CHANGE IN SOIL CHARACTERISTICS AFTER INCORPORATING COVER CROPS IN THE CROP ROTATION

By

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ABSTRACT

Planting cover crop mixtures in the cash crop rotation has been proposed to overcome possible cash crop productivity losses during the organic transition (OT) period. The first objective of this study was to examine the effects of cover cropping systems on soil properties of potential importance for crop performance and cash crop yield following three years of an OT period prior to planting organic corn (*Zea mays* L.). The 3-year crop rotation was a corn - soybean (*Glycine max* L.)-winter wheat (*Triticum aestivum* L.) rotation (CSWR). Four cover cropping systems were studied including a traditional cover crop system (TR), a mixture of cold susceptible cover crop species (WK), a mixture of cold tolerant species (WH), and a no-cover control (NC). Split block experiments were setup in four fields with contrasting topographical positions, namely depression, slope, and summit in each field. There was no difference in particulate organic matter, microbial biomass carbon, nitrogen mineralization rate, soil ammonium contents, and organic corn yield following the transition period, across the cover crop treatments. However, soil moisture, nitrate content, carbon mineralization rate, and aboveground plant biomass were significantly affected by cover crop treatments. Field topography influenced organic corn yield after the transition period, with the highest yields in field depressions. The second chapter focused on a meta-analysis from 15 field studies that estimated the change in soil carbon sequestration due to cover crops and tillage systems. Four systems were compared: conventional tillage without cover crop (CT-NC), conventional tillage with cover crop (CT-CC), no-tillage without cover crop (NT-NC), and no-tillage with cover crop (NT-CC). NT-CC systems had the highest whereas CT-NC had the lowest soil carbon sequestration. Tillage had stronger influence on carbon sequestration compared to cover crops.

To my family and friends

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CHAPTER 1
INTRODUCTION

The Food and Agriculture Organization of the United Nations says that total food production needs to increase by 70% by 2050 to feed the growing population (FAO, 2009). The amount of land available for farming is decreasing. The environmental impact of agriculture on limited water resources in a warming climate also limits food production (Foley et al., 2011). To increase agricultural productivity and to ensure the long-term viability of food supply, sustainable and resilient agricultural practices must be followed (Scoones and Toulmin, 1999; Ajayi, 2007; Wilson and Lovell, 2016).

Organic farming systems are increasing in the United States because of favorable organic food pricing and profitability and because of farmer's choice in how they would like to farm. Farmers may prefer to only use organic-certified pesticides, no crop varieties or hybrids that are GMO, and/or prefer to use only animal manure or plant-based residues as fertilizers. Enhancing soil fertility, biodiversity, reducing groundwater pollution, and combating global warming are just a few of the significant environmental advantages that organic farming offers (Mondelaers et al., 2009; Tuomisto et al., 2012; Gattinger et al., 2012). Certifiying farm fields as organic takes 36 months (Webber et al., 2009). During this time, chemical fertilizers and pesticides cannot be applied and crops cannot be marketed as organic, potentially diminishing crop yields and profits (Delbridge et al., 2017). Adding cover crops to a farming system transitioning to organic production may increase nitrogen mineralization and other nutrient availability and may influence weed management (Mitchell et al., 2017; Ghimire et al., 2019) These factors may help mitigate any potential yield decreases during the organic transition when crop prices remain lower than organic prices (Kim et al., 2020).

Cover crops improve soil structure, minimize erosion and nutrient loss, increase soil carbon (C) and nitrogen (N), and improve water infiltration and storage and microbial activity (Blanco-Canqui et al., 2015; Vukicevich et al., 2016; Schmidt et al., 2018). Cover crops suppress weeds by competing for light, nutrients, and water or by releasing allelopathic compounds (Brust et al., 2014; Cordeau et al., 2015). Leguminous cover crops supply N to crops; high-biomass non-legumes minimize soil erosion, suppress weeds, and improve soil organic matter; and tap-rooted species like *Brassica* spp. reduce soil compaction (Kasper et al., 2019; Chen and Weil, 2010; Ebelhar et al., 1984; Kaspar et al., 2001). Farmers establish and manage cover crops to maximize soil-based ecosystem services during the organic transition period (Plastina et al., 2018). Mixtures of of cover crop species may provide multifunctional advantages to agrosystems because no single species can provide all the benefits a farmer may be interested in (Kramberger et al., 2014; Tosti et al., 2014). Cover crop mixtures can enhance plant biomass and N-fixation (Hooper et al., 2005). Finney et al. (2016) found that combining cover crop species reduced nitrate leaching and improved weed control. Grass-legume combinations generated biomass similar to or greater than single species (Ranells and Wagger, 1996; Hayden et al., 2014), suggesting that combining cover crops with different N functionality may improve plant biomass. Cover crop mixtures and their effect on soil properties is an important topic of research, and how cover crops influence soil properties following the transition period has not been researched.

Therefore, the first objective of this thesis was to study the effects of cover crops on soil characteristics following the organic transition period.

Soil organic carbon (SOC), a key marker of soil health, is crucial for food production, greenhouse gas balance, and the mitigation and adaptation to climate change (Lorenz and Lal, 2016). Due to land conversion and exploitation, agricultural soils often have lower SOC levels than soils with wild vegetation (Hassink, 1997; Poeplau and Don, 2015). Compared to natural or semi-natural vegetation, SOC losses through agriculture are 30–40% higher (Don et al., 2011; Poeplau et al., 2011). Thus, it is crucial to raise SOC stocks.

Tilled soils are viewed as a depleted C reservoir that can be refilled, and plowing the soil is primarily blamed for C loss (Reicosky, 2003). In the United States, Lal and Bruce (1999) estimate that croplands have lost 5 Gt C, or 36 t ha^{-1}, and that with careful management, much of this can be restored over 50 years. Boosting carbon inputs and decreasing carbon outputs are the keys to increasing soil carbon sequestration (Campbell et al., 2014). For SOC sequestration, it is commonly recommended to implement conservation agriculture techniques such as including cover crops into the crop rotation, applying biochar to soils, and decreasing soil tillage (West and Post, 2002; Smith, 2004; Lal, 2004a; Luo et al., 2010a; b). In the US, widespread adoption of conservation tillage might capture 24–40 Mt of carbon per year (Lal et al., 2003). One of the most significant global actions for stabilizing atmospheric CO_2 concentrations would be to convert all croplands to conservation tillage, which has the potential to sequester 25 Gt C over the next 50 years (Pacala and Socolow, 2004). One of the potential options was to switch from conventional tillage (CT) to no-tillage (NT), with an annual rate of C sequestration of 100–1000 kg ha-1 (Smith et al., 1998; Paustian et al., 2000; Six et al., 2004; Lal, 2004a).

Cover crops can also help soils adsorb carbon. (Mazzoncini et al., 2011; Verhulst et al., 2012; Poeplau and Don, 2015; Muhammad et al., 2019). In agroecosystems, cover crops add carbon, nitrogen, biodiversity, and above- and belowground biomass (Lal, 2004b; Blanco-Canqui et al., 2011). Cover crops improve soil structure and aggregation (Sainju et al., 2011), reducing carbon loss from soil erosion (De Baets et al., 2011). Cover crops can counterbalance SOC losses and soil property deterioration by raising C concentration and stocks (Ruis and Blanco-Canqui, 2017). In water-limited places, cover crops reduce soil erosion and drought stress for the next crop while increasing carbon input (Lal, 2004b; Frye et al., 2015). Studies show cover crops can raise SOC storage and prevent climate change (Lal, 2004b). A meta-analysis study found that C input driven SOC sequestration with cover crops was effective (Poeplau and Don, 2015), with a potential anticipated worldwide SOC sequestration of 0.12 Pg C yr^{-1}, which would offset 8% of the direct yearly greenhouse gas emissions from agriculture.

Most research on soil carbon sequestration focuses on single practices (conversion to no-tillage or cover crops), and there are few studies measuring the combined effect of conservation tillage and cover crops. Cover crops and conservation tillage increase SOC, according to recent studies (Blanco-Canqui et al., 2013; Higashi et al., 2014). This highlights the need to statistically examine the combined impacts of no-tillage and cover crops on SOC sequestration under varied climate and soil conditions.

Therefore, the second objective of this thesis was to evaluate the combined effects of conservation tillage (no-till) and cover crops compared to conventional tillage with and without cover crops on soil carbon sequestration using a meta-analysis.

CHAPTER 2
EFFECTS OF INTERSEEDED COVER CROP MIXTURES ON SOIL PROPERTIES IN TOPOGRAPHICALLY DIVERSE AGRICULTURAL LANDSCAPES DURING A THREE-YEAR ORGANIC TRANSITION PERIOD

Introduction

Agricultural producers are becoming increasingly interested in developing organic farming systems in response to growing pricing of organic products and concerns over environmental sustainability. A 36-month waiting time is necessary to get official organic certification (Webber et al., 2009). There may be a drop in agricultural yields and profit if farmers are unable to use synthetic inputs like chemical fertilizers and pesticides at this time (Delbridge et al., 2017). One way to counteract the possible yield drop during the organic transition is to incorporate cover crops into the rotation (Ghimire et al., 2019; Mitchell et al., 2017). Cover crops can improve soil quality by improving soil aggregation and soil structure, decreasing erosion and nutrient loss, increasing soil carbon (C) and nitrogen (N), improving drainage, enriching soil microbial communities, and reducing weed growth (Blanco-Canqui et al., 2015; Schmidt et al., 2018). These benefits of cover crops may enhance crop productivity during the 36 month transition to an organic system (Tribouillois et al., 2016; Florence and McGuire, 2020). Planting mixtures of diverse cover crop species has been suggested as a potential way to enhance cover crop benefits (Treadwell et al., 2010; Wortman et al., 2012b; Robertson et al., 2014) (Vukicevich et al., 2016; Finney and Kaye, 2017; Smith et al., 2020). Cover crop mixtures with higher functional diversity can mitigate cover crop disservices and favor multifunctionality of cover crops (Finney et al., 2017; Finney and Kaye, 2017).

The effect of cover crops on cash crop yields have been inconclusive. Increasing the number of species in the cover crop mixture resulted in higher biomass with enhanced ecosystem services such as better weed suppression, reduced nitrate leaching, however, the corn (Zea mays

L.) yield in the following cropping season was negatively affected (Finney et al., 2016; Lapierre et al., 2022). Legumes, grasses, and Brassica spp. mixture resulted in higher soybean (Glycine max L.) yield, increased soil gravimetric water content and soil inorganic nitrogen as compared to monoculture and no-cover crop treatments in a 3-year study (Chu et al., 2017a). According to a meta-analysis , leguminous winter cover crops increased corn yield by 37%, biculture winter cover crops increased yield by roughly 21%, and grass winter cover crops had no effect on corn yield as compared to no cover crops (Miguez and Bollero, 2005), which concluded that winter cover crops with proper management does not result in reduced corn productivity (Marcillo and Miguez, 2017).

While cover crops can affect soil characteristics and production, research data indicate that cover crop effects on soil characteristics are inconsistent and can vary over time (Blanco-Canqui and Jasa, 2019). The short-term investigations demonstrated limited and variable cover crop effects on soil parameters (Wegner et al., 2015; Rorick and Kladivko, 2017; Blanco-Canqui et al., 2017; Dozier et al., 2017). The few long-term research on cover crops reveal more consistent effects of cover crops, indicating that cover crops may change soil properties more over the long term (Basche et al., 2016b; Blanco-Canqui et al., 2011a; Olson et al., 2014b). Long term cover crop experiments will provide more accurate assessment of the cover crops benefits for boosting soil ecosystem services (Blanco-Canqui et al., 2015; Liebig et al., 2015).

Topography also impacts the soil properties and crop yield, including cover crops and their benefits to the soil (Ladoni et al., 2015; Muñoz et al., 2014). Landscape topographic patterns affect soil nutrients, plant growth, water flow distribution, and microbial biomass (Corre et al., 2002; Kay et al., 2006). Soil N availability has been reported to be different among different topographical positions due to variations in soil organic matter (Chan et al., 2011).

Cover crops and topographical positions had an interactive effect on corn yields (Muñoz et al., 2014), where a red clover (Trifolium pretense) cover crop had a positive effect on corn yields at summit and slope positions. Thus, topography can mediate influences of cover crops on soil properties. Understanding the role of topography in mediating cover crop effects on soil properties and plant performance is necessary to develop effective management strategies during the organic transition period.

Cover crop-based organic transition in row crop rotation systems, such as corn-soybean-winter wheat (Triticum aestivum L.) rotation (CSWR), has grown in popularity in the Midwest of the United States because of low financial risk in the transition years (Silva and Delate, 2017). However, there are uncertainties in the selection of cover crop mixtures, the seeding times during the transition, and the termination timing of winter hardy cover crop species (Belfry and Van Eerd, 2016; Finney et al., 2016, 2017; Wortman et al., 2012a). The length of the cover crop growth season and the potential C, nutritional, and microbial diversity contributions from the cover crop are reduced when cover crops are seeded during corn senescence or after corn is harvested. Cover crops sown in corn at the V8–V10 growth stage won't compete with the corn crop, but they might struggle to establish in this shadowed environment (Belfry and Van Eerd, 2016). On the other hand, sowing a cover crop at the same time as corn could result in competition for resources with the developing crop (Uchino et al., 2009). A different strategy is to interseed a cover crop into corn at V4-V6 stage, which can be done in conjunction with another field activity and extends the cover crop's growing season and ecosystem services (Noland et al., 2018). Interseeding cover crops in to corn at V4-V6 stage did reduce corn grain yield (Baributsa et al., 2008; Jeranyama et al., 1998). Although, the use of cover crop mixtures grows in popularity due to their potentially greater biodiversity benefits, there is less information is available on cover crop mixtures interseeded in corn in June or early July during the organic transition period. The ideal cover crop species, planting, and termination times that would maximize ecosystem benefits during the CSWR transition to organic production are still unknown.

In 2018, we initiated a field study to investigate the benefits of incorporating cover crops during the three-year transition period of CSWR system to organic production The cover crop systems included no-cover control (NC); a traditional cover crop system (TR) used in CSWR in the US Midwest consisting of cereal rye (Secale cereal L.) planted after corn harvest in the first year and red clover (Trifolium pretense) frost-seeded into the winter wheat in the third year of the rotation; and a mixture of either cold tolerant (WH, short for winter-hardiness) or winter killed (WK, short for winter-killed) cover crop species interseeded into corn in the first year and planted after wheat harvest in the third year. The study addressed the system performances across a topographically diverse terrain, targeting three contrasting topographical positions: namely depressions, representing footslope and toeslope positions; slopes, representing backslope and shoulder positions; and summits, representing summit positions. Nguyen et al., 2022 highlighted the experimental results of the study after the first year of the transition. They found that WK cover crops led to higher soil NO3- content, especially in the depression and summits. WH covers crops had higher microbial biomass in depressions and higher soil N mineralization rate in slopes as compared to other cover crop systems, whereas the cover crops did not have any effect on soil C mineralization. The study showed positive effects of cover crops in improving soil characteristics as well as in decreasing soil N leaching risks. Here we follow up on that work by measuring soil properties in the spring prior to planting organic corn after the three-year organic transition.

The advantages of legume cover crops have been known for decades and red clover is one of the most popular and advantageous when frost-seeded under winter wheat prior to planting corn in the rotation the year after wheat (Gaudin et al., 2013). Red clover that has been frost-seeded into winter wheat fixes a significant amount of nitrogen, builds a strong root system, and produces a significant quantity of plant biomass without harming winter wheat yield (Stute and Posner, 1993; Thiessen Martens et al., 2001; Tiffin and Hesterman, 1998). Addiingf red clover to the cropping system is reported to positively impact SOM, improve soil quality, improve water use efficiency and nitrogen use efficiency, reduce nitrate leaching, increasing subsequent crop biomass production and corn grain yields (Gaudin et al., 2013).

The objectives of this research were to explore the effects of three cover crop-based transition scenarios, first, on soil characteristics of potential importance to plant performance at the end of the three-year organic transition period and, second, on the yield of the first organic cash crop (corn). The soil properties studied included soil moisture, soil mineral N, soil C and N mineralization, and microbial biomass C. The performance of the cover-crop systems was assessed in topographically diverse landscapes at four field sites, three of them transitioned to organic in 2021 and one field in 2022.

Materials and Methods

Field site description

The study was conducted at 4 experimental fields located at Kellogg Biological Station (KBS) (42° 24′ N, 85° 24′ W), Michigan. The region’s mean annual temperature and annual rainfall is 10.1^{o}C and 1005 mm, respectively, with about half of the precipitation received as snow (Robertson and Hamilton, 2015). The soils of the studied fields are well-drained Typic Hapludalfs of Kalamazoo (fine loamy, mixed, mesic) and Oshtemo (coarse loamy, mixed, mesic) series, which developed on glacial washout from the last Wisconsin glaciation (Crum and

Collins, 1995). The experiment started in May 2018 at three of the fields, referred to as fields A-1, 82-1, and MP, and in May 2019 for an additional field, namely field 85 (Appendix Supplementary Figure 1). Prior to the start of the experiment soil samples for baseline soil characterization were collected from 0-20 cm depth and analyzed for soil texture and total C and N contents. The results were reported previously by Nguyen et al., 2022.

Experimental design

The crop rotation consisted of corn, soybean, and winter wheat in the first, second, and third years of transition, respectively. The two studied factors were topographical position and type of cover crop. The topography factor had three levels: depression, slope, and summit. The four types of cover crop systems include no cover control (NC); traditional cereal rye planted after corn harvest in the first year and red clover frost seeded into wheat in the third year (TR); a mixture of cold susceptible species: oats *(Avena sativa*), winter pea (*Pisum sativum*), and radish (*Raphanus sativus*) interseeded into corn at the V5-V6 growth stage in the first year and seeded again after wheat harvest in the third year (WK); a mixture of cold tolerant species: annual ryegrass (*Lolium multiflorum*), Dwarf essex rapeseed (*Brassica napus*) and crimson clover (*Trifolium incarnatum*) interseeded into corn at the V5-V6 growth stage in the first year and terminated to the following May prior to soybean planting. The mixture was seeded again after wheat harvest in the third year and terminated the following May prior to corn planting (WH). In three of the studied fields the experiment was setup as a split block design with either four (fields A-1 and 82-1) or three (field 85) replications. The blocks were placed across three topographical positions. The cover crop treatments were assigned at random to four experimental plots within each block. The plots were 4.5 m in width and ranged from 19 to 372 m in length depending on the field size (Appendix, Supplementary Figure 2 A-C). The fourth field, MP, consisted of two blocks located within each topographical position. Each block was further divided into four subblocks. The sub-block had four experimental plots (8.5 by 19.5 ft), and the four cover crop treatments were randomly assigned to these four experimental plots within each sub-block (Appendix, Supplementary Figure 2 D).

Crop rotation and cover crops during organic transition

Transition year 1 (Corn): Corn was planted in May 2018 in fields A-1, 82-1, and MP, and in May 2019 for field 85, in 30-inch rows at a seeding rate of 75,000 seeds/ha. The WH and WK cover crop mixtures were interseeded by broadcasting onto the fields when corn was at V5-V6 developmental stage, in June 2018 for fields A-1, 82-1, and MP, and June 2019 for the field 85. The WH mixture consists of annual ryegrass, Dwarf essex rapeseed and crimson clover at seeding rates of 9, 2, and 5 Kg/ha, respectively. Whereas the WK mixture is made up of oats, winter pea and radish at seeding rates of 28, 23 and 2 kg/ha, respectively. Corn was harvested in November 2018 (fields A-1, 82-1, and MP) and November 2019 (field 85), followed by cereal rye seeded at125 kg/ha using a no-till drill in the Traditional (TR) cover crop system.

Transition year 2 (Soybean-wheat): The fields were chisel-plowed to terminate cereal rye and the WH mixture at the end of May next year. Soybean was then planted in 30-inch rows at a seeding rate of 370,000 seeds/ha in June 2019 for the fields A-1, 82-1, and MP and in June 2020 for the field 85. The soybean was harvested in first week of November in 2019 in fields A-1, 82-1, and MP and in November 2020 for the field 85. Following the soybean harvest, wheat was seeded at 5,500,000 seeds/ha.

Transition year 3 (Wheat/cover crop): The red clover was frost seeded into the wheat at 15 kg/ha in TR cover crop systems in late March 2020 for the fields A-1, 82-1, and MP and in March 2021 for the field 85. Wheat was harvested in August 2020 for the fields A-1, 82-1, and MP and in August 2021 for the field 85, using a farm combine and straw was not removed. Following wheat harvest, the plots (except TR treatment plots consisting of red clover) were chisel-plowed to terminate any volunteer weed growth. Two cover crop mixtures, WK (oats: 28 kg/ha, winter pea: 23 kg/ha, and radish: 2kg/ha) and WH (annual ryegrass: 9 kg/ha, Dwarf Essex rapeseed: 2 kg/ha, and crimson clover: 5 kg/ha) were interseeded into wheat stubble. The fields were chisel plowed at the end of May in the following spring to terminate red clover and WH cover crops before the first organic corn planting.

Year 4 (Organic corn): Corn was planted at 75,000 seeds/ha in 30-inch rows on late May 2021 in the fields A-1, 82-1, and MP and in early June 2022 in the field 85.

Soil sampling

Soil samples were collected in spring (end of April) at the end of the transition, prior to organic corn planting. Soil samples were taken in 2021 from fields A1, 82-1, and MP and in 2022 from field 85. Three soil samples were taken using a push probe (2 cm diameter and 20 cm in length) from each plot within each topographical position and mixed into a single composite soil sample. Soil samples were kept at 4^{o}C prior to analyses.

Soil analyses

Gravimetric soil water content was determined by oven-drying a 10 g sub-sample at 104oC for 24h. Soil particulate organic matter (POM) was measured using the method described by Cambardella and Elliott (1992). Specifically, a 30 g soil sample was mixed with 90 ml of 0.05% $(NaPO3)_6$ solution and shaken at 200 rpm for 15 hours. The solution was then decanted onto a 3” #270 (53 µm) sieve and the material on the sievewas rinsed with DI water. The rinsed material in the sieve was transferred to an aluminum container and dried at 60˚C for two days and then ashed in a muffle furnace at 400 oC for 1h. The ashed material was weighed (POM) and is shown as POM g/kg dry soil.

Soil inorganic N, namely NO_3^- and NH_4^+, was measured using a micro plate method (Keeney and Nelson, 1982; Doane and Horwath, 2003). Briefly, NO_3^- and NH_4^+ was extracted with 2M potassium chloride solution, then filtered by Whatman no.1 filter paper. Salicylate and cyanurate for NH_4^+ and vanadium (III), sulfanilamide and N-(1-naphthyl) –ethylenediamine dihydrochloride for NO_3^-were added to 100 µl of each sample to form calorimetric solutions. The absorbance of solutions was measured at wavelengths of 630 and 540 nm to determine NH_4^+ and NO_3^-, respectively on a Synergy H1 spectrophotometer (Biotek, Vermont, USA). Standard curves were constructed from a series of known concentrations of $(NH_4)_2SO_4$ and KNO_3, to convert absorbance values into NH_4^+ and NO_3^- concentrations, respectively.

Soil microbial biomass C was measured using a fumigation-incubation method (Jenkinson and Powlson 1976), following the protocol reported by Nguyen et al., 2022. In brief, we used 10 g soil samples, either subjected to chloroform fumigation or as non-fumigated controls. Production of CO2 from the control and fumigated samples after a 10-day incubation was measured by using infrared Photoacoustic Spectroscopy (PAS) (1412 Photoacoustic multi-gas monitor; INNOVA Air Tech Instruments, Ballerup, Denmark) to calculate the microbial biomass C.

Measurements of short-term C mineralization were conducted by following the protocol mentioned in Nguyen et al., 2022 as per Franzluebbers et al. (2000) and Culman et al. (2013). Briefly, 10 g of soil samples in jars were incubated for 10 days in the dark. Infrared Photoacoustic Spectroscopy (PAS) was used to measure soil CO2 concentration within the jar at the end of the incubation to calculate soil mineralizable C.

Net amounts of NO_3^- and NH_4^+ produced in the soil incubated at room temperature over a period of 10 days were measured to determine soil N mineralization (Hart et al., 1994), following the protocol explained by Nguyen et al., 2022. Briefly, 10 g of soil sample was incubated at constant room temperature for 10 days. Soil NH_4^+ and NO_3^- concentrations were measured from incubated soil samples as explained above. The rate of N mineralization was determined as the difference in NO_3^- and NH_4^+ concentrations between soil samples without incubation (as mentioned in above soil inorganic N section) and the incubated samples.

Aboveground plant biomass measurement and cash crop yield

The fall cover crop and weed biomass was collected in mid-November (2020 for fields A1, 82-1, and MP, and 2021 for field 85). Prior to cover crop termination and corn planting, we collected spring cover crop and weed biomass (2021 for fields A1, 82-1, and MP, and 2022 for field 85). Two quadrats (50 x 50 cm) were randomly placed within each plot, but 1.5 m away from the plot margins to eliminate border effects (Wortman et al., 2012). Plants were collected from each quadrat separately for each species. After oven drying at 60 °C for at least a week, total plant biomass, the biomass of the cover crops and all weeds were calculated.

The organic corn (fourth year of rotation) was collected from two center rows of each plot using a plot combine and the weight of the harvested grain was recorded (. The corn yield was adjusted to 15.5% moisture and expressed as kg/ha.

Statistical analysis

The data were analyzed using a mixed effect model approach in *proc glimmix* procedure of SAS 9.4 (SAS Institute, Cary, NC, USA). Topography, cover crop systems, and their two-way interactions were used as fixed effects in the statistical model. The model also included the following random effects: (i) fields; (ii) block nested within topographical positions and fields, which was used as an error term for testing the effects of topography; and (iii) interaction of block and cover nested within fields, which was used as an error term for testing the effects of cover crop treatments.

The statistically significant interactions (*P* <0.05) between topography and cover crops were subjected to simple F-tests, after slicing, to explore differences among the cover crop treatments within each topography. Subsequently, multiple comparisons were conducted using t-tests for statistically significant sliced F-tests. The means with standard errors are reported for each cover crop system within three topographical positions separately. When the interaction between topography and cover crops was not statistically significant, yet the main effects were significant (*P* <0. 05), the marginal means were also compared using t-tests. In individual field analysis, we included blocks, the interaction between blocks and cover crops, and interaction between blocks and topography in the model as random effects fields A1, 82-1, and 85. The interaction between blocks and topography was used as an error term for testing the topography effect for field MP.

The equal variance and normality assumptions were checked using (i) plots of the residuals vs. predicted values and (ii) normal probability plots of the residuals. No violations in the assumptions were found for any of the studied data. The *P*-values for all the analysis are given in Appendix supplementary Table 1.

Pearson correlation analysis was executed using *proc corr* procedure of SAS 9.4 (SAS Institute, Cary, NC, USA) to examine the relationship between measured soil properties, total plant biomass, weed biomass, and cover crop biomass. The analysis of covariance (ANCOVA) for standardised yield was performed using *proc glimmix* procedure of SAS 9.4 (SAS Institute, Cary, NC, USA) to examine the effect of different soil properties as covariate on the yield at different topographical levels and cover crop treatments. The original yield data was standardised for individual fields using the following formula:

$$\text{Standardized Yield} = (\text{Yield} - \text{Mean yield}_{(field)}) / \text{Standard deviation}_{(field)}$$

Results

Soil analyses

Gravimetric soil water content at 0-20 cm depth in April prior to the first organic crop planting was significantly affected by both topography ($P < 0.01$) and cover crop treatments ($P = 0.02$), whereas the interaction between the two factors was not significant (Figure 2.1A). The soil water content was the highest at depressions, followed by summits and then slopes. The plots with WK cover crops interseeded in corn and following winter wheat harvest retained more soil moisture compared to the TR and NC cover crop treatments, whereas the soil moisture content in the WH plots were not statistically different from other cover cropping treatments (Table 2.1). Soil POM was not affected by cover crops or topographical positions across all fields (Figure 2.1B, $P > 0.05$).

Across all fields, the topography did not affect the soil NO_3^- contents ($P > 0.05$), whereas cover crop treatments had a significant effect on the soil NO_3^- content ($P = 0.07$), irrespective of the topographical position (Figure 2.2A). The TR cover crop treatment where red clover was frost-seeded into winter wheat the year prior to corn planting had the highest soil NO_3^- content; whereas the WH treatment where annual ryegrass, crimson clover, and Dwarf Essex rape were seeded after winter wheat harvest had the lowest soil NO_3^- content (Table 2.1).

Soil NH_4^+ content was mildly influenced by topography across all fields (Figure 2.4, $P = 0.05$), with depression having higher soil NH_4^+ content than both slopes and summits. However, there was no effect of cover crops on soil NH_4^+ content (Figure 2.2B, $P > 0.05$).

Across all fields, topography had a significant effect on soil microbial biomass C (Figure 2.3, $P < 0.01$), but microbial biomass was not significantly affected by cover crops (Figure 2.3, $P = 0.09$). The depressions had more soil microbial biomass C compared to both slopes and summits. Also, there was no significant interaction between topography and cover crops.

Cover crops affected the soil C mineralization rate irrespective of topographies across all fields (Figure 2.4A, $P = 0.02$). Soil C mineralization was highest in the WK and WH treatments and lowest in the NC treatment, whereas the TR treatment did not differ from the other cover crop treatments (Table 2.1). Topography did not have any effect on soil C mineralization rate across all fields (Figure 2.4A, $P > 0.05$).

No statistically significant effect of cover crops or topography on soil N mineralization rate were found across all fields (Figure 2.4B, $P > 0.05$).

Aboveground biomass and organic corn yield

In Fall, total plant biomass consisiting of cover crops and weeds were significantly effected by topography (Figure 2.5A, $P = 0.05$) as well as cover crop treatments (Figure 2.5A, $P = 0.0023$). Depression and summit had highest total plant biomass followed by the slopes (Figure 2.5A), whereas WK and WH had greater total plant biomass than NC and TR cover crop treatments in fall (Table 2.1).

Fall weed biomass did not vary among topographical positions (Figure 2.5B, $P > 0.05$), but cover crop treatmnets had significant effect on the weed biomass in fall (Figure 2.5B, $P < 0.0001$). The TR, WH, WK cover crop treatments had the lowest weed biomass as compared to the no cover crop treatment (Table 2.1).

Cover crop biomass was significantly affected by both topography and cover crop traetaments in fall (Figure 2.5C, $P = 0.027$ for topography and $P = 0.003$ for cover crops). The cover crop biomass was highest at depression and summit followed by the slope. Among cover crop treatments, WH had the highest cover crop biomass followed by WK and then TR treatment (Table 2.1).

In spring, total plant biomass was significantly affected by both topography and cover crops treatments across all fields (Figure 2.6A, $P < 0.01$ for topography and $P < 0.001$ for cover crops), and there was no significant interaction. Depression and summits had significantly higher total plant biomass as compared to slopes. The TR cover crop treatment had the highest total plant biomass, followed by the WH treatment. The WK and NC had the lowest total biomass at the time of collection (Table 2.1).

The spring weed biomass was significantly affected by the cover crop treatments ($P < 0.001$), where WK and NC treatment had more weeds (about three times) as compared to the WH and TR cover crop treatments (Table 2.1). There was a significant interaction between topography and cover crops affecting weed biomass (Figure 2.6B, $P < 0.05$). In depressions and summits, the WH and TR treatments had the lowest weed biomass as compared to the WK and NC treatments, whereas the cover crops treatments were not different at slopes.

Topography did not affect the aboveground cover crop biomass in spring (Figure 2.6C, $P > 0.05$). A significant difference in spring cover crop biomass was found between cover crop treatments (Table 2.1 $P < 0.0001$). The TR treatment had the highest cover crop biomass followed by WH, and WK had the lowest of the cover crop biomass. The Appendix Supplementary Table 3 shows the mean aboveground biomass of individual cover crop species in different cover crop treatments and topographical positions.

There was no effect of cover crops on the yield of organic corn, but topography significantly affected the yield (Figure 2.7, $P = 0.0007$). Across all fields, the corn yield was the highest in depressions followed by summits, and then by slopes.

Correlation and ANCOVA

Soil moisture was significantly correlated with soil NO_3^- (r = 0.248), soil N mineralization (r = 0.465), soil microbial biomass (r = 0.389), soil C mineralization (r = 0.389), and negatively correlated with total plant biomass (r = -0.160), and cover crop biomass (r = -0.163) (Appendix, Supplementary Table 4). POM was negatively correlated with soil C mineralization rate (r = -0.164) (Appendix, Supplementary Table 4). Soil NH_4^+ was positively correlated with soil NO_3^- (r = 0.2), il N mineralization, and soil C mineralization (r = 0.287), but negatively correlated with total plant biomass (r = -0.152) (Appendix, Supplementary Table 4). Soil NO_3^- was positively correlated with microbial biomass (r = 0.312) and soil C mineralization (r = 0.137) (Appendix, Supplementary Table 4). Soil N mineralization was positively correlated with C mineralization (r = 0.572), but negatively correlated with total plant biomass (r = -0.284) and cover crop biomass (r = -0.224) (Appendix, Supplementary Table 4). Soil microbial biomass C was significantly correlated with soil C mineralization rate (r = 0.248), whereas soil C mineralization was negatively correlated with total plant biomass (r = -0.252) and cover crop biomass (r = -0.185) (Appendix, Supplementary Table 4). Total plant biomass significantly correlated with cover crop biomass (r = 0.928) and cover crop biomass was significantly correlated with weed biomass (r = -0.415) (Appendix, Supplementary Table 4).

The analysis of covariance shows that spring soil properties such as POM, soil NH_4^+, N mineralization rate, C mineralization rate, and total plant biomass did not have any significant effect on the subsequent corn yield in fall, but soil microbial biomass C significantly affected the corn yield at different topographies (*P* = 0.019) (Appendix, Supplementary Table 5). The corn yield increases significantly with increasing microbial biomass at depression and slope whereas, the yield decreases with increasing microbial biomass C (Figure 2.8A, Appendix Supplementary Table 5). The yield also followed the similar pattern of increasing in the depression and slope, whereas yield decreased in the summit with increasing soil NO_3^- content, but the difference was not significantly different (*P* = 0.107) (Figure 2.8B, Appendix Supplementary Table 5).

Discussion

We studied the effect of different cover cropping treatments (NC, TR, WH, and WK) at different topographical conditions (depression, slope, and summit) on soil properties and crop yields of CSWR during the organic transition period. This study presents the soil properties following three years of transition (2018-2021 for three fields, and 2019-2022 for the fourth field).

As expected, topography influenced the spring soil moisture distribution, with depressions being the wettest and slopes the driest. However, the cover crop treatments also influenced the spring soil moisture. Soil under the WK treatment had higher soil moisture as compared to the TR treatment. Nguyen et al., (2022) also saw similar effects of topography on soil moisture after the first year of the transition period, but there were no effects of cover crops at that time following cover crop interseeding in corn in the WH and WK treatments and cereal rye seeded after corn in the TR treatment. The lower soil moisture in the TR treatment could be due to a longer duration of the live cover crops as red clover was actively growing up until the soil sampling date. Due to water uptake and transpiration, live cover crops have the potential to reduce the amount of water in the soil (Nielsen et al., 2002; Unger and Vigil, 1998). This is supported by the aboveground biomass data in our research, where the TR treatment had higher total biomass and higher cover crop biomass as compared to WK treatment, suggesting greater water uptake. Previous research examining soil water in response to cover crops also reported that cover crops can affect soil moisture during the cover crop season (Acharya et al., 2019; Kahimba et al., 2008).

The TR treatment had higher NO_3^- content when compared to the other cover crop treatments. The higher NO_3^- contents in the TR treatment could be attributed to red clover (legume). The aboveground biomass of red clover in the TR treatment was higher than that of the legume (crimson clover) in the WH treatment, while the legume in the WK treatment (winter pea) did not survive the winter. Nguyen et al., (2022) found higher NO_3^- contents in the WK treatment after the first year of the organic transition. If interseeded red clover is allowed to develop through the winter after winter wheat harvest, the clover can supply enough nitrogen for a subsequent corn crop, while also suppressing weeds and providing soil cover (Bruulsema and Christie, 1987; Ebelhar et al., 1984; Vyn et al., 1999). It is in line with previous research showing that legumes have good impacts on soil NO_3^- because of their high N content and low C/N ratio (Finney et al., 2016; Jahanzad et al., 2016; Utomo et al., 1990). Cover crop treatments across topography did not influence soil NH_4^+ contents and N mineralization following the three-year organic transition. The lack of cover crop effect on N mineralization observed in this study is inconsistent with some of the previous findings, where cover crops were shown to affect N mineralization (Chu et al., 2017b). Nguyen et al., (2022) found higher N mineralization in WH and WK treatments following the first year of organic transition as compared to the NC treatment. Cover crops are known to have variable short term and long term effects on soil N availability and N mineralization (Kuo et al., 1997). In other research, potential N mineralization did not differ among cover crop treatments when measured in the spring (Steenwerth and Belina, 2008). Soil N mineralization is known to be regulated by the chemical composition and nature of soil organic matter (SOM) (Sano et al., 2010). Higher SOM content, specifically labile SOM fraction, results in N mineralization because it provides microorganisms with an easily accessible energy source (Ros et al., 2011). Particulate organic matter (POM), a form of SOM fraction (particle size analysis >53 μm), is the most degraded plant leftovers in the early stages of humification which include readily mineralizable C and N (Besnard et al., 1996; Zeller and Dambrine, 2011). Potential N mineralization rate were reported to be highly correlated with POM contents in soil (Bu et al., 2015), which could also be the reason why we did not see any difference in N mineralization. However, we did not observe a significant correlation of POM content and N mineralization in our study (Appendix, Supplementary Table 3). In our study, POM did not differ among topographical positions or cover crop treatments. Our findings were in accordance with previous studies examining soil POM in response to cover crop treatments, where no differences in POM were reported (Franzluebbers and Stuedemann, 2014; Motta et al., 2007).

Cover crop treatments affected soil C mineralization, where WK and WH treatments had higher C mineralization rate as compared to NC. This is supported by the previous studies which reported the increase in soil C mineralization due to incorporation of cover crops in the rotation (Ghimire et al., 2017; Tian et al., 2011). The higher mineralization rate could be due to the presence of legume and brassicas with their low C/N ratio having a higher decomposition rate (Finney et al., 2016). Carbon mineralization is strongly connected to the release of mineral nitrogen, phosphorus, and sulfur and can be driven by microbial needs for C and nutrients for maintenance, growth, and extracellular metabolites like enzymes (Jonasson et al., 1999; LeBauer and Treseder, 2008; Vitousek et al., 2010). Higher C mineralization rate in the WK treatment could possibly lead to higher availability of nutrients to subsequent corn crop, however we did not find a significant effect of C mineralization rate on the subsequent corn yield (Appendix, Supplementary Table 3). Also, increases in C mineralization rate can be due to the increased microbial activity in the soil because of higher microbial biomass C added by cover crops (Wang et al., 2007). In this study, soil microbial biomass C was following a trend of being highest in WK and TR treatments, and lowest in WH and NC treatments (although it was statistically not significant), irrespective of the topographical position. However, in one of the four fields (A-1), WK had statistically higher microbial biomass C than other cover crop treatments. Higher soil microbial biomass C was reported in cover crop treatments as compared to no cover crops (Muhammad et al., 2021; Steenwerth and Belina, 2008). Nguyen et al., (2022) also reported higher microbial biomass C in the WK treatment as compared to NC. The microbial biomass C was higher in the depression compared to slope and summit, possibly because of higher soil moisture in depression, which is suitable for greater microbial growth (Borowik and Wyszkowska, 2016).

Corn yield was greatest in the depression, followed by the summit and lowest in slope. Our research supports previous studies which showed higher yields in field depressions (Denys et al., 2006; Soon and Malhi, 2005). Also, microbial biomass was greater in the depressions. had significant effect on corn yield at different topographies. Corn yield increases with increasing microbial biomass at depression and slope, whereas the yield followed the trend of decreasing at summit with increasing microbial biomass. Such variation in crop yield at different topographies could be due to the inherent differences in soil biological properties among topographical positions (Wickings et al., 2016). Cover crops did not influence the yield of the first organic crop (fourth year). Numerically, the corn yields were following the pattern TR > WH > NC > WK, but the differences were not statistically significant. The higher (statistically non-significant) yield in the TR treatment could be due to higher soil N availability because of the high biomass of the red clover (legume) cover crop terminated just prior to corn planting. However, there was no significant relation of corn yield with soil NO_3^- and NH_4^+ contents or N mineralization rate. Red clover biomass increased corn yield, although its impact varied temporally and spatially. Red clover had the greatest positive impact on corn yields in years with minimal precipitation and at summit and slope topographical positions (Muñoz et al., 2014) suggesting summit and slope corn crops benefit most from good red clover cover crop stands. There are different reports on variable effect of cover crops on corn yield in organic production, where cover crops may reduce, increase, or have no effect on subsequent corn crop yield. For example, (Basche et al., 2016a) reported that there was no cover crop effect on corn yields. Crimson clover resulted in poorer corn yields, whereas hairy vetch and rye mixture increased the corn yield in no-till organic farming (Parr et al., 2011). Gentry et al. (2013) found that a single season of cover crop integration boosted corn aboveground biomass N, grain quality, and corn yield in the first of two research years, however there was no change in corn yield in the second year. Another study reported reduction in corn yield because of hairy vetch cover crop in the rotation due to increased weed competition, increased insect pests, and possibly inadequate N supply (Mischler et al., 2010). Since, corn yield is also affected by insects and diseases, it impossible to control these under organic farming with cover crops. Also, another point to be noted is that cover crop treatments did not reduce corn yield significantly from NC treatment, as they could have used soil resources during their growth period.

In conclusion, the use of cover crops during the transition to organic farming in row crop rotation systems like CSWR has gained popularity in the Midwest of the United States due to its viability and low financial risk. This study investigated the effects of three cover crop-based transition scenarios on the yield of the first organic corn cash crop and on soil characteristics that may be important for crop growth and yield at the end of the three-year organic transition phase. The results showed that the WH cover crop mixture improved biological, chemical, and physical soil health in the CSWR organic transition system across varied agricultural landscapes. However, the effect on cash crop yield remains inconclusive, and field topography had a significant effect on yield. The TR and WH treatments were beneficial in improving soil characteristics, but further research is needed to determine their effect on cash crop yield.

Table 2.1. Marginal means of soil properties, aboveground plant biomass, and corn yield for different cover crops systems across topographies for all fields together. Letters in a column mark the significant differences between cover crops (P <0.05). ns means no significant difference between cover crops.

Cover crop	Soil moisture	POM	Soil NO_3^-	Soil NH_4^+	Soil microbial biomass C	Soil C minerali-zation rate	Soil N mineral-ization rate	Total plant biomass		Weed biomass		Cover crop biomass		Organic corn yield (Year 4)
								Fall	Spring	Fall	Spring	Fall	Spring	
NC	13.14 b	0.36	7.84 ab	1.01	332.93 b	5.47 b	0.56	1445 b	429 c	1445 a	429 a	-	-	6338
TR	13.51 b	0.40	7.98 a	0.83	357.97 a	6.37 ab	0.57	1583 b	2112 a	294 b	180 b	1292 b	1932 a	6433
WH	13.83 ab	0.42	5.85 b	0.87	358.52 ab	6.92 a	0.52	2242 a	1363 b	202 b	181 b	2040 a	1182 b	6233
WK	14.01 a	0.39	5.89 ab	0.71	354.09 b	6.89 a	0.58	2213 a	694 c	473 b	612 a	1784 a	82 b	6176
P-value	0.019	ns	0.07	ns	0.09	0.025	ns	0.0023	< 0.0001	< 0.0001	0.0001	0.0001	0.003	ns

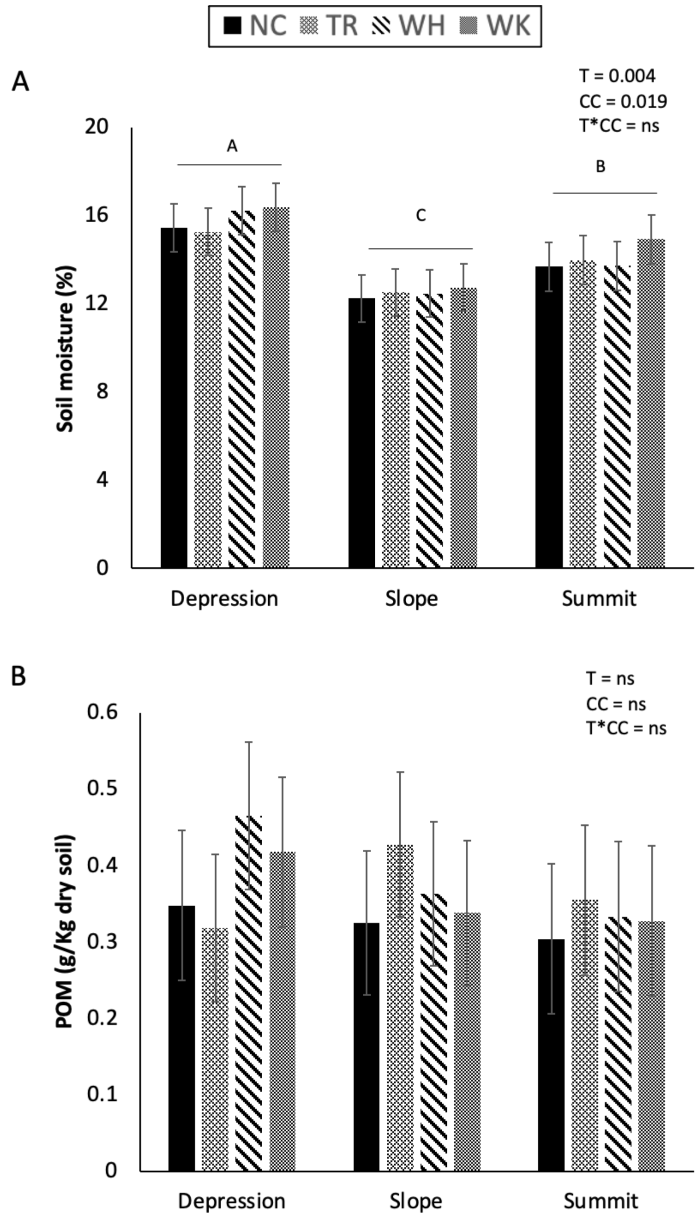

Figure 2.1. Means and standard errors of A) soil moisture and B) soil particulate organic matter (POM) contents at 20 cm depth for the studied cover crop systems within each topographical position across all fields. *P*-values for topography (T), cover crops (CC), and interaction for topography and cover crops (T*CC) are shown in top-right corner for each variable, where ns means there is no significant difference. Letters mark significant differences among marginal means of topographical positions ($P < 0.05$).

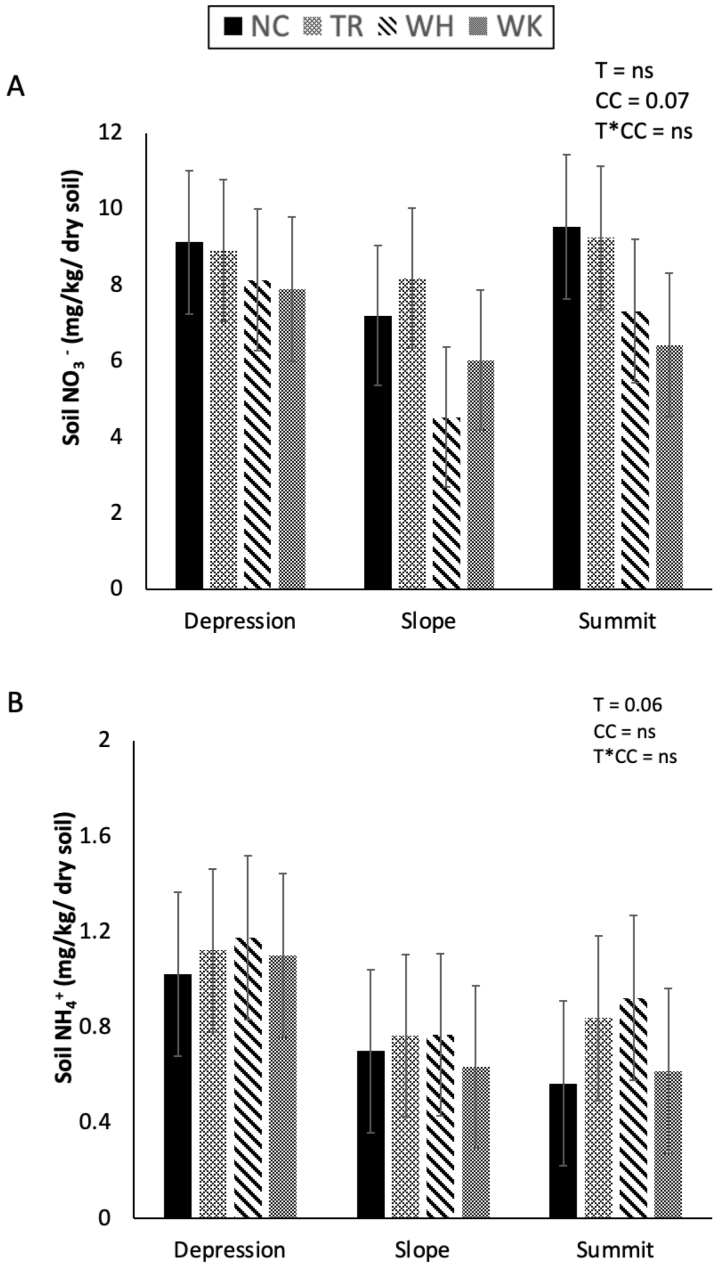

Figure 2.2. Means and standard errors of A) soil nitrate contents and B) soil ammonium contents at 20 cm depth for the studied cover crop systems within each topographical position across all fields. P-values for topography (T), cover crops (CC), and interaction for topography and cover crops (T*CC) are shown in top-right corner for each variable, where ns means there is no significant difference.

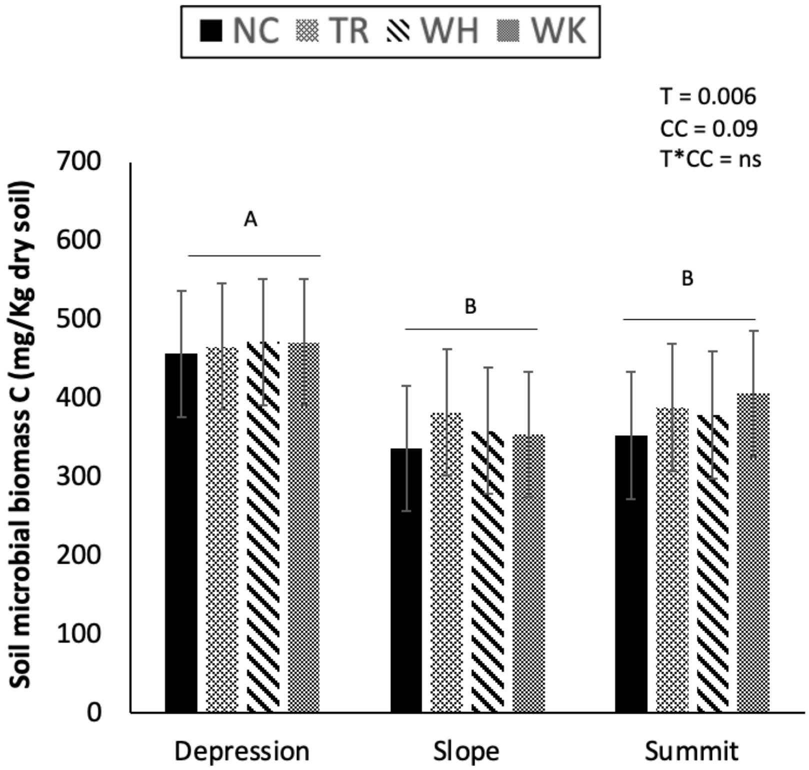

Figure 2.3. Means and standard errors of soil microbial biomass carbon contents at 20 cm depth for the studied cover crop systems within each topographical position across all fields. *P*-values for topography (T), cover crops (CC), and interaction for topography and cover crops (T*CC) are shown in top-right corner for each variable, where ns means there is no significant difference. Letters mark significant differences among marginal means of topographical positions ($P < 0.05$).

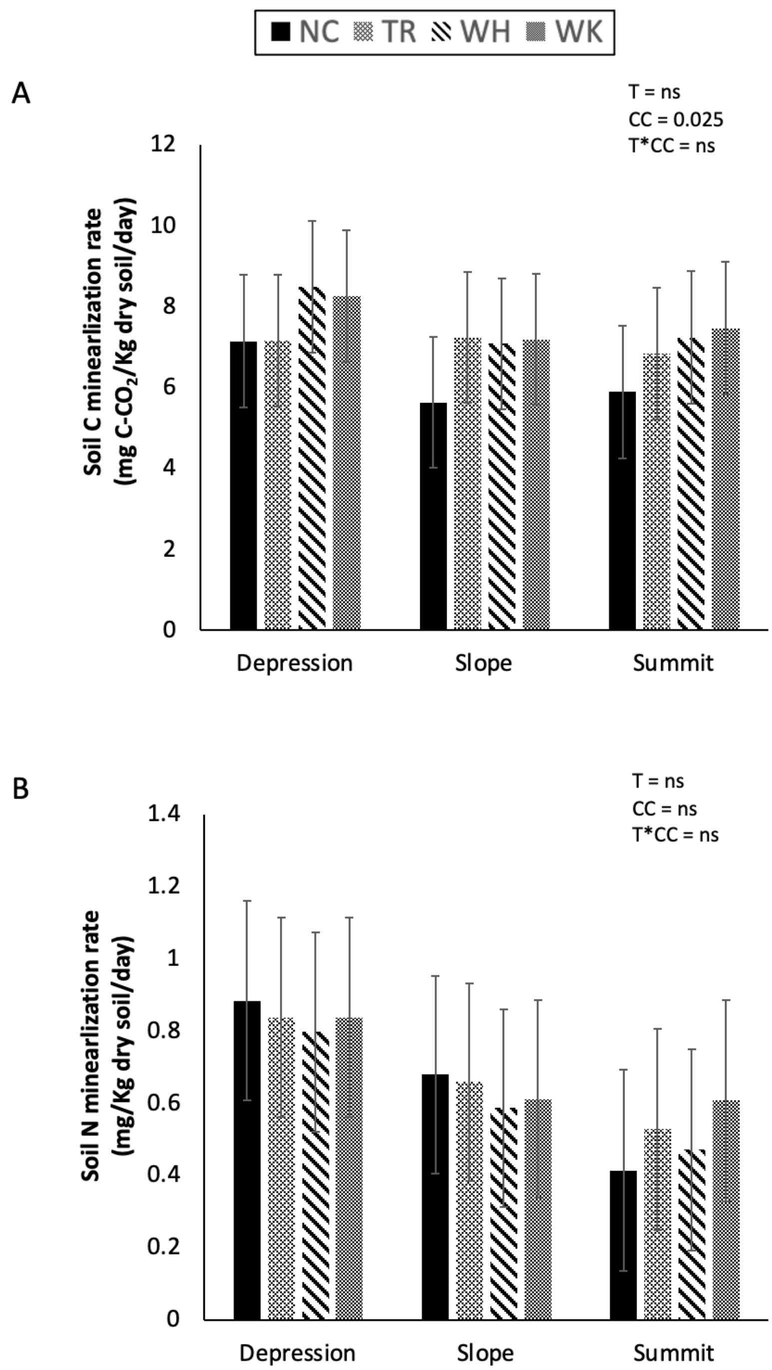

Figure 2.4. Means and standard errors of A) soil carbon mineralization rate and B) soil nitrogen mineralization rate at 20 cm depth for the studied cover crop systems within each topographical position across all fields. P-values for topography (T), cover crops (CC), and interaction for topography and cover crops (T*CC) are shown in top-right corner for each variable, where ns means there is no significant difference

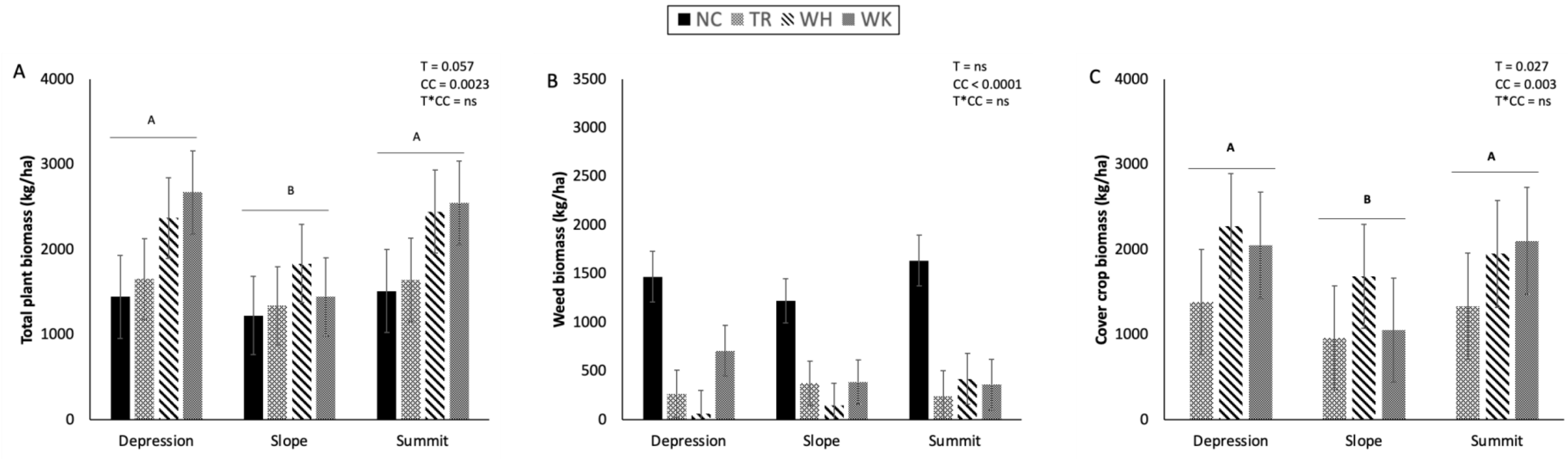

Figure 2.5. Means and standard errors of fall A) total biomass, B) weed biomass, and C) cover crop biomass for the studied cover crop systems within each topographical position across all fields. P-values for topography (T), cover crops (CC), and interaction for topography and cover crops (T*CC) are shown in top-right corner for each variable, where ns means there is no significant difference. Capital letters mark significant differences among marginal means of topographical positions ($P < 0.05$) and small letters mark significant differences among cell means of cover crops at individual topographical positions after slicing the significant interaction ($P < 0.05$).

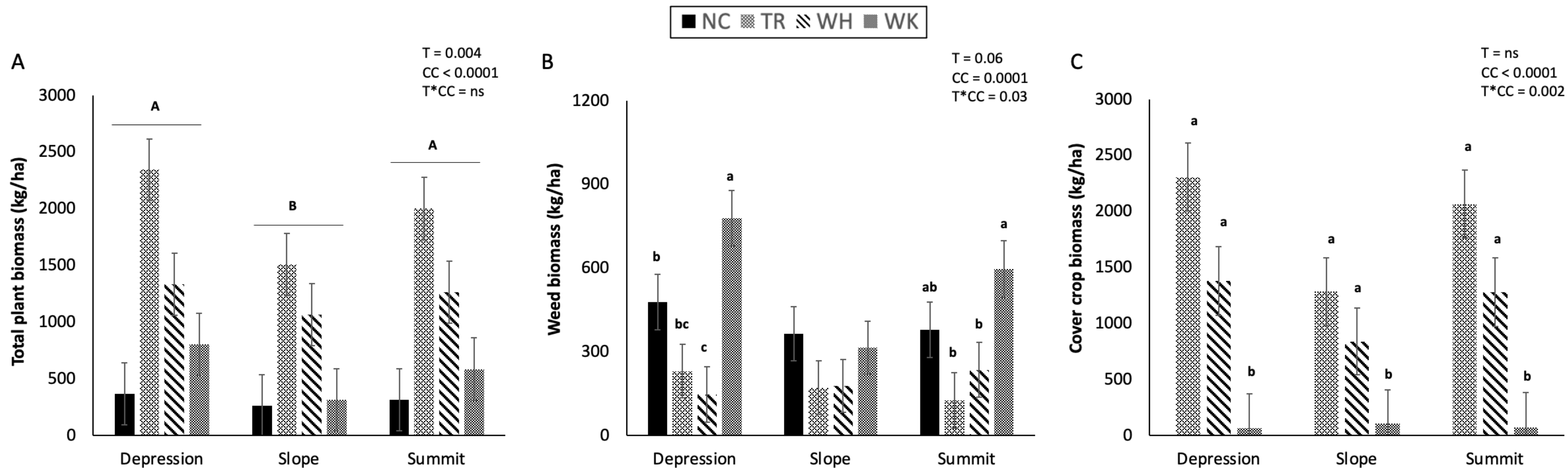

Figure 2.6. Means and standard errors of spring A) total biomass, B) weed biomass, and C) cover crop biomass for the studied cover crop systems within each topographical position across all fields. *P*-values for topography (T), cover crops (CC), and interaction for topography and cover crops (T*CC) are shown in top-right corner for each variable, where ns means there is no significant difference. Capital letters mark significant differences among marginal means of topographical positions ($P < 0.05$) and small letters mark significant differences among cell means of cover crops at individual topographical positions after slicing the significant interaction ($P < 0.05$).

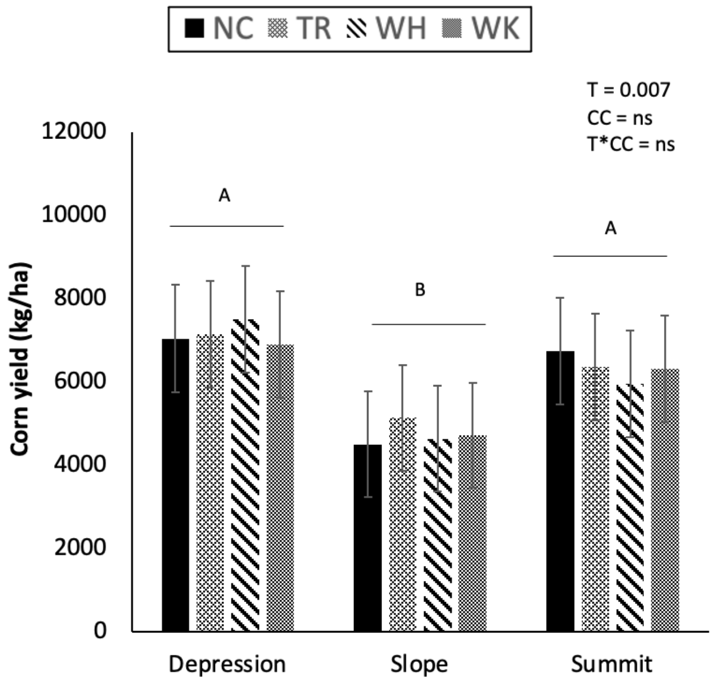

Figure 2.7. Means and standard errors of first organic corn yield (15.5% moisture) for the studied cover crop systems within each topographical position across all fields. P-values for topography (T), cover crops (CC), and interaction for topography and cover crops (T*CC) are shown in top-right corner for each variable, where ns means there is no significant difference. Letters mark significant differences among marginal means of topographical positions ($P < 0.05$).

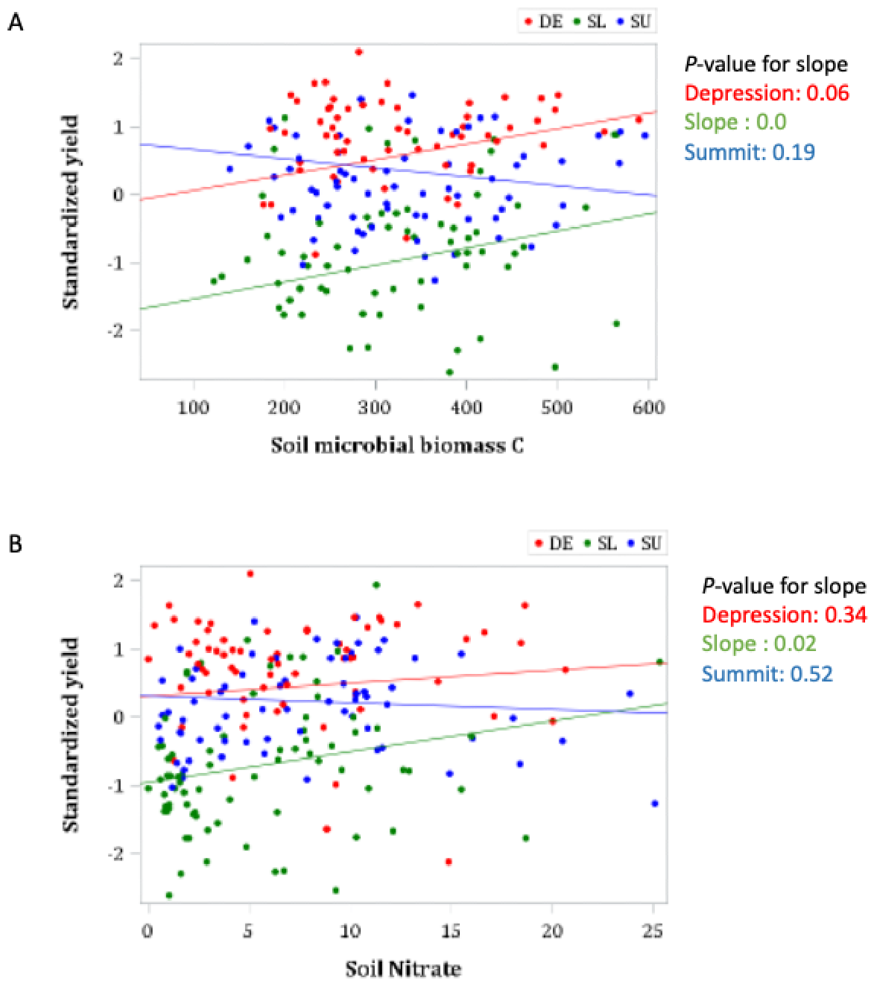

Figure 2.8. Scatterplot of Standardized yield data vs A) microbial biomass carbon, and B) Soil Nitrate contents for three different level of topographies: depression (DE), slope (SL), and summit (SU). *P*-values for the slope of line for topographical positions is given in the top right corner of each figure.

CHAPTER 3
SOIL CARBON SEQUESTRATION: META-ANALYSIS OF THE ADDITIVE EFFECT OF NO-TILL AND COVER CROPS

Introduction

Food production, greenhouse gas balance, and climate change mitigation and adaptation all rely heavily on soil organic carbon (SOC), a primary indicator of soil health (Lorenz and Lal, 2016). Soil organic carbon consists of plant and animal tissues and microbial biomass at different stages of decomposition and is a crucial component of soil solids. What is meant by "soil carbon sequestration"? It is "the process of transferring CO2 from the atmosphere into the soil or a land unit through unit plants, plant residues and other organic solids, which are stored or retained in the unit as part of the soil organic matter (humus). Retention time of sequestered carbon in the soil (terrestrial pool) can range from short-term (not immediately released back to atmosphere) to long-term (millennia) storage. The sequestrated SOC process should increase the net SOC storage during and at the end of a study to above the previous pre-treatment baseline" (Olson, 2013; Olson et al., 2014c).

Soil organic carbon is typically lower in agricultural soils than in soils under natural vegetation due to land conversion and cultivation (Hassink, 1997; Poeplau and Don, 2015). Soil organic carbon levels are significantly lower in cropland than in forested areas. SOC losses from cultivation are 30–40% higher than those from natural or semi-natural vegetation (Don et al., 2011; Poeplau et al., 2011). As a result, cropland soils are a massive worldwide carbon sink. Due to a growing population and emerging economy, food production is needed worldwide. Thus, converting cultivated land to grassland or wild vegetation is limited. Southern hemisphere agricultural land is still increasing (McGuire et al., 2001). Thus, increasing SOC stocks while increasing agricultural yield is essential. When environmental and management factors are held constant over long periods of time, the dynamic of agricultural SOC is governed by the balance between carbon inputs (such as crop residues and organic fertilizers) and outputs (such as decomposition and erosion). Climate change, however, is expected to increase SOC decomposition and weaken the capacity of soil to sequester carbon, altering this balance (Wiesmeier et al., 2016). However, with the right management, agricultural soils can sequester carbon from the atmosphere (Lal and Rattan Lal, 2018). As a result, it is essential to look for ways to improve agricultural SOC sequestration without reducing the delivery of ecosystem services like food, feed, fiber, or other agricultural products.

Increasing carbon inputs and decreasing carbon outputs are the keys to increasing soil carbon sequestration (Campbell et al., 2014). Adding cover crops to the crop rotation, adding biochar to soils, reducing soil tillage, and other conservation agricultural practices are frequently suggested for SOC sequestration (Lal, 2004a; Luo et al., 2010a, 2010b; Smith, 2004; West and Post, 2002). Many observations and data have been gathered as a result of the use of these management techniques in important agricultural regions around the world in recent decades (Chen et al., 2009; Clark et al., 2017; Spokas et al., 2009). Plowing the soil is mainly blamed for loss of C (Reicosky, 2003), and tilled soils are seen as a depleted C reservoir that can be recharged. Lal and Bruce (1999) estimate that US croplands have lost 5 Gt C, or 36 t ha^{-1}, and indicate that with proper management, much of this can be regained over 50 years. Conservation tillage is any process that leaves enough crop residue to cover at least 30% of the soil surface following planting (Lal, 2010). By reducing soil disturbance, slowing SOC breakdown, increasing biomass of fungi and earthworms, conservation tillage promotes SOC stabilization (Briones and Schmidt, 2017; Lavelle et al., 1999; Liang and Balser, 2012; Salinas-Garcia et al., 1997). A broad implementation of conservation tillage in the US might sequester 24–40 Mt C year−1 (Lal et al., 2003). Converting all croplands to conservation tillage might sequester 25 Gt

C over the next 50 years, making it one of the most important worldwide measures for stabilizing atmospheric CO2 concentrations (Pacala and Socolow, 2004). Conversion from conventional tillage (CT) to no-tillage (NT) is considered to be one of the potentially effective solutions, with a rate of C sequestration of 100–1000 kg ha-1 each year (Lal, 2004a; Paustian et al., 2000; Six et al., 2004; Smith et al., 1998). Angers and Eriksen-Hamel (2008) found that NT increased C in surface soil. Twelve paired NT and CT experiments in three US states showed that after adopting NT for 5–23 years, soil C stock in the surface 60 cm ranged from 22.8 to −20.3 t ha-1 (Christopher et al., 2009).

Cover crops, legume or non-legume, also preserve soil by avoiding bare soil periods where there are no plants growing actively converting carbon dioxide in the atmosphere to plant carbon (Valkama et al., 2020). Cover crops have been studied for their potential to grow in these ‘spaces’ where there is no green cash crop growing. Cover crops sequester C (Mazzoncini et al., 2011; Muhammad et al., 2019; Poeplau and Don, 2015; Verhulst et al., 2012). In agroecosystems, cover crops increase inputs of carbon, nitrogen, and biodiversity through above and belowground biomass (Blanco-Canqui et al., 2011b; Lal, 2004b). In addition, cover crops improve soil structure and increase soil aggregation (Sainju et al., 2011), which reduces carbon loss due to soil erosion (De Baets et al., 2011). Cover crops may offset residue removal-induced SOC losses by increasing C concentration and stocks (Ruis and Blanco-Canqui, 2017). When utilized as mulch cover in water-limited areas, cover crops have been proven to reduce soil erosion and drought stress for the subsequent crop in addition to increasing carbon input (Frye et al., 2015; Lal, 2004b). Cover crops can be grown in the fall and winter to absorb more nitrogen from the soil and lessen leaching (Blombäck et al., 2003). Until now, cover crops have mostly been studied from a scientific perspective for their ability to enhance soil quality and consequently promote cash crop output. Studies have emphasized the capacity of cover crops to raise SOC stores and hence reduce climate change (Lal, 2004b). A meta-analysis study showed that the introduction of cover crops was effective in increasing C input driven SOC sequestration (Poeplau and Don, 2015), with a potential predicted worldwide SOC sequestration of 0.12 Pg C yr-1, which would offset 8% of the direct yearly greenhouse gas emissions from agriculture.

Most of the research on soil carbon sequestration is focused on the effects of single practice (either conversion from conventional to no-tillage or inclusion of cover crops), however, there are few studies estimating the combined effect of conservation tillage and cover crops. Recent research found that combining cover crops and conservation tillage increased SOC considerably (Bai et al., 2019; Blanco-Canqui et al., 2013; Duval et al., 2016; Higashi et al., 2014). Soil carbon sequestration increased 0.267 Mg C ha−1 year−1 when no-till was combined with a cover crop of hairy vetch (*Vicia villosa*) and rye (*Secale cereale*) but when only no-till was utilized, carbon loss was 0.967 Mg C ha−1 year−1 (Sainju et al., 2006). These findings emphasize the need of statistically examining the combined impacts of no-tillage and cover crops over conventional agricultural practices on SOC sequestration under diverse climate and soil conditions. We conducted a meta-analysis to examine the combined effects of two management practices (i.e., no-till (NT), and cover crops), as compared to conventional tillage (CT) with no cover crops. This study will focus on the effects of these practices on the sequestration of soil organic carbon.

Materials and methods

Data Collection

This meta-analysis is based on studies of the effects of two tillage practices (CT and NT) and cover crops (with cover crops (CC) and no cover crop (NC) on soil carbon sequestration. We included 15 field studies conducted over a period of 3-50 years published between 2000 and

2022 (Table 3.1). All studies included two tillage practices and cover crops, and the reported response variable was total organic carbon. We carried out a comprehensive keyword search across Web of Science and Google Scholar. The terms that were searched for were ‘cover crops’, ‘tillage’ ‘soil carbon sequestration’, ‘soil organic carbon’. The original data were taken from the tables and figures using the WebPlotDigitizer (https://apps.automeris.io/wpd/) that were published in the articles.

All chosen studies satisfy the following criteria for inclusion: (a) SOC was measured in field experiments to determine the potential of tillage and cover crops in increasing soil carbon; (b) observations were made on croplands only, excluding orchards and pastures; (c) ancillary information, such as experiment duration, replication, and sampling depth; and (d) crop rotation was listed.

We combined the two tillage practices (CT and NT), and cover crops (CC and NC) into one factor, which gives us one factor with 4 levels: 1) conventional tillage with no cover crop (CT-NC); 2) conventional tillage with cover crop (CT-CC); 3) no-tillage with no cover crop crop (NT-NC); and 4) no-tillage with cover crop crop (NT-CC). Fixing CT-NC as a control, the effect of converting conventional tillage without cover crop to no-tillage without cover crop, to conventional tillage with cover crop, and to no-tillage with cover crop was examined. The effect of converting from conventional tillage with cover crop to no-tillage without cover crop and to no-tillage with cover crop was studied by taking CT-CC as control. In the end, the effect of conversion from no-tillage without cover crop to no-tillage with cover crop was studied by taking NT-NC as control.

Meta-analysis

Each study's measurements were standardized to common units. Mg C ha^{-1} units of SOC were reported. According to Rosenberg et al., (2000), the response ratio is a measure of outcome that compares the experimental group to the control group. It has the benefit of assessing the effect as a proportionate change brought on by experimental manipulation. The natural log of response ratio (RR) of SOC in the treatment group to TOC in the control group served as the effect sizes/dependent variable in this meta-analysis (Hedges et al., 1999).

$RR = (SOC_{treatment}) / (SOC_{control})$

$L_i = ln\ (RR)$

where,

$SOC_{treatment}$ and $SOC_{control}$ are the mean values of response variable (soil organic carbon for treatment and control, respectively, in the study) and *ln* is the natural logarithm.

More than one *ln*(RR) was calculated for the same study when results from multiple cover crop were reported, and effect sizes were considered independent observations for meta-analysis. For ease of interpretation, *ln*(R) values were back-transformed to mean effect sizes and expressed as percentage change in response due to treatment group:

$\%$ change in response $= [e^{ln(RR)} – 1] *100\ \%$

Positive values suggest an increase because of treatments, whereas negative percentage changes denote a reduction in the variable relative to the control.

Individual effect sizes are often weighted in a traditional meta-analysis by the inverse of pooled variances to give more weight to studies with higher precision or lower within-study variability (Philibert et al., 2012). However, several of the papers that were considered for this analysis did not provide data on within-study variability (SDs, SEs, or CV), which is necessary to calculate pooled variances. So, based on experimental replications, we employed a different weighting method (Adams et al., 1997):

$W_i = (N_{treatment} * N_{control}) / (N_{treatment} + N_{control})$

Where W_i is weight for ith observation and $N_{treatment}$ and $N_{control}$ are the number of replications for the treatment and control group, respectively. A mixed-effects model was employed using meta package in RStudio 2022.07.1 because it accounts for the variety of variables that could affect the principal treatment effects related to the study location, management procedures, and cropping system. We calculated the 95% confidence interval (CI) for the weighted natural log mean effect sizes [*ln*(RR)]. If the 95% confidence interval (CI) for the effect size for groups (treatments) did not contain zero, the effect size was considered significant (null hypothesis).

Results

When cover crops are added to conventional tillage systems (CT-CC vs. CT-NC), SOC increases by 1.13% (P = 0.02) (Figure 3.1). The change from conventional tillage/no-cover crops to no-tillage systems/no-cover crops (NT-NC vs CT-NC) also contriibuted a 4.5% increase in SOC (P < 0.0001). The greatest increase in SOC (7%) was found in no-till systems that also used cover crops (NT-CC vs CT-NC; P < 0.0001).

The % change in SOC in a) no-tillage/no-cover crops systems (NT-NC) compared to conventional tillage with cover crops (CT-CC) and b) adding cover crops to the no-tillage/no-cover crops systems is shown in Figure 3.2. The SOC was reduced by 4% when conventional tillage with cover crop systems was compared tot no-tillage/no-cover crop systems (CT-CC vs NT-NC) (P < 0.0001). SOC significantly increased by 1.6% (P < 0.0001) when cover crops were added to the no-tillage/no-cover crop systems (NT-CC vs NT-NC).

Figure 3.3 depicts a no-tillage with cover crop system (NT-CC) as the treatment and conventional tillage with cover crop (CT-CC) as the control. When compared to the CT-CC, the NT-CC increased the SOC by 5.2% (P < 0.0001).

Discussion

Intensive tillage has consistently been identified as a potential factor in raising greenhouse gases (GHGs) emissions under conventional agriculture techniques, which increases global warming (Gupta et al., 2015). Additionally, based on the geography of the soil, intensive tillage practices cause soil disintegration and loss of soil nutrients and organic carbon (Jain et al., 2014; Shrestha et al., 2013). Organic matter is the principal source of carbon in an agricultural ecosystem. Another way to describe the function of organic matter is as a "source" and "sink" of carbon. Enhancing SOC levels in agricultural systems creates a win-win situation by improving soil fertility, reducing CO_2 emissions, and increasing crop output (Bhattacharyya et al., 2015; Navarro-Pedreño et al., 2021). Therefore, to lessen soil disturbance and GHG emissions, conversion from conventional to conservational agricultural practices is needed.

In order to increase SOC, it is common practice to either increase carbon inputs, decrease losses, or do both. Soil carbon sequestration is enhanced using any of the conservation agricultural management strategies brought up in this study, including the use of cover crops, conversion to conservation tillage which includes strip tillage, vertical tillage and no tillage, and the combination of cover crops and conservation tillage. Conservation tillage and cover crops increased SOC stocks by 3-10% (Abdalla et al., 2016; Aguilera et al., 2013; Du et al., 2017; Luo et al., 2010b; Zhao et al., 2017). We confirmed these earlier findings in the meta-analysis, showing that no-tillage raised SOC by 4.5%, followed by cover crops increasing SOC by 1.12% as compared to conventional tillage systems (Figure 3.1). However, the greatest increase of 7% in SOC was found in systems which included both no-tillage and cover crops as compared to conventional tillage practices.

Soil organic carbon stocks are dependent on tillage practices (Hussain et al., 2021). In our meta-analysis, SOC increased by 4.5% in no-till systems as compared to conventional tillage systems (Figure 1, NT-NC vs CT-NC), and 5.25% in no-till with cover crop systems (NT-CC) as compared with conventional tillage with cover crop systems (Ct-CC) (Figure 3.3). Conservation tillage techniques prevent SOC from leaving farm fields by wind and water erosion and by reducing microbial populations that respirate CO2.(Lal, 2005; Six et al., 2000). When crop residues are left on the soil's surface under conservation tillage systems there is less soil disturbance, which slows the incorporation of residues and lowers the rates at which organic matter is mineralized (Mikha and Rice, 2004). Increased soil cover, less soil disturbance, and increased soil aggregation and structure result from the switch to no-tillage farming. No-tillage also decreases the downward migration of surface soil C (Lampurlanés and Cantero-Martínez, 2003; Martínez et al., 2008; Qin et al., 2004). Additionally, crop residues on the soil surface lower summertime soil temperatures which slows microbial population growth, resulting in less soil C mineralization (Duiker and Lal, 2000).

Cover crops are a type of green manure that adds more carbon to the soil, increasing SOC (Poeplau and Don, 2015). Including cover crops enhances the SOC stock of farmland soils, which can be a useful strategy to offset human-caused greenhouse gas emissions (Lal, 2004). This is supported by the results from this study, where incorporation of cover crops increased SOC by 1.12% in conventional tillage system (Figure 3.1), and 1.6% in no-tillage systems (Figure 3.2). By substituting an additional period of carbon assimilation when the cash crop is not in the field (a carbon source), cover cropping improves the net ecosystem carbon balance of a cropland (Lal, 2001). A considerable portion of the carbon input from cover crops is added as roots, which contribute to the relatively stable carbon pool more effectively than above ground C-input (Kätterer et al., 2011). Additionally, an increase in SOC may have a positive feedback effect on plant development, increasing the primary crop's input of C. (Brock et al., 2011).

Shifting from conventional tillage to no-till and incorporating cover crops in crop rotations can have additive effect on soil carbon sequestration, as cover crops will add more organic matter to the soil through plant residues and no-tillage will result in a reduced mineralization rate of organic matter (Alvarez et al., 1995; Chahal et al., 2020; Hussain et al., 2021). This is supported by the highest increase in SOC in NT-CC systems when compared to CT-NC systems in this meta-analysis (Figure 3.1). No-tillage and cover crop systems increase SOC and soil physical, chemical, and biological properties. No-tillage with a cover crop is better at improving SOC, microbial activity, soil health, and quality (Balota et al., 2014; Higashi et al., 2014; Mitchell et al., 2017; Veloso et al., 2018; Wulanningtyas et al., 2021). However, when conventional tillage systems with cover crops (CT-CC) were compared with no-till/no-cover system (NT-NC), the SOC was reduced by 4% (Figure 3.2), indicating that tillage has a stronger effect on changing SOC as compared to cover crops. Cover crops increase SOC sequestration in conservation tillage, and under temperate circumstances, the impacts of no-till are enhanced with the addition of cover crop (Lal, 2004b; Nunes et al., 2018). Winter cover crops increase the amount of carbon that is annually captured from the atmosphere (via photosynthesis), and some of this carbon is retained in the soil as organic matter (Moebius-Clune, 2016). To increase agricultural production and sustainability, cover crop management and no-tillage systems are being adopted. Combining these two conservation techniques, however, may have positive synergistic effects that enhance soil services (Acharya et al., 2019). A long-term combination of no-tillage and cover crops improves soil quality, according to another author (Mitchell et al., 2017; Nouri et al., 2019). The combination of both has a stronger influence on SOC storage, and the cover crop contributes significantly to NT techniques (Veloso et al., 2018).

The results from meta-analyses of cover crops and no-tillage studies are directly related to the carbon credit system because these practices are known to sequester carbon in soil, which can generate carbon credits. The carbon credit system is a market-based approach designed to incentivize and reward activities that reduce greenhouse gas (GHG) emissions or sequester carbon. Under this system, carbon credits are generated by implementing activities that reduce emissions or increase carbon sequestration, such as using renewable energy, implementing energy efficiency measures, or adopting conservation practices like no-tillage and cover crops. These carbon credits can be sold to entities looking to offset their emissions, creating a financial incentive for individuals and organizations to reduce their carbon footprint. Farmers who adopt these practices can potentially benefit financially by selling their carbon credits on carbon markets. At the same time, the carbon credit system incentivizes farmers to adopt these practices, providing a financial reward for their efforts to reduce emissions and sequester carbon.

Table 3.1. List of studies used to get the data for meta-analysis.

S. No.	Citation	Publication Title	Geographic location	Study duration	Main Crop
1	(Huang et al., 2020)	Assessing synergistic effects of no-tillage and cover crops on soil carbon dynamics in a long-term maize cropping system under climate change	Kentucky	48 years	Maize
2	(Halvorson et al., 2002)	Tillage, Nitrogen, and Cropping System Effects on Soil Carbon Sequestration	North Dakota	12 years	Spring wheat, Spring wheat- Winter wheat - Sunflower
3	(Higashi et al., 2014)	Tillage and cover crop species affect soil organic carbon in Andosol, Kanto, Japan	Andosol, Kanto, Japan	9 years	Rice, Soybean
4	(Haruna and Nkongolo, 2019)	Tillage, Cover Crop and Crop Rotation Effects on Selected Soil Chemical Properties	Missouri	3 years	Maize, soybean

Table 3.1. (cont'd).

S. No.	Citation	Publication Title	Geographic location	Study duration	Main Crop
5	(Mazzoncini et al., 2011)	Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content	University of Pisa, Central Italy	16 years	Maize, wheat, sunflower
6	(Nouri et al., 2019)	Thirty-four years of no-tillage and cover crops improve soil quality and increase cotton yield in Alfisols, Southeastern USA	Jackson, Tennessee	34 years	Cotton
7	(Veloso et al., 2019)	Legume cover crops under no-tillage favor organomineral association in microaggregates and soil C accumulation	Eldurado do Sul, Brazil	30 years	Maize, Cowpea
8	(Conceição et al., 2013)Co	Combined role of no-tillage and cropping systems in soil carbon stocks and stabilization	Eldurado do Sul, Brazil	18 years	Maize

Table 3.1. (cont'd).

S. No.	Citation	Publication Title	Geographic location	Study duration	Main Crop
9	(Sainju et al., 2006)	Carbon Supply and Storage in Tilled and Nontilled Soils as Influenced by Cover Crops and Nitrogen Fertilization.	Fort Valley, GA	4 years	Cotton and Sorghum
10	(Veloso et al., 2018)	High carbon storage in a previously degraded subtropical soil under no-T tillage with legume cover crops	Eldurado do Sul, Brazil	30 years	Maize, Cowpea
11	(Sainju et al., 2002)	Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentrations in sandy loam soil in Georgia, USA	Georgia	6 years	Tomato, Corn
12	(Sainju et al., 2005)	Carbon accumulation in cotton, sorghum, and underlying soil as influenced by tillage, cover crops, and nitrogen fertilization	Fort Valley, GA	3 years	Cotton and Sorghum

Table 3.1. (cont'd).

S. No.	Citation	Publication Title	Geographic location	Study duration	Main Crop
13	(Mbuthia et al., 2015)	Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality	Jackson, TN	32 years	Cotton
14	(Sainju et al., 2008)	Soil carbon and nitrogen sequestration as affected by long-term tillage-cropping systems and nitrogen fertilizer sources	Belle Mina, Alabama, US	10 years	Cotton and Corn
15	(Olson et al., 2014a)	Long-Term Effects of Cover Crops on Crop Yields, Soil Organic Carbon Stocks and Sequestration	Southern illinois	12 years	Corn-Soybean

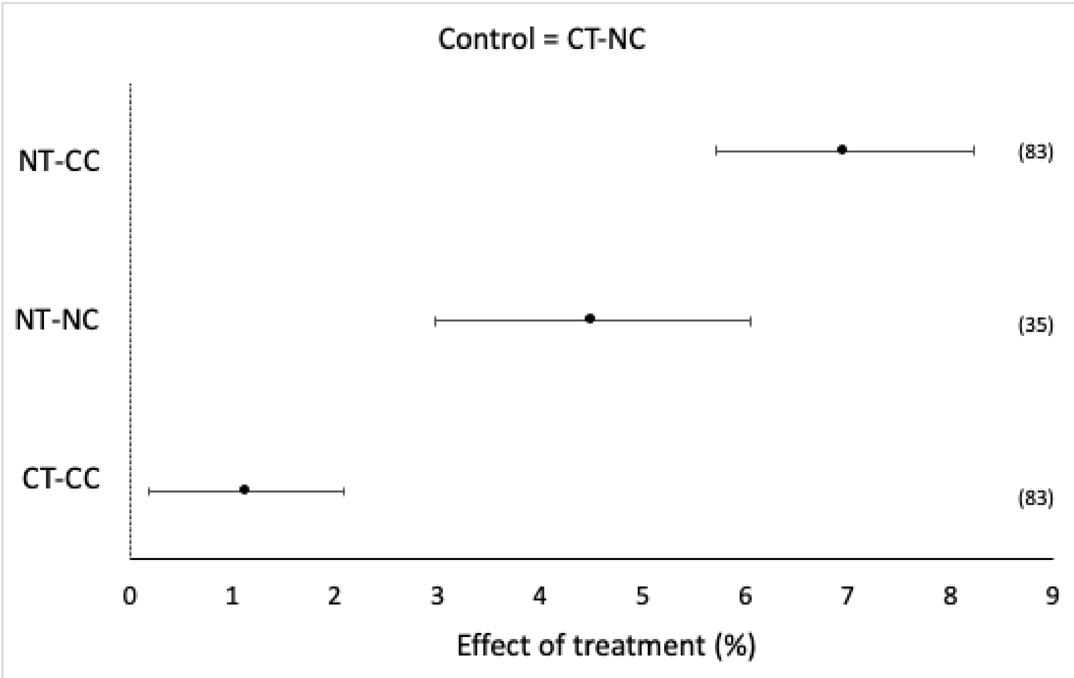

Figure 3.1. Effect of treatments (CT-CC, NT-NC, and NT-CC) on Soil organic carbon (SOC) content (Mg C ha^{-1}) as compared to CT-NC. Means and 95% confidence intervals are depicted. Numbers of experimental observations are in parentheses.

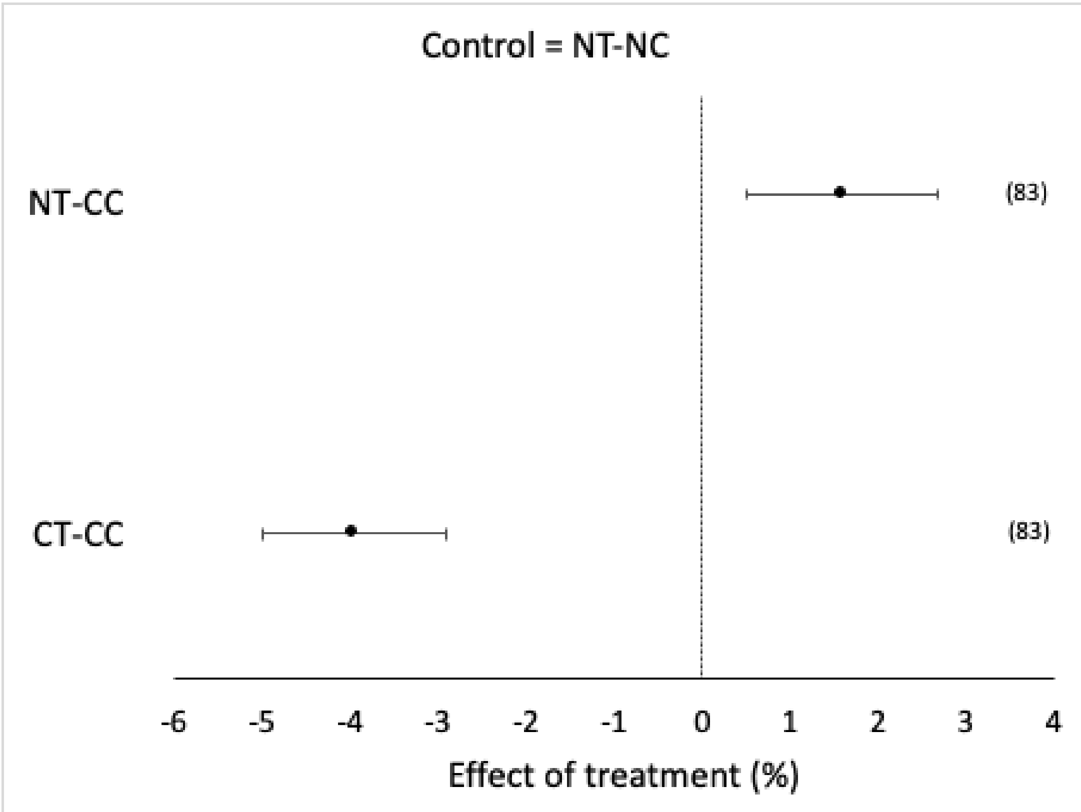

Figure 3.2. Effect of treatments (CT-CC and NT-CC) on Soil organic carbon (SOC) content (Mg C ha-1) as compared to NT-NC. Means and 95% confidence intervals are depicted. Numbers of experimental observations are in parentheses.

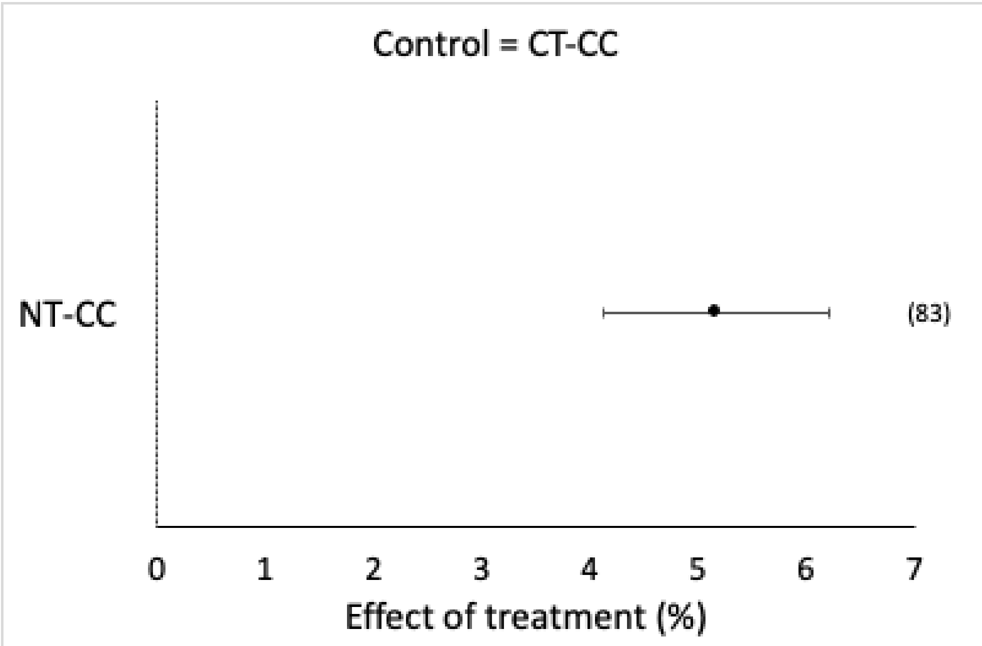

Figure 3.3. Effect of treatments (NT-CC) on Soil organic carbon (SOC) content (Mg C ha^{-1}) as compared to CT-CC. Means and 95% confidence intervals are depicted. Numbers of experimental observations are in parentheses.

CHAPTER 4
SUMMARY AND CONCLUSIONS

Obtaining official certification for organic crops requires a 36-month transition period. If farmers are unable to add chemical fertilizers and pesticides at this time, agricultural production and profits may suffer. Incorporating cover crops into the rotation is one strategy to prevent the potential yield decline during the transition to organic farming. Due to its viability and low financial risk, cover crop-based organic transition in row crop rotation systems, such as corn-soybean-winter wheat rotation (CSWR) has gained favor in the Midwest of the United States. There were, however, questions regarding the selection of cover crop combinations, the viability of establishing highly productive cover crops, the length of cover crop cultivation, and the soil quality benefits provided by cover crops, particularly during the organic transition.

In 2018, we began field research to examine the benefits of cover crops throughout CSWR's three-year transition to organic farming. The cover crop systems included no-cover control (NC), a traditional cover crop system (TR) used in CSWR in the US Midwest consisting of cereal rye planted after corn harvested in the first year and red clover frost-seeded into the winter wheat in the third year of the rotation, and a mixture of cold tolerant (WH) or winter killed (WK) cover crop species interseeded into corn in first year and again planted after wheat harvest in the third year. The study targeted three topographical positions: depression, slope, and summit. The studied responses included soil properties: soil moisture, soil inorganic N, soil C and N mineralization, microbial biomass C, and yield of the first organic corn. Nguyen et al., (2022) highlighted the differences in soil properties after the first year of corn planting and cover crop seeding and concluded that the WH cover crop mixture improved biological, chemical, and physical soil health in the CSWR organic transition system across varied agricultural landscapes.

The first objective of this thesis was to investigate the effects of three cover crop-based transition scenarios on the yield of the first organic corn cash crop and on soil chracateristics that may be important for crop growth and yield at the conclusion of the three-year organic transition phase. We found that cover crops altered spring soil moisture. WK had more soil moisture than NC in the spring prior to planting corn, suggesting that including a WK cover crop in the organic transition CSWR system will not likely deplete soil water or compete with cash crops for water. Cover crop treatments did not differ in particulate organic matter content, soil NH_4^+ contents, and N mineralization but TR treatmnets had higher soil NO_3^- contents, probably because of the presence of the red clover cover crop frost seeded into wheat and terminated prior to planting the organic corn. Soil microbial biomass was found to be numerically highest in the WH and TR treatments, whereas soil C mineralization rate was statistically higher in the WH and WK treatments as compared to the NC treatment. This implies that the WH and TR system would probably have higher microbial activity as compared to the NC treatment, and may result in more availability of soil nutrients to the subsequesnt cash crop, resulting in higher yields. However, we saw no difference in the organic corn yield in year 4 across cover crop treatments. Field topography had a significant effect on yield. Field depressions had highest yield, and the yield at different topographies were also significantly regulated by soil microbial biomass C. In conclusion, the TR and WH treatments were beneficial in improving soil characteristics, but the effect on cash crop yield remains inconclusive.

Enhancing soil organic carbon (SOC) levels in agricultural systems creates a win-win situation by improving soil fertility, reducing CO_2 emissions, and increasing crop output. Soil carbon sequestration is enhanced by the use of conservation agricultural management strategies. The use of no tillage (and the use of cover crops has been intensively studied in terms of soil carbon sequestration. Previous meta-analysis showed that the conversion to no-tillage and cover crops systems individually, both increased SOC compared to conventional systems. Here, we did a meta-analysis study to estimate the additive effect of both systems when they are combined together. This meta-analysis compares two tillage techniques (CT and NT) and cover crops (CC and NC) on soil carbon sequestration. Fifteen field studies published between 2000 and 2022 were selected and the response variable in all investigations was total organic carbon. However, it is to be noted that the duration of experiment, soil type, and other soil properties were different in the studies used in this meta-analysis. These factors may influence the change in SOC, but due to the limited number of studies found, these factors were not taken into consideration.

No tillage increased SOC by 4.5% compared to conventional tillage. Adding cover crops to conventional tillage system increased SOC only by 1.12%. The greatest increase in SOC (7%) was found to be in the no-tillage systems which also included cover crops in the crop rotation. Thus, changing from conventional tillage to no-till and introducing cover crops into crop rotations can have an additive effect on soil carbon sequestration, as cover crops supply additional organic matter to the soil through plant residues and no-till reduces organic matter mineralization, resulting in lower CO_2 loss from the soil.

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APPENDIX

Supplementary Tables and Figures for Chapter 2

Supplementary Table 1. *P*-values for the studied variables for factors: topography (T), cover crops (CC), and their interaction T x CC, compared across fields together and each field individually. ns means *P*-value > 0.1 and the difference is non-significant.

Variable	Factor	Across all fields*	Individual field*			
			A-1	82-1	MP	85
Soil Moisture	T	0.0004	ns	0.046	0.02	ns
	CC	0.019	0.034	ns	ns	ns
	T x CC	ns	ns	ns	ns	ns
POM	T	ns	ns	ns	ns	ns
	CC	ns	ns	ns	ns	ns
	T x CC	ns	ns	ns	ns	ns
Soil NO_3^-	T	ns	ns	ns	ns	ns
	CC	0.075	ns	ns	0.031	ns
	T x CC	ns	ns	ns	ns	ns
Soil NH_4^+	T	0.057	ns	ns	ns	0.039
	CC	ns	ns	ns	ns	ns
	T x CC	ns	ns	ns	ns	ns
Soil microbial biomass C	T	0.006	0.085	0.017	ns	ns
	CC	0.091	0.042	ns	ns	ns
	T x CC	ns	ns	ns	ns	ns
Soil C mineralization rate	T	ns	ns	0.088	ns	ns
	CC	0.025	0.012	ns	0.066	ns
	T x CC	ns	ns	ns	ns	ns
Soil N Mineralization rate	T	ns	ns	ns	ns	0.014
	CC	ns	ns	0.082	ns	ns
	T x CC	ns	ns	ns	ns	ns

Supplementary Table 1. (cont'd).

Variable	Factor	Across all fields*	Individual field*			
			A-1	82-1	MP	85
Total plant biomass	T	0.004	ns	ns	0.02	ns
	CC	<0.0001	<0.0001	0.0002	<0.0001	0.0024
	T x CC	ns	ns	ns	ns	0.021
Weed biomass	T	0.057	ns	ns	0.044	ns
	CC	0.0001	0.0045	ns	<0.0001	0.023
	T x CC	0.031	0.053	ns	0.0008	0.061
Cover crop biomass	T	ns	ns	ns	ns	ns
	CC	< 0.0001	0.0006	0.003	< 0.0001	ns
	T x CC	0.002	ns	ns	ns	0.0172
Corn yield	T	0.0007	0.008	ns	0.007	0.081
	CC	ns	ns	ns	ns	ns
	T x CC	ns	ns	ns	ns	ns

Supplementary Table 2. Means of aboveground biomass of individual cover crop species collected in fall from different cover crop treatments.

Topography	Cover crop	Annual ryegrass	Clover	Rape	Oat	Pea	Radish
		Kg/ha					
Depression							
	NC	-	-	-	-	-	-
	TR	-	1455	-	-	-	-
	WH	1542	379	431	-	-	-
	WK	-	-	-	1474	159	482
Slope							
	NC	-	-	-	-	-	-
	TR	-	994	-	-	-	-
	WH	1009	419	122	-	-	-
	WK	-	-	-	1162	180	335
Summit							
	NC	-	-	-	-	-	-
	TR	-	1526	-	-	-	-
	WH	1473	280	398	-	-	-
	WK	-	-	-	1713	166	397

Supplementary Table 3. Means of aboveground biomass of individual cover crop species collected in spring from different cover crop treatments.

Topography	Cover crop	Pea	Radish	Rapeseed	Clover	Annual ryegrass
		Kg/ha				
Depression						
	NC	-	-	-	-	-
	TR	-	-	-	2271.80	-
	WH	-	-	100.89	300.04	503.09
	WK	3.34	8.14	-	-	-
Slope						
	NC	-	-	-	-	-
	TR	-	-	-	1284.44	-
	WH	-	-	18.40	523.01	150.03
	WK	7.69	7.47	-	31.15	-
Summit						
	NC	-	-	-	-	-
	TR	-	-	-	2057.77	-
	WH	-	-	110.60	458.73	356.18
	WK	5.41	13.6875	-	-	-

Supplementary Table 4. Pearson's correlation coefficients between soil properties, total, weed, and cover crop aboveground biomass and corn yield across all topographies for all fields together. Italic bold, bold, and italic fonts marks correlation coefficients significantly different from zero at P < 0.01, P < 0.05, and P < 0.1, respectively.

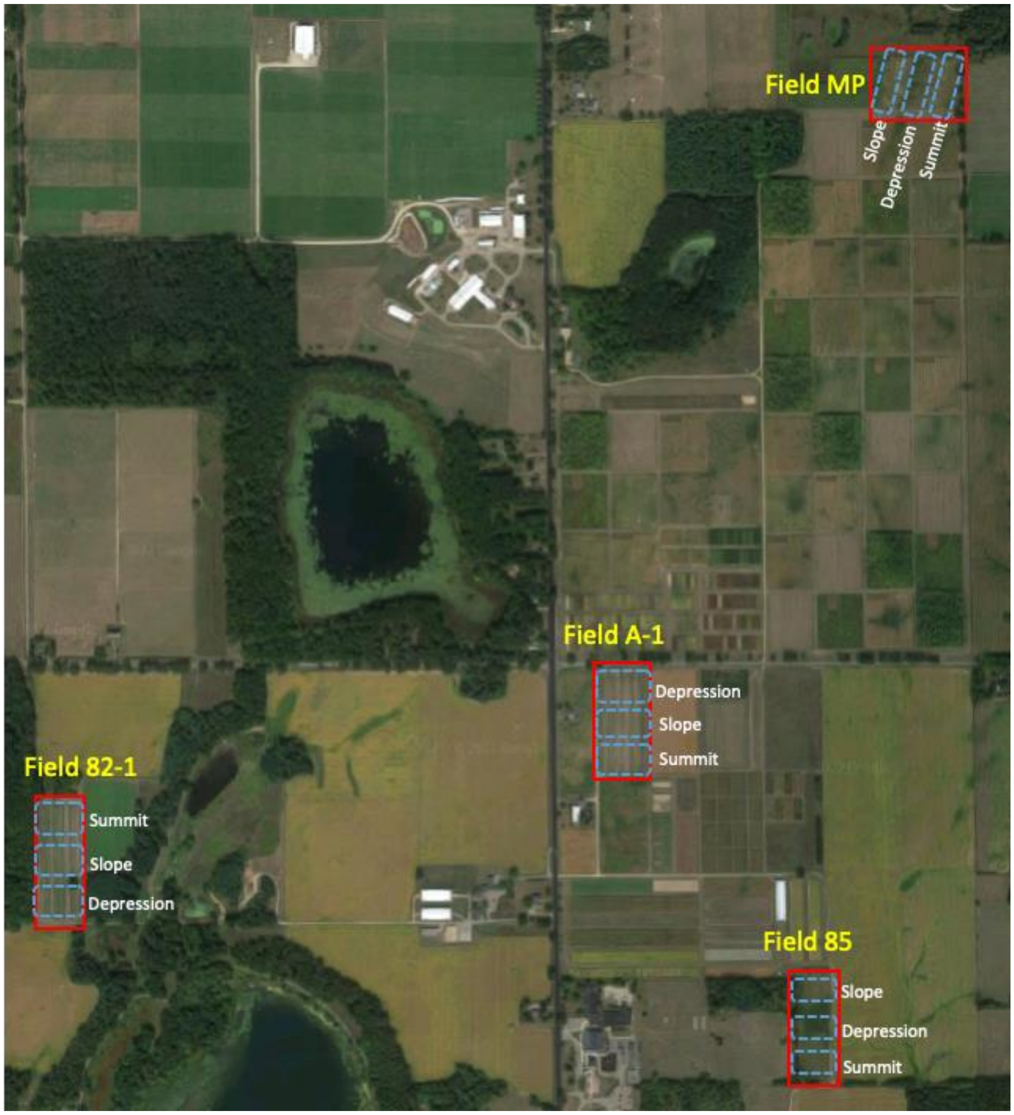
Variable	Soil moisture	POM	Soil NH_4^+	Soil NO_3^-	Soil N mineralization rate	Soil microbial biomass C	Soil C mineralization rate	Total plant biomass	Cover crop biomass
POM	0.101								
Soil NH_4^+	0.097	0.006							
Soil NO_3^-	***0.248***	-0.012	***0.200***						
Soil N mineralization rate	***0.465***	-0.045	***0.200***	0.059					
Soil microbial biomass C	***0.389***	-0.034	-0.062	***0.312***	0.068				
Soil C mineralization rate	***0.470***	**-0.164**	***0.287***	***0.317***	***0.572***	***0.248***			
Total plant biomass	**-0.160**	*0.125*	**-0.152**	-0.050	***-0.284***	-0.025	***-0.252***		
Cover crop biomass	**-0.163**	0.109	-0.094	0.004	***-0.224***	-0.043	***-0.185***	***0.928***	
Weed biomass	0.048	0.012	*-0.121*	*-0.132*	-0.094	0.055	*-0.119*	-0.045	***-0.415***

Supplementary Table 5. P-values from Analysis of Covariance (ANCOVA) for standardized corn yield with different soil properties as covariates. “-“ in the column means interaction term was removed from ANCOVA model because P-value was higher than 0.3 for the interaction term.

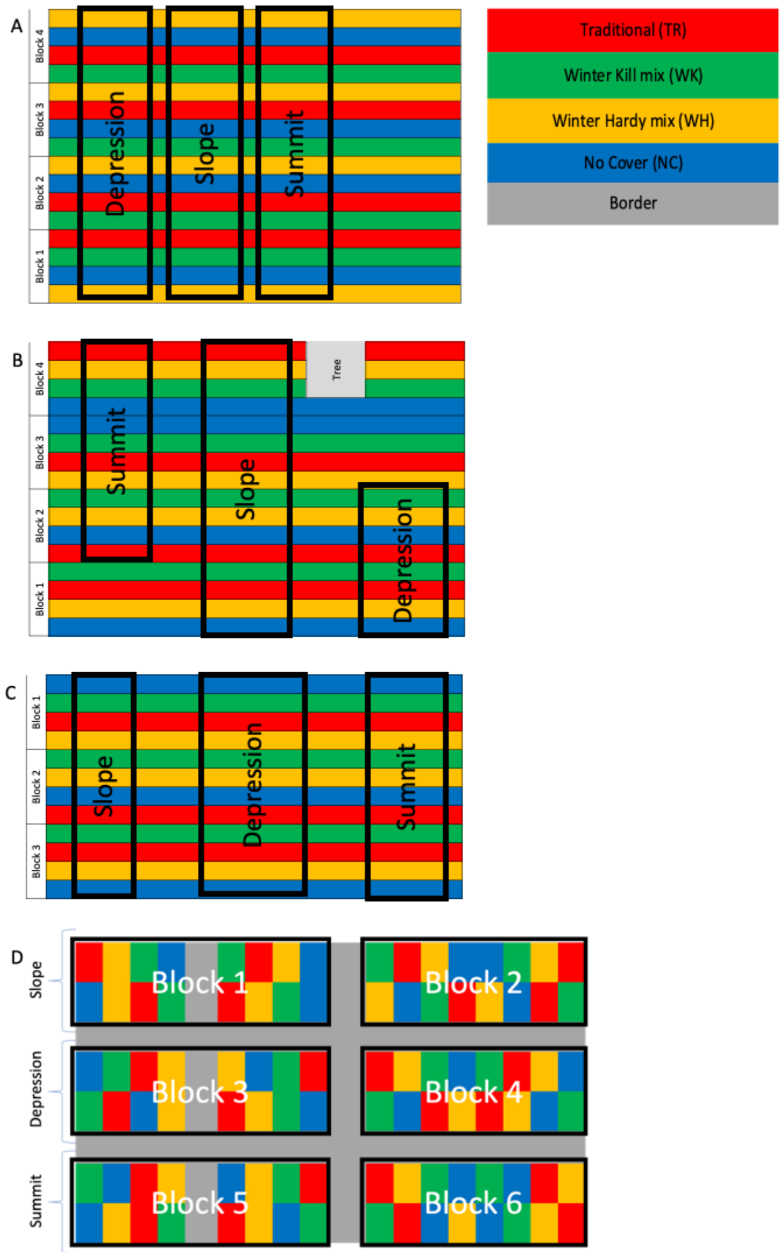
Covariate	T	CC	T*CC	Covariate	Covariate*T	Covariate*CC
POM	< 0.0001	0.075	0.060	0.417	-	0.130
Soil NH_4^+	0.0004	0.569	0.267	0.422	-	-
Soil NO_3^-	0.0004	0.728	-	0.146	0.107	-
Soil N mineralization rate	0.0004	0.569	0.260	0.397	-	-
Soil microbial biomass C	0.0022	0.378	-	0.087	0.019	-
Soil C mineralization rate	0.0003	0.600	0.259	0.775	-	-
Total plant biomass	0.0007	0.561	0.295	0.487	-	-

Supplementary Table 6. Means of total carbon (%), and total nitrogen (%) for different cover crops systems, topographies, and their interaction for all fields together measured at the end of experiment (after three years). Letters in a column mark the significant differences between cover crops (P <0.05). ns means no significant difference between cover crops.

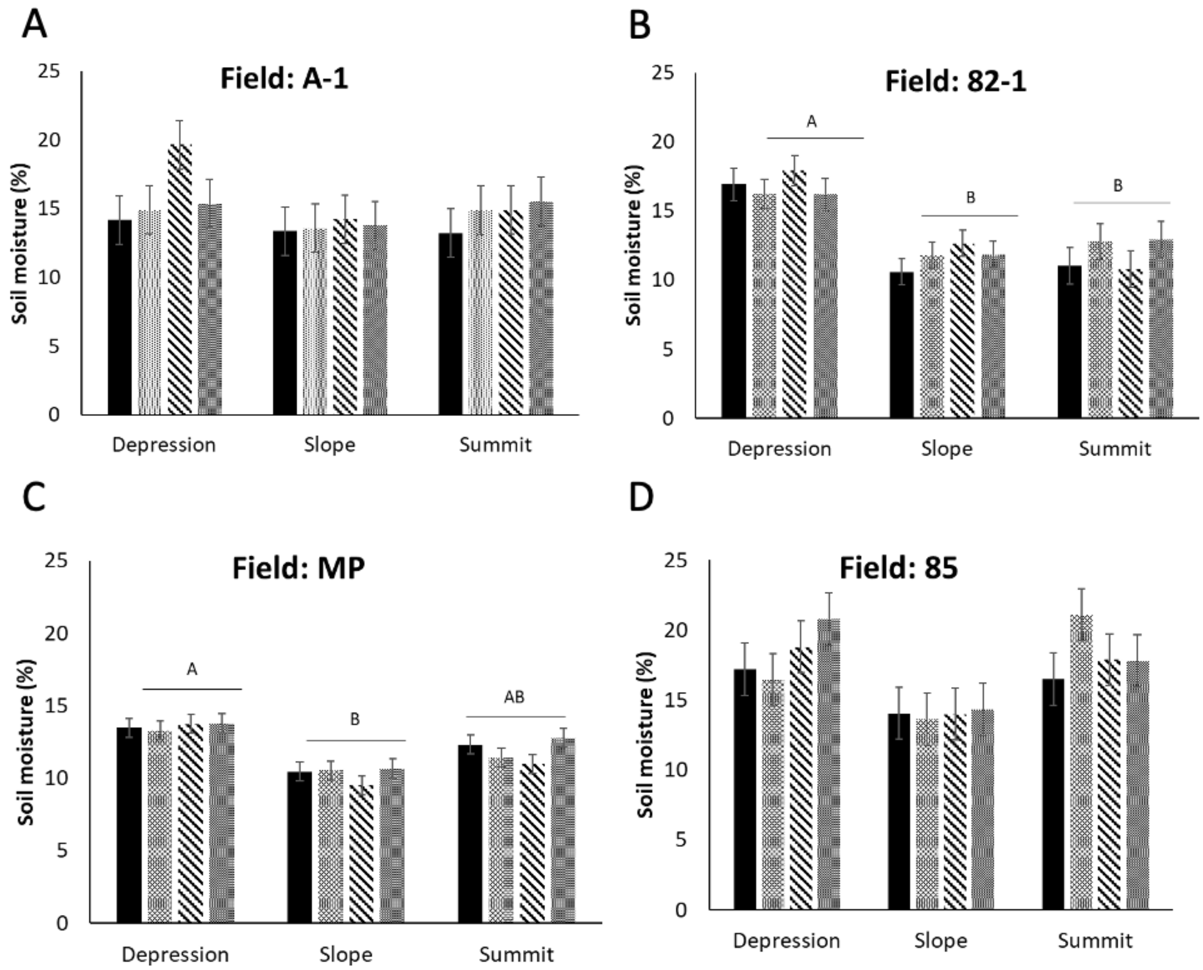
Topography	**Cover Crop**	**Total C (%)**	**Total N (%)**
Depression		0.89 A	0.09 a
Slope		0.72 B	0.08 b
Summit		0.78 AB	0.08 b
	NC	0.82	0.09
	TR	0.81	0.09
	WH	0.79	0.08
	WK	0.77	0.08
Depression	NC	0.96	0.10 ab
	TR	0.75	0.08 b
	WH	0.89	0.09 ab
	WK	0.99	0.10 a
Slope	NC	0.70 ab	0.08 ab
	TR	0.87 a	0.09 a
	WH	0.76 ab	0.08 ab
	WK	0.56 b	0.07 b
Summit	NC	0.82	0.08
	TR	0.80	0.08
	WH	0.72	0.08
	WK	0.79	0.08
P-value	Top	0.01	0.01
	CC	ns	ns
	Top*CC	0.02	0.03



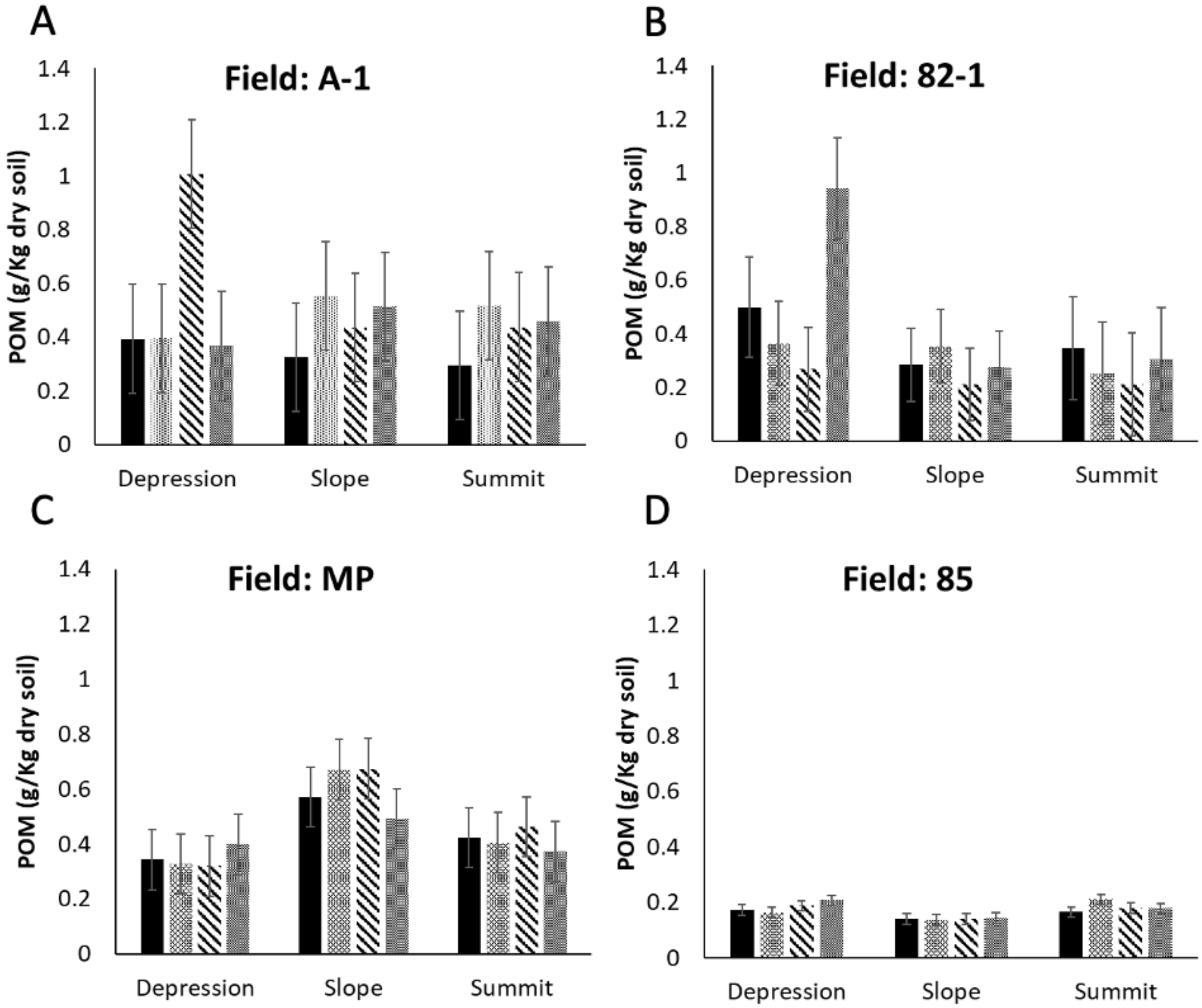
Supplementary Figure 1. The Google Maps view of the study area with positions of the four studied fields (A-1, 82-2, 85, and MP) outlined in red A-1, 82-2, 85, and MP. Schematic representations of the topographical positions (depression, slope, and summit) within each field are shown with dashed-blue outlines, the exact locations of the topographical positions within the fields are shown on Supplementary Figure 2.



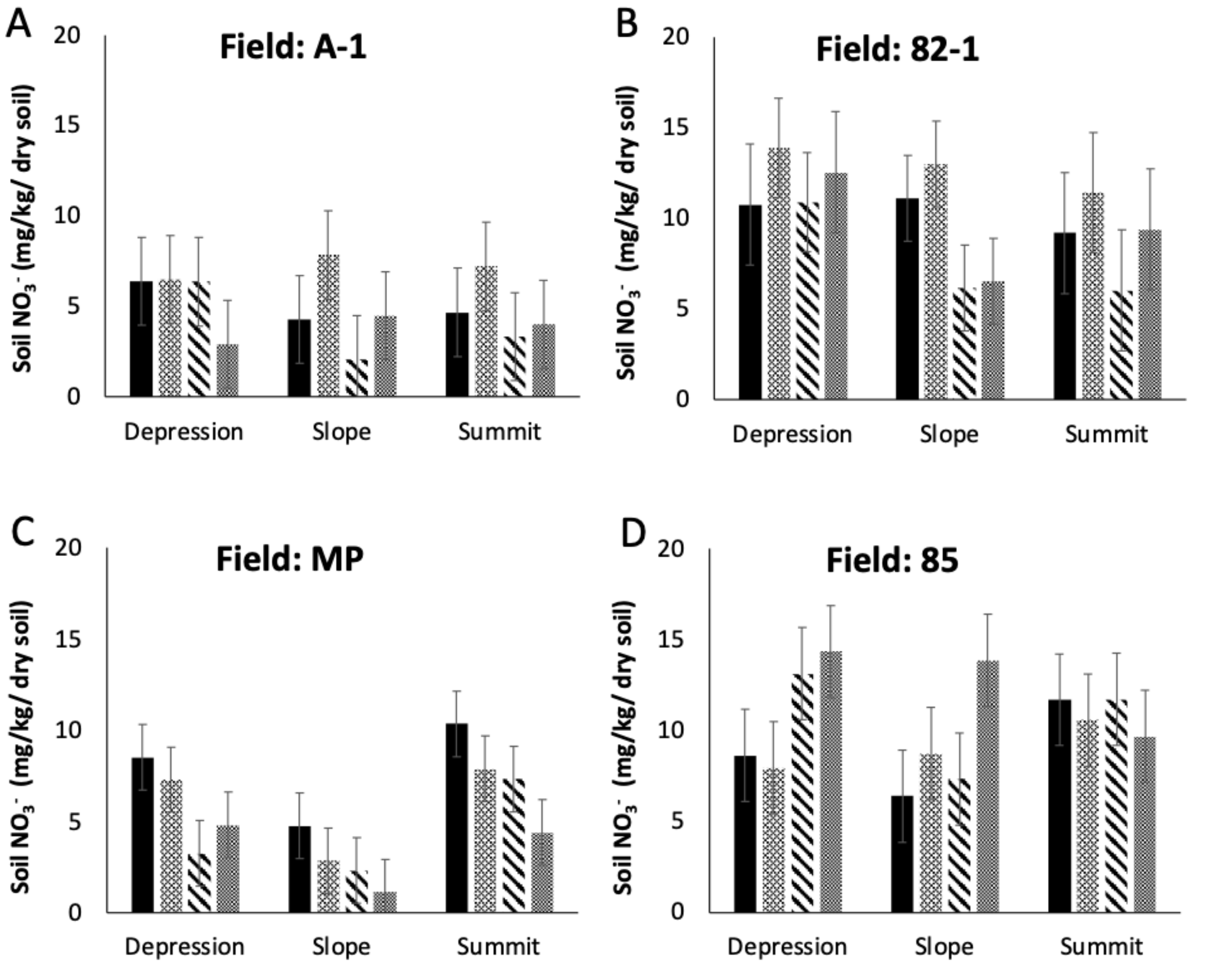
Supplementary Figure 2. Layouts of the blocks, topographical position areas, and experimental plots assigned to the studied organic transition systems in the four fields A) A-1, B) 82-1, C) 85, and D) MP.



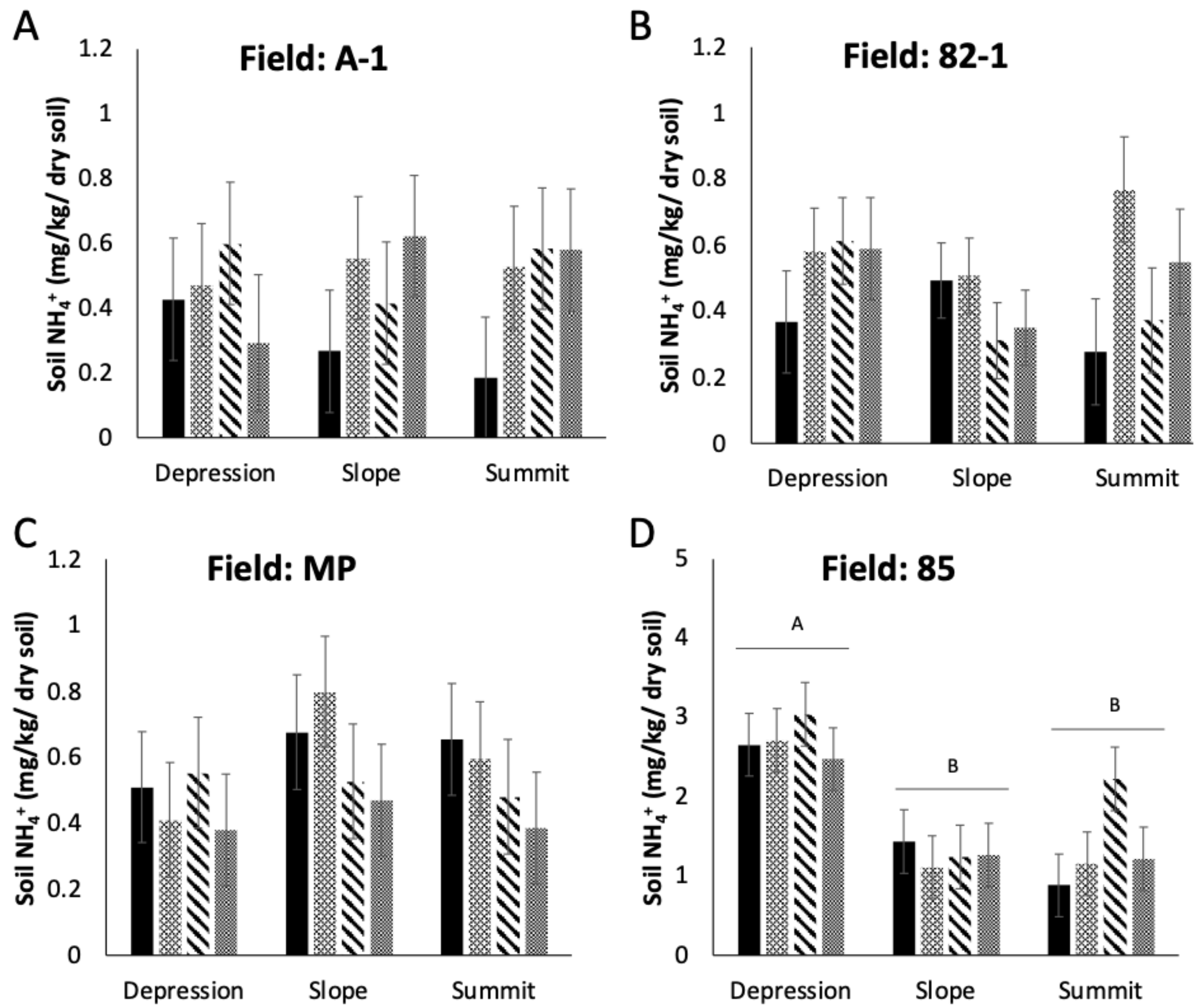
Supplementary Figure 3. Means and standard errors of soil moisture contents at 20 cm depth for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85. Capital letters mark significant differences among marginal means of topographical positions ($P < 0.05$).



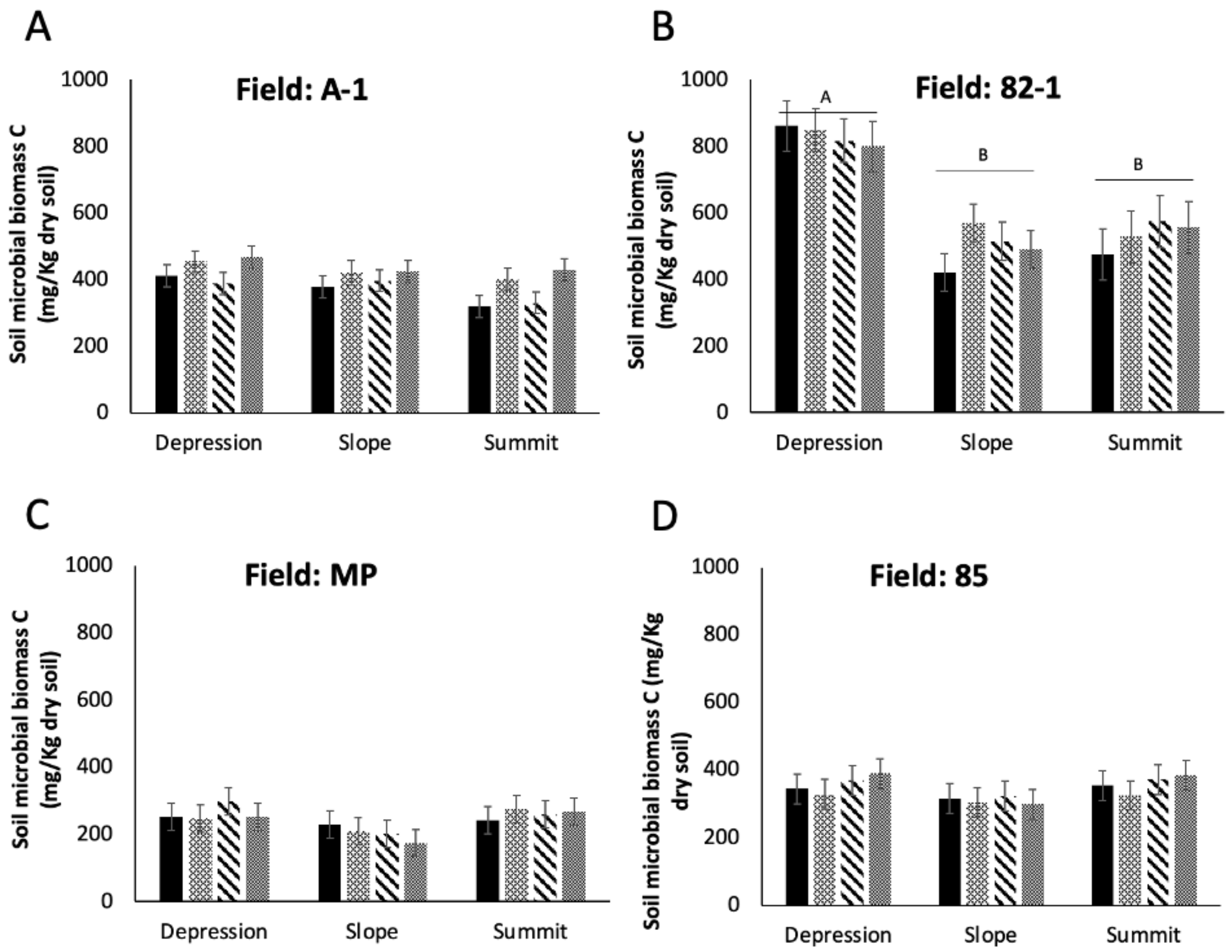
Supplementary Figure 4. Means and standard errors of soil particulate organic matter (POM) contents at 20 cm depth for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85.



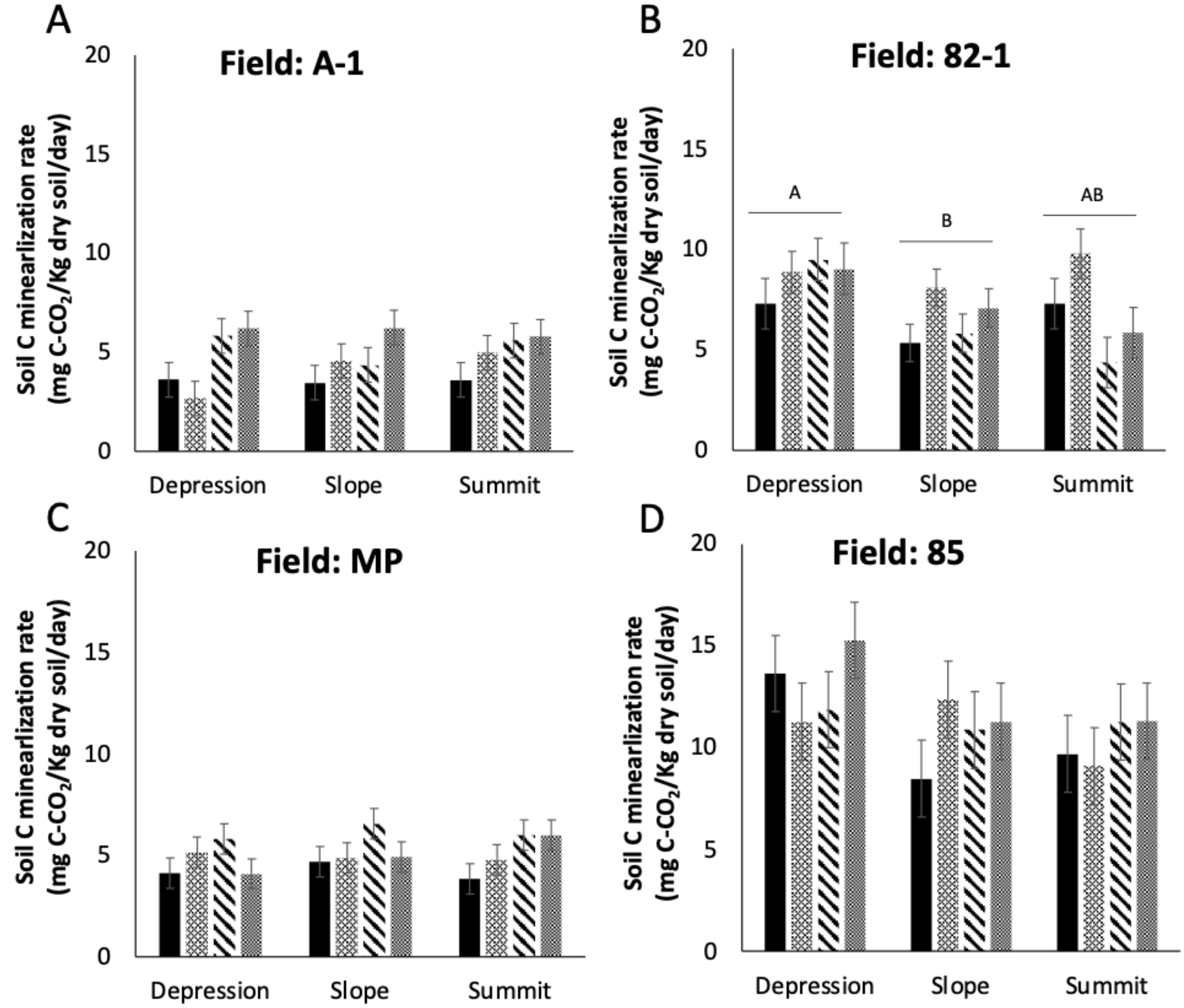
Supplementary Figure 5. Means and standard errors of soil nitrate contents at 20 cm depth for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85.



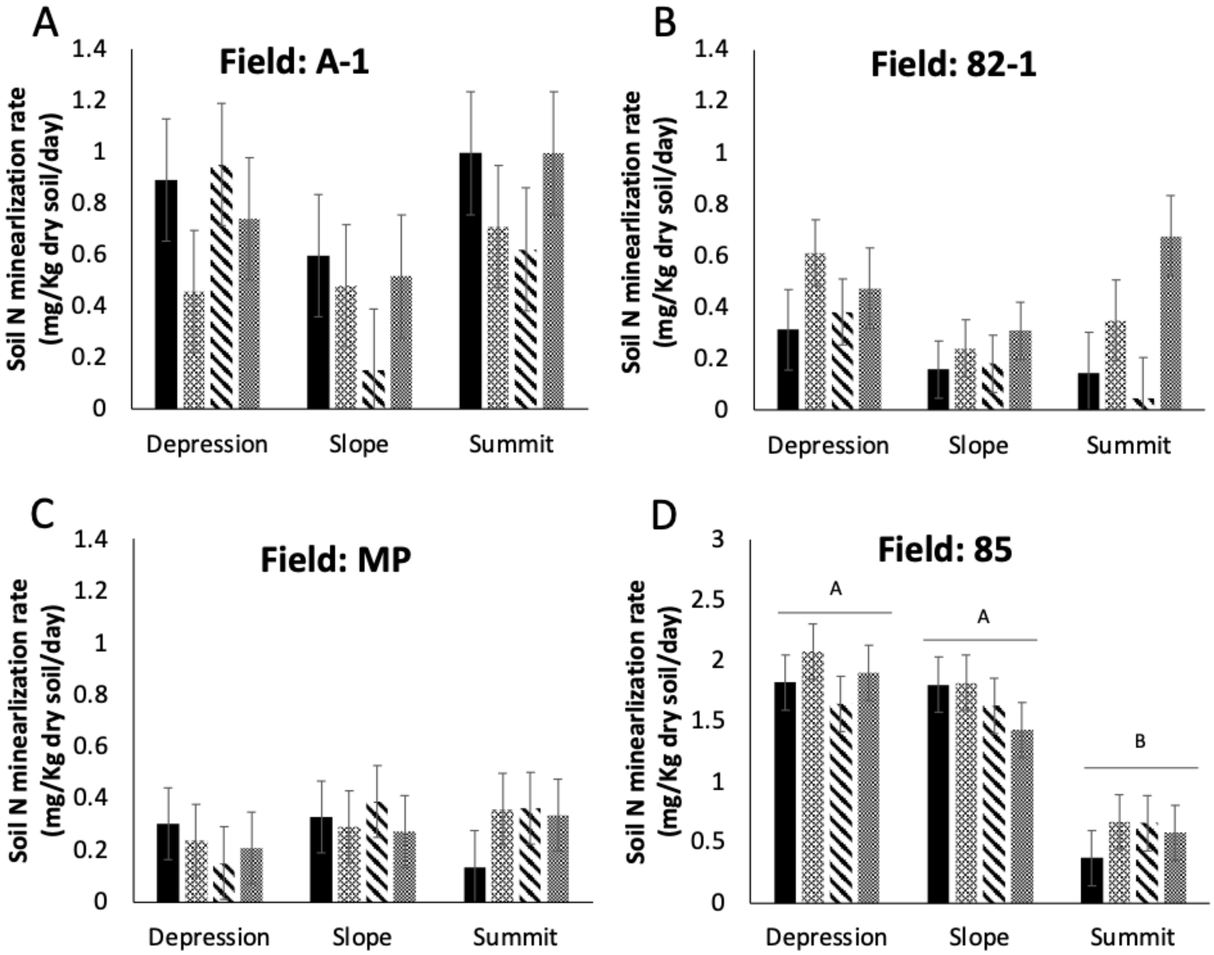
Supplementary Figure 6. Means and standard errors of soil ammonium contents at 20 cm depth for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85. Capital letters mark significant differences among marginal means of topographical positions (P < 0.05).



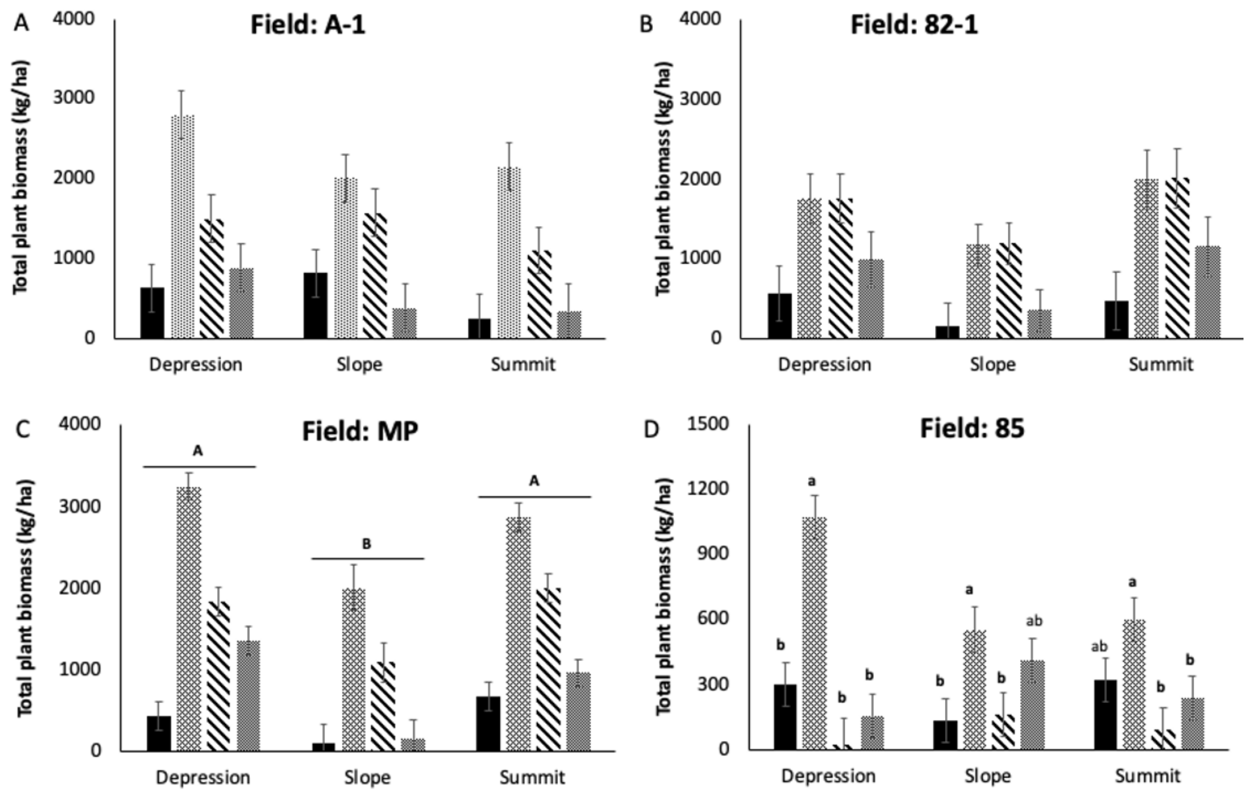
Supplementary Figure 7. Means and standard errors of soil microbial biomass carbon contents at 20 cm depth for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85. Capital black letters mark significant differences among marginal means of topographical positions ($P < 0.05$).



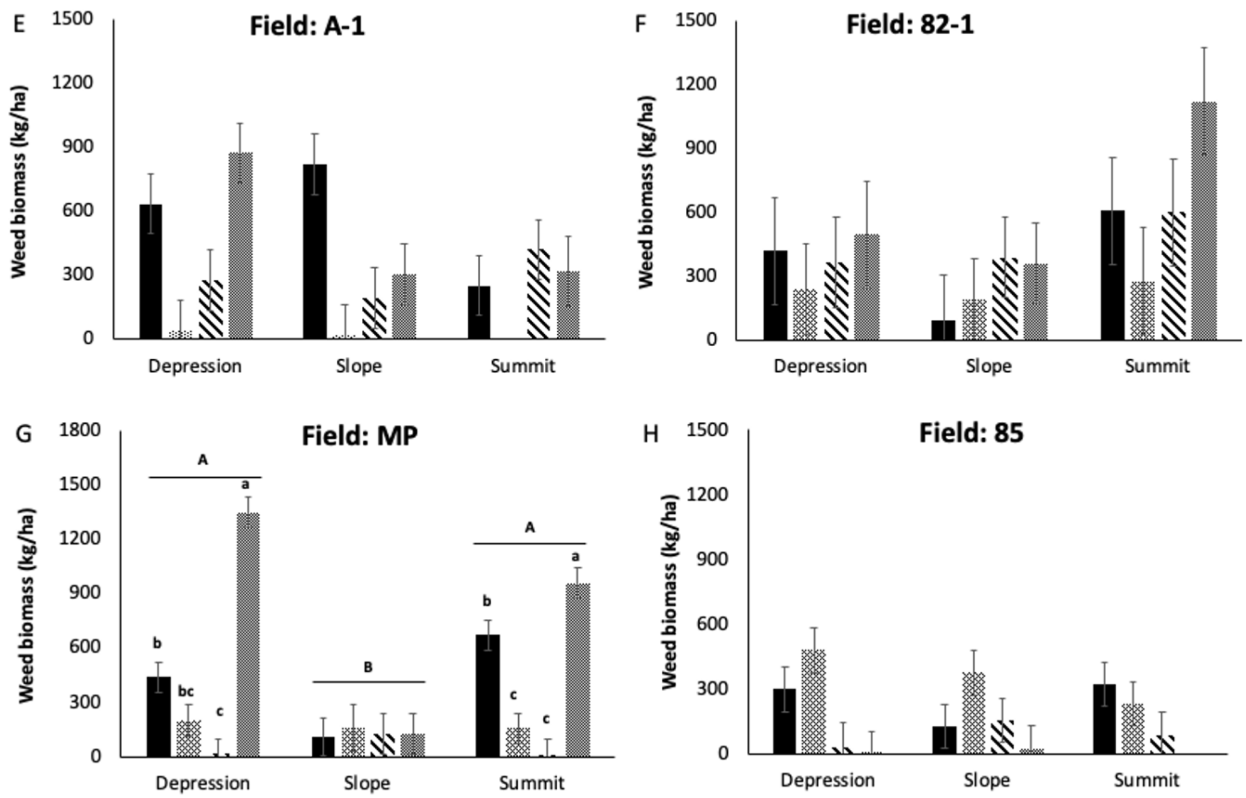
Supplementary Figure 8. Means and standard errors of soil carbon mineralization rate at 20 cm depth for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85. Capital black letters mark significant differences among marginal means of topographical positions ($P < 0.05$).



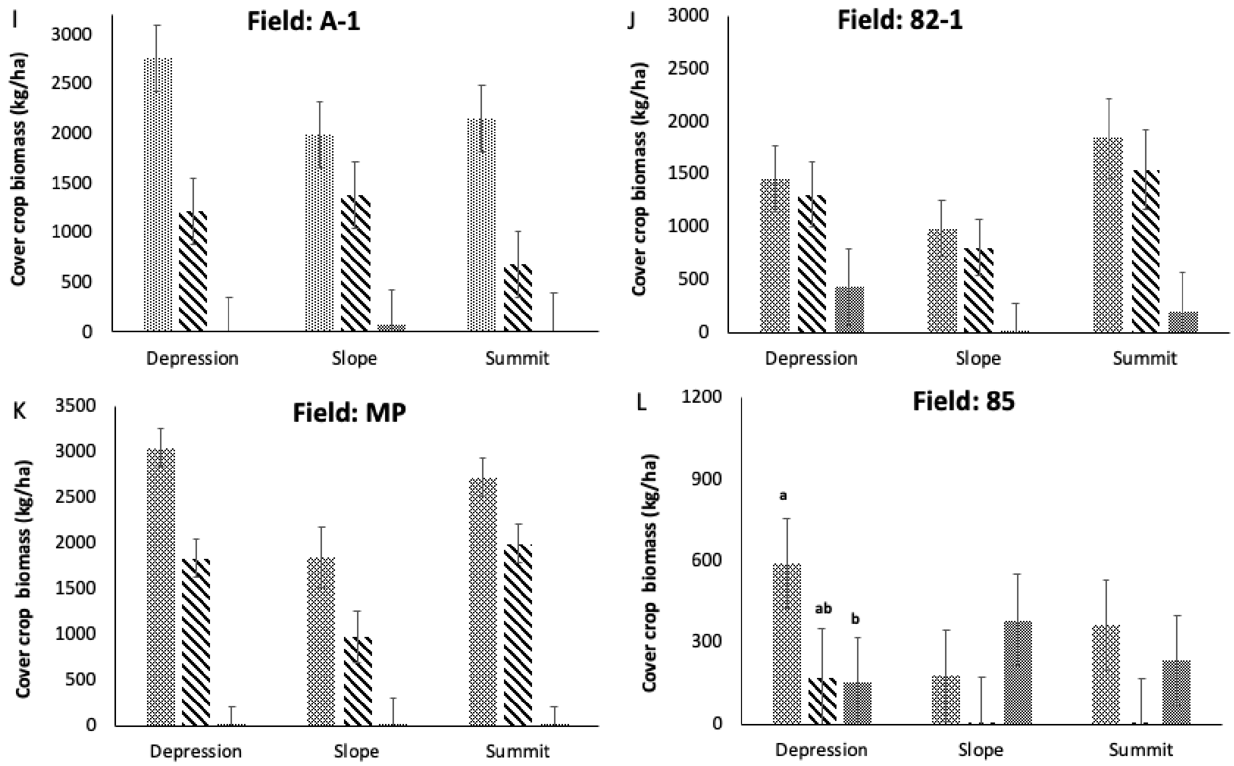
Supplementary Figure 9. Means and standard errors of soil nitrogen mineralization rate at 20 cm depth for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85. Capital black letters mark significant differences among marginal means of topographical positions ($P < 0.05$).



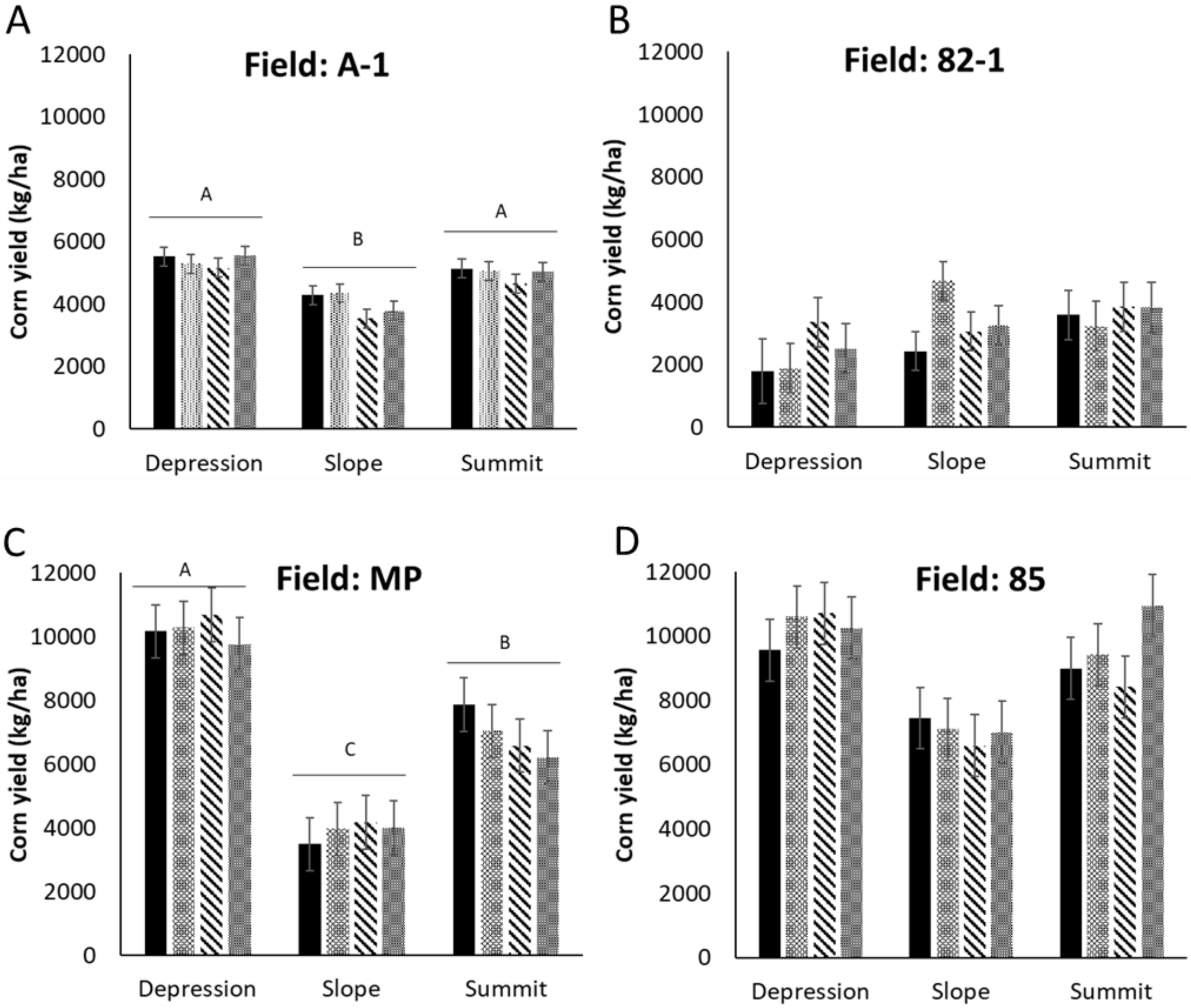
Supplementary Figure 10. Means and standard errors of A-D: total biomass, E-H: weed biomass, and I-L cover crop biomass for the studied cover crop systems within each topographical position for each individual field: A-1, 82-1, MP, and 85. Capital black letters mark significant differences among marginal means of topographical positions ($P < 0.05$). Small letters marks the significant differences among cover crop systems within each topographical position after slicing the significant interaction ($P < 0.05$).



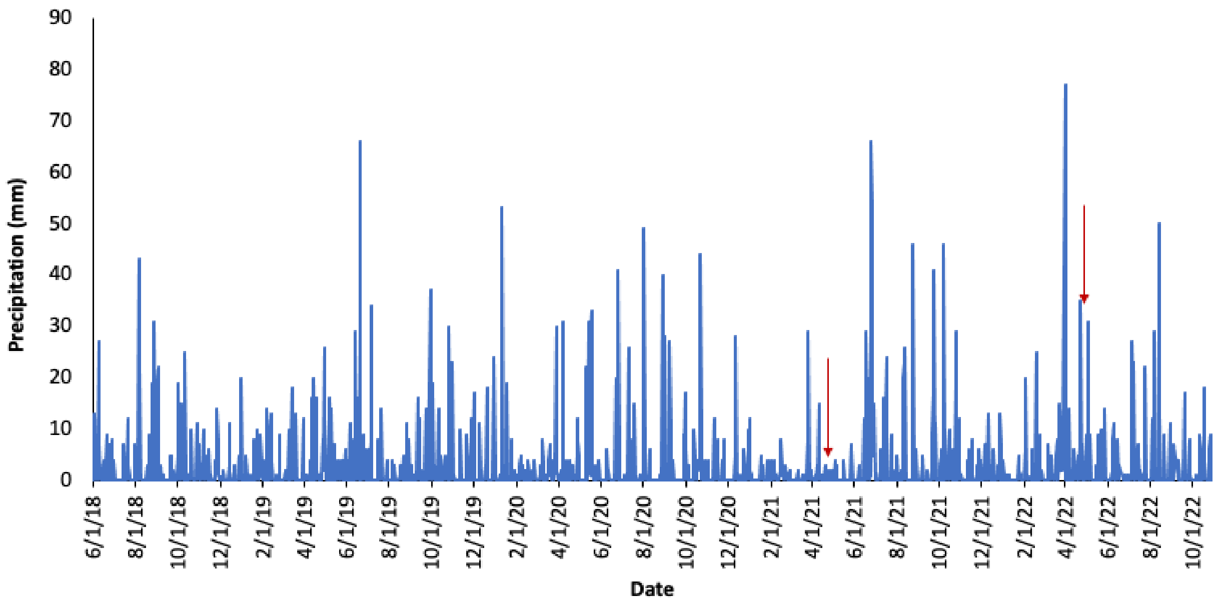
Supplementary Figure 10. (cont'd).



Supplementary Figure 10. (cont'd).



Supplementary Figure 11. Means and standard errors of first organic corn yield (15.5% moisture) for the studied cover crop systems within each topographical position for each individual field: A) A-1, B) 82-1, C) MP, and D) 85. Capital black letters mark significant differences among marginal means of topographical positions ($P < 0.05$).



Supplementary Figure 12. Precipitation at KBS during the three years of organic transition (2018-2021 for fields A1, 82-1 and MP; 2019-2022 for field 85). Red arrows indicate spring sampling dates in 2021 for fields A1, 82-1 and MP; and in 2022 for field 85.