analyses of topographical influences we combined the data from organic
346 and low-input management treatments, referred to from now on as management with
347 cover crops.

348 In topographical depressions the conventional management had numerically
349 higher levels of all three studied C fractions as compared to the management with cover
350 crops (Fig. 2). On topographical slopes the cover crop management tended to have higher
351 values of POC ($p < 0.05$) and SMC (numerical difference only) than the conventional
352 management.

354 *Contribution of cover crop to POC and SMC across topography*

355 The aboveground inputs of C from both red clover and rye residue in depressions
356 were significantly larger than that on slopes (Fig. 3; $p < 0.05$).

357 The results of micro-plot data analyses indicated that soil covered with red clover
358 had higher SMC levels in all topographical positions ($p < 0.1$ and $p < 0.05$) (Fig. 4a). Soil
359 in rye cover micro-plots had significantly higher SMC only on summits (Fig. 5a)
360 ($p < 0.05$). POC in cover microplots tended to be numerically higher than that in bare
361 plots, but the differences were not statistically significant (Figs. 4b and 5b).

362 The regression analysis between SMC differences in cover-bare microplots and
363 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 ($p < 0.1$). For both red clover and rye, the relationship between cover crop biomass and the increase in SMC was not statistically significant in depressions. The regression analysis between POC differences in cover-bare microplots and red clover biomass indicated presence of positive association in depressions, while the difference in POC between bare and cover was significantly related to rye biomass across all topographical positions (Table 3).

Significantly larger SMC was observed in the soil under red clover compared to bare soil at the beginning of the growing season before red clover termination, as well as two months after red clover termination and after the main crop harvest ($p < 0.1$) (Fig. 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. 6b); however, the differences were not statistically significant. The effects of cover crops on POC during the growing season were not statistically significant (data not shown).

DISCUSSION

Despite the large differences in the C inputs between conventional and cover crop-based management practices the overall effects of management on soil organic C fractions across entire studied fields were not statistically significant. However, the presence of cover crops differentially affected POC and SMC at different topographical positions. Thus the results supported our hypothesis that topography influences the magnitude of cover crop effects on soil C. Nevertheless, contrary to our expectations, the effects of cover crop presence tended to be greater in topographical summits and slopes

as compared to depressions. This was despite the substantially higher amounts of cover crop biomass produced in depression areas.

Effects of management on TOC, POC and SMC

The studied management practices differed from each other in terms of several components relevant to soil C, including differences in C inputs due to cover crop presence and due to differences in main crop growth, as well as differences in the fertilizer inputs. Specifically, from the start of the Scale-up experiment in 2006 to the first soil sampling of this study in 2011 the low input and organic management systems had received substantial C inputs from their cover crops, e.g. 3.7 Mg C ha⁻¹ and 4.5 Mg C ha⁻¹, respectively. On the other, hand, the total amount of C in the aboveground biomass of main crops during the 2006-2011 period was 13.5, 10.5, and 8.2 Mg C ha⁻¹ for conventional, low-input and organic management, respectively. While it was not possible to estimate the belowground C inputs if these systems, still it is safe to assume that overall C inputs from main crops of conventional system exceeded those of low-input and organic.

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). Yet Grandy et al. (2013) reported no effects from N fertilizer on residue decomposition in the same study area.

409 Reports from the adjacent LTER site convincingly demonstrated that, after >10
410 years of implementation, the organic management resulted in higher soil TOC levels as
411 compared to those of the conventional management (Robertson et al., 2000; Grandy and
412 Robertson, 2007; Hao and Kravchenko, 2007; Senthilkumar et al., 2009; Syswerda et al.,
413 2011); while TOC in low-input management was numerically but not statistically
414 significantly higher than that of the conventional management (Syswerda et al., 2011).
415 Syswerda et al. (2011) reported that after 12 years of continuous cover cropping the
416 change in TOC constituted only 20%. Thus, only 5 years (2006 to 2011) duration of this
417 study probably was not sufficient to generate detectable differences in TOC. Still we did
418 expect to observe differences in POC and SMC, since these C variables are known for
419 their more rapid response to management changes (Haynes, 2005; Culman et al., 2013).

420 However, as suggested by power analysis, the sizes of the changes that could be
421 detected for TOC and POC were much greater than the effects of cover crop presence
422 expected to take place in 5 years in the studied area (Table 2). The most likely possible
423 cause for the large MDD values is extremely large variability of the C fractions due to
424 high soil and topographical diversity of the large fields used in our study. Large
425 variability translates into smaller statistical power and larger MDD values.

426 VandenBygaart (2006) also reported that one of the difficulties of studying soil C at the
427 landscape scale is multiple sources of variation contributing to greater variability, and
428 thus to low power of statistical comparisons. Large topographical variations typical of
429 agricultural fields of the Great Lakes region is one of the main sources of variations in
430 soil properties (Kravchenko and Bullock, 2000; Yoo et al., 2006), including soil C

(VandenBygaart and Kay, 2004; Senthilkumar et al., 2009), and crop biomass (Jiang and Thelen, 2004; Munoz et al., 2014) across agricultural fields.

Topography modifications of management effects on soil C

The observed differences in the magnitudes of topographical trends under the conventional and cover crop based managements (Fig. 2) probably resulted from a combination of the 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 eroded in agricultural fields under a tillage system similar to ours in northern Ohio with terrain slopes comparable in steepness with those here. Our 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. Jacinthe et al. (2002) reported erosion to have a greater effect on redistribution of POC as compared to other C variables, likely due to erosion-driven movement of smaller pieces of plant residue that constitute a large component of POM. A greater difference between POC in slopes and depressions under conventional management observed in our study thus can be an indirect indication of erosion effects. Conversely, somewhat smaller differences between depressions and slopes in the management with cover crops could be seen as an indicator of lower erosion under cover crops (Fig. 2b). These observations are

consistent with the 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.

Spatial and temporal cover crop contributions to POC and SMC

We 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. One advantage of our innovative micro-plot based experimental design with the micro-plot pairs located in close proximity to each other was the ability to directly 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 use of the micro-plots allowed us to specifically focus on the effect of the cover crop presence. This is in contrast to the soil C trends observed from the entire fields, which were affected both by the cover crop growth patterns and by erosion. Indeed, in depressions both the bare and the cover micro-plots were equally influenced by the influx, if any, of eroded material from the higher located areas. Thus, the differences between the micro-plot pair members there can be safely attributed to cover crop presence. While for the micro-plot pairs on summits and slopes it could be assumed that the magnitude of losses of soil from the bare plots due to erosion, if any, was much smaller than what could be expected from similar topographical positions in fields entirely under conventional management without cover crops. During the study period we observed no visual indications of soil erosion occurring at the bare plots of summits and slopes. Thus, again, it can be safely assumed that the differences

between cover and bare micro-plots of each pair are driven primarily by the cover crop presence.

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 labile C pools (Fig. 4a). Our results are in agreement with those of Steenwerth and Belina (2008) who observed positive effects from cover crops on mineralizable pools of soil C and with those of Franzlubers (2002) and Culman et al. (2013) who found SMC to be reflective of management effects. However, our observations are in contrast to observations 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 the lack of statistical significance (Ladoni et al., 2015).

As mentioned earlier, in our study 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 managements.

The larger cover crop biomass returned to soil in depression areas (Fig. 3) is driven by better growing conditions there, including higher soil organic C (Table 2), lower sand content (Table 1), and better soil structure in depressions as compared to slopes and summits. Thus we assumed a presence of a feed-forward mechanism of better cover crop growth because of, in part, higher soil C, followed by a still 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. 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). In a near-by experimental site, depressions under cover crops management had higher soil CO₂ emissions than depressions under conventional management, while no differences in CO₂ fluxes between cover and conventional managements were observed in summits (Negassa et al., 2015). 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.

The temporal patterns in the effects of cover crop presence on SMC and POC observed here are similar to those reported earlier. For example, Steenwerth and Belina (2008) observed an increased potential microbial respiration during the entire growing season, with an increase appearing in spring even before cover crop termination. Relatively weak relationships between the aboveground cover crops biomass and SMC (Table 3), fast decomposition of cover crop residue, and increases in SMC observed even before the aboveground biomass was returned to the soil indicate that the roots of cover crops play an important role in increasing labile pools of soil C. Experimental studies of cover crop roots are rare due to difficulties associated with accurate measurements of root activity, however, those conducted showed that cover crops produced larger belowground than aboveground biomass (Gardner and Sarrantonio 2012). Using C isotopes Steinbeiss et al. (2008) reported that soil C was derived more from roots than from the aboveground biomass. Gleixner (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 (McDaniel et al., 2014).

Another interesting observation was that larger SMC values in red clover microplots as compared to bare microplots 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. Our 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, Culman et al. (2013) reported significant differences in SMC between managements during growing season but no significant differences at the end of growing season. Our observations suggested that red clover presence could gradually increase labile pools of soil C and increase the soil quality in the long-term. This observation is consistent with current understanding of the enhanced role that plant residues of high-quality, e.g., legumes, can play in C sequestration (Cotrufo et al., 2013; Castellano et al., 2015). The positive effect of high-quality plant inputs is expected to be particularly notable in soils that are far from the state of C saturation (Castellano et al., 2015), which the soils of the current study is an example of.

Management Implications

Recommendations for cover cropping in agricultural landscapes on diverse terrain should consider two factors that control the magnitude of effects of cover crops on soil organic C. The first factor is how much of cover crop residue is returned to soil when

cover crops are terminated. Management practices should allow for good establishment of cover crops before their termination in the spring. However, aboveground biomass of cover crops may have a smaller role compared to their belowground growth and activity of cover crop roots. This suggests that farmers can still benefit from cover crops even if the aboveground biomass is harvested or grazed.

The second factor is the extent of the erosion in the field. The short-term and long-term effects of cover crops are magnified in an agricultural field that is prone to water and sediment redistribution via surface runoff. Cover crops limit the depletion of soil organic C on slopes by controlling erosion. Observed tendencies for positive response of soil SMC and POC to cover crop inputs on slopes and summits are especially important. These are the areas within the fields with the lowest soil fertility and the lowest crop yields, therefore increasing organic C there would be of interest to farmers. The greater benefits of cover crops on slopes and summits open interesting perspectives for site-specific cover crop management. A differential planting of cover crops across topography can be practiced to meet the specific production needs. Planting cover crops only on slopes and summits will be sufficient to increase soil quality via controlling erosion. This can be an important consideration in some geographical areas for the farmers to decide on using winter cover crops. For example, wet depressions in spring can delay main crop planting, especially when they have cover crops grown there. Presence of cover crops only on slopes and summits can speed up spring field operations, thus facilitate adoption.

CONCLUSIONS

- 1- The effects of five years of contrasting, i.e., conventional vs cover-crop based, agricultural managements on TOC, POC and SMC were not detected in the data analyses conducted across large agricultural fields, likely because of extremely high variability of the entire field data. However, the conventional management tended to have lower POC on the topographical slopes compared to managements with cover crops, indicating a gradual loss of C, possibly as a result of greater soil erosion in conventionally managed fields.
- 2- The magnitude of cover crop effects on SMC and POC varied across topography. However, despite greater aboveground cover crop biomass values in depression areas, the contributions of cover crop's presence to soil C variables there were not greater, and in some cases even lower, than that on topographical slopes and summits. Greater decomposition of the cover crop biomass in depression areas and reduced C depletion by soil erosion on slopes and summits are hypothesized reasons for the observed result, which require further testing.
- 3- The observed results indirectly point to greater importance of the cover crop roots, as opposed to the aboveground biomass, for soil C processes. Specifically, the aboveground biomass was primarily only weakly correlated with the increases in SMC and POC. Most of aboveground biomass of cover crops decomposed within two months after they were returned to soil. Moreover, the positive effects of red clover on SMC were observed even before its aboveground biomass was returned to soil.
- 4- Both POC and SMC could reflect the changes induced by management, however the spatial and temporal effects of cover crops on soil organic C were more

detectable when assessed via SMC. The larger lab measurement variability of POC compared to SMC decreased the statistical power of detecting the changes in POC. SMC is a suitable measure of soil organic C changes due to its rapid response to management modifications and its low measurement variability.

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Figure Captions

Figure 1. a) The study area showing locations of the monitored fields from the three studied treatments with the years on which the field was sampled; b) a schematic representation 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.

Figure 2: Total organic carbon (TOC) (a), particulate organic carbon (POC) (b), and short-term mineralizable carbon (SMC) (c) in the studied fields after five years of conventional and cover crop managements. Error bars represent standard errors. Different letters above the bars indicate significant differences among topographical positions within the same management type ($p < 0.05$). The * shows significant difference between conventional and cover crop based managements ($p < 0.05$).

Figure 3. C returned to soil from the 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. Different letters above the bars indicate significant differences between the topographical positions for each of the cover crops ($p < 0.05$).

Figure 4. Short-term mineralizable carbon (a) and particulate organic carbon (b) 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.

Figure 5. Short-term mineralizable carbon (a) and particulate organic carbon (b) 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 $p<0.05$.

Figure 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.

886 Table 1. Means and standard errors (in parentheses) of topographical features and soil
 887 properties of the studied fields.

	Agricultural management		
	Conventional	Low-input	Organic
Slope, %	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

888 Different lowercase letters mark significant differences among the topographical
 889 positions (p<0.05); when there were no significant differences among positions the letters
 890 were not shown.

891

892 Table 2. Means and standard errors (in parentheses) of total organic carbon (TOC),
893 particulate organic carbon (POC) and short-term mineralizable carbon (SMC) after five
894 years of practicing the three studied managements in the studied fields across all three
895 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

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Table 3. Relationships between the cover crop biomass and the cover vs. bare micro-plot differences in terms of short-term mineralizable carbon (SMC) and particulate organic C (POC).

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	
		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
	Red clover biomass	Depression	0.02	0.03
		Slope	0.01	0.10
		Summit	0.00	
POC difference between cover and bare (%)	Rye biomass	Depression	0.08	0.01
		Slope	0.10	0.03
		Summit	0.14	0.01

* p-values greater than 0.15 are not shown

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6