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**Core ideas (3-5 impact statements, 85 char max for each)**

* Effects of cover cropping on soil water parameters at 14 cm depth varied by site
* Cover crops increased water held at field capacity in two of four trials
* Cover crops did not affect water held at saturation or increase percentage of macropores (>30 um) at any trial
* Proposed causal model shows cover crop root measurements may be key to understanding site-specific effects

Effects of Winter Cover Cropping on Soil Water-holding Capacity Varies by Site

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Abbreviations:

CC, cover crop

USDA, United States Department of Agriculture

Abstract

*The abstract should be a single paragraph of 250 words or less. It should be specific, telling why and how the study was made, what the results were, and why they were important. The abstract should read like a “mini-manuscript” with 1 to 2 sentences each for a justification/rationale, objective(s), methods, results, and conclusion.*

Addition of an over-wintering cereal rye (*Secale cereal*) cover crop (CC) to Midwestern maize (*Zea mays*)-based systems offers several environmental benefits, but the long-term effects on soil hydrological properties is not well-understood. We utilized four long-term (10+ year) no-till trials in Iowa, USA that included a replicated winter rye CC and no-cover treatment in systems with a maize crop (grain or silage) rotated with soybean (*Glycine max*). At each trial, we took intact 7.62 diameter soil samples from a 10-18 cm depth increment shortly after cash crop planting in the spring of 2019. We measured the volumetric soil water content at saturation and matric potentials of -2.5, -10, -25, -50, -100, -200 and -500 cm water. Additionally, we measured organic matter, soil texture, and bulk densities of the samples. Pore-size distribution indices and air-entry potentials were estimated from non-linear models fit to the soil water retention curves, and percent macropores (>30 um) were estimated using capillary rise equations. Water contents at saturation and at field capacity (-100 cm water) were taken directly from the data. Neither pore-size distribution nor air-entry potential (model parameters) were affected by CCs. At this depth, CCs did not meaningfully affect bulk density or water contents at saturation at any trial, nor did CCs increase the percent macropores. At two trials, soil water content at field capacity was increased by 2.5% (SE: 1.2%) and 2.4% (SE:1.3%), respectively. The presence or absence of a CC effect on field capacity was not related to CC above-ground biomass production or trial texture. We propose a causal model relating CCs to soil properties relevant to soil water, which indicates root measurements may be key to understanding variable effects of CCs on soil water storage. Our results demonstrate more research is needed on the exact mechanisms by which CCs can improve soil water storage, as well as when and where those benefits may be most easily realized.

Introduction

Addition of an over-wintering cereal rye (*Secale cereal*) cover crop (CC) to Midwestern maize (*Zea mays*)-based systems offers several environmental benefits including reduced soil erosion and nutrient pollution (Kaspar et al. 2001, Strock et al. 2004, Kaspar et al. 2007, Kladivko et al. 2014 ). On average, rye CCs have no effect on maize yields compared to winter fallow in the short term (Marcillo and Miguez 2017), but it is possible long-term cover cropping could stabilize crop yields. In Midwestern rain-fed systems, crops rely on stored soil water and often suffer from terminal drought stress (Campos et al. 2006). In these systems, CCs may induce soil changes such as increased organic matter, lower bulk densities, and more macro-pores (Haruna et al. 2020). In theory, these changes could result in more water storage capacity (Hudson 1994, Minasni et al. 2018, King et al. 2020), and therefore buffer crop yields against drought stress (Williams et al. 2016, Kane et al. 2021). Cover crops may also benefit crop-water relations by increasing water infiltration or through a mulching effect (Unger and Vigil 1998, Leuthold et al. 2021). Two global meta-analyses suggest CCs increase soil’s capacity to store water and increase infiltration rates (Basche and DeLonge 2017, Basche and DeLonge 2019). However, to our knowledge there are few studies supporting these findings in Midwestern cover cropping contexts, and the few existing studies report contradicting results (Villamil et al. 2006, Haruna and Nkongolo 2015, Basche et al. 2016, Rorick and Kladivko 2017, Irmak et al. 2018). Region-specific studies are needed, as climatic and managerial constraints of maize-soybean rotations can limit CC options and growth potential (Strock et al. 2004, Baker and Griffis 2009, Nichols and Martinez-Feria 2021).

In addition to regional differences in CC effects, the duration of CCing may also influence whether changes in soil are detected. Long-term studies on tillage have shown significant, but slow changes to the soil after implementing no-till (al-Kaisi et al. 2014, Cusser et al. 2020). Addition of CCs may likewise require several years before improved soil hydrological properties can be detected, necessitating data collection from long-term experiments. Furthermore, while shallow soil depths (0-10cm) may be more responsive to CC effects (e.g. Kaspar et al. 2006, Moore et al. 2014, Atwood and Wood 2020), deeper depths may be more important when considering the soil’s contribution to the crop’s water supply (Asbjornsen et al 2008, Williams et al. 2008, Rizzo et al. 2018).

Lastly, to our knowledge the causal relationships between CCs and soil water storage have not been explicitly presented. Causal models can help identify data needs, and when sufficient data is available can be used to construct structural equation models that estimate the relative strength of causal paths. It can also be used to frame hypotheses, resulting in more targeted research questions that directly test the presence or absence of causal links. Therefore, a proposed causal model can greatly aid in advancing future research.

Given the need to quantify long-term benefits of cover cropping, the scarcity of Midwest-specific data, and lack of a framework for organizing knowledge, the objectives of our study were to (1) determine what aspects of a soil’s hydrological profile are affected by long-term cover cropping at a depth relevant to crop production, and (2) use our findings to propose a causal model connecting CCs to changes in soil properties to aid in targeting future research. We collected soil samples at a 10-18 cm depth increment from four long-term (10+ years) no-till cover crop trials located in Iowa, USA. Two trials were on-farm production fields, and two trials were part of a larger research experiment. We assessed the effects of long-term cover cropping on soil water content at saturation, soil water content at matric potentials approximating field capacity (-100 cm H2O, Moore 2021), and pore-size distributions as estimated by the soil water retention curve shape. To complement and contextualize these data, we also measured soil texture, soil organic matter, and bulk densities of the soil samples. We used our results in combination with previous literature to construct a proposed causal model (Pearl 2008).

Materials and Methods

## *Site descriptions*

Three long-term sites were used for this study (**Figure 1**), with one site having two trials. Therefore, a total of four trials were sampled (**Table 1**). Each trial consisted of two treatments that had been in place for at least 10 years: (1) a maize/soybean rotation (either grain- or silage-based) with a winter rye CC planted yearly in the fall following cash crop harvest and terminated in the spring, and (2) the same rotation without a CC. Every trial was arranged in a randomized complete block design with four (West and East) or five (Central) replicates. The plots within each trial were managed identically save for the planting of the CC in the fall. The exact herbicide and nutrient programs varied by site, reflective of their particular managers and contexts (**supplementary material**). More detailed accounts of agronomic management have been published elsewhere for the research site (Moore et al., 2014) and production sites (Nichols et al. 2020). All sites had sub-surface tile drainage and were managed without tillage since initiation.

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| **Plot Size and Average Slope\*** | **Treatment Replications** | **Dominant Soil Types\*** | **Average Depth to Water Table\*** | **30-year Annual Mean** | |  | **Mean Cover Crop Biomass** | | **2018 Crop** | **2019 Sampling Date** |
| ***Air Temperature*** | ***Precipitation*** |  | ***5-year*** | ***10-year*** |
| m |  |  | cm | ⁰C | mm |  | Mg ha-1 | Mg ha-1 |  |  |
| *West-grain (commercial farm),* 41⁰55’N 94⁰36’W, initiated in 2008 | | | | | | | | | | |
| 25 x 250 (1-3%) | 4 | Nicollet loam | 67 | 9.5 | 880 |  | 0.24 | 0.45 | Soybean | May 23 |
| *Central-silage (research plots);* 42⁰00’N 93⁰48’W; initiated in 2002 | | | | | | | | | | |
| 3.8 x 55  (1-3%) | 5 | Clarion loam | 100 | 9.8 | 907 |  | 2.38 | 1.98 | Maize | June 6 |
| *Central-grain (research plots);* 42⁰00’N 93⁰48’W; initiated in 2009 | | | | | | | | | | |
| 3.8 x 55  (1-3%) | 5 | Clarion loam | 100 | 9.8 | 907 |  | 1.53 | 0.88 | Maize | June 6 |
| *East-grain (commercial farm);* 41⁰18’N 92⁰48’W; initiated in 2009 | | | | | | | | | | |
| 25 x 275  (0-2%) | 4 | Taintor silty clay loam | 0 | 10.2 | 947 |  | 1.73 | 1.32 | Maize | June 10 |

*\* From Web Soil Survey data, see supplementary material for field maps*

The West-grain and East-grain trials were production fields on commercial farms, and only one phase of the maize/soybean rotation was present each year. The Central site was a larger research study managed by the United States Department of Agriculture (USDA) and included both phases of each rotation (Kaspar et al., 2007, Kaspar et al. 2012). For the present study, only the soybean phase of the USDA site was sampled due to time constraints. Cover crop biomass sampling occurred each spring at every trial; details about methodology are reported elsewhere (Nichols et al. 2020) and historical values are available in supplementary material.

## *Soil Sampling*

An aluminum ring 7.62 cm in diameter and 7.62 cm tall was used to take intact soil samples. Sampling occurred in May or June of 2019 after maize (West) or soybean (East, Central-grain, Central-silage) emergence at each site. Sampling was conducted immediately following crop emergence to minimize the effects of live roots in the samples, and a few days following a rain to ensure the soil was fully drained, but wet enough to remain in the ring during sampling (Al-Shammary et al. 2018).

At all trials, samples were taken in the middle of the plots between planted rows. To get intact soil cores, a hole 10 cm deep was dug, and soil was smoothed by hand to create a flat area approximately 30 cm square. The ring was placed on the soil surface in the center of the flat area, a hollow metal cap was placed on it, and a 15 kg weight was used to evenly drive the ring into the undisturbed soil. Once the ring was fully inserted into the soil, a hole was dug around the ring. A flat sheet of metal was slid under the ring to extract it, and a knife was used to remove soil from the top and bottom of the ring using a Z-cutting motion. The ring was wrapped in aluminum foil with the soil orientation (top, bottom) marked. The foil-wrapped ring was then placed in an individual plastic container, then placed in a cooler. This process was repeated for each plot. Samples remained in the cooler for no more than four hours before being placed in a refrigerator.

### *Soil-water-retention curve*

## The equipment could accommodate 12 samples at a time, so each trial’s samples were run together in a batch. Our interest was in comparing relative effects within a trial, so variation between runs was experimentally included in variation between trials. The samples were measured in the order they were sampled. A given trial’s cores had cheesecloth taped to the bottom of each core and an additional ring taped to the top. The full batch of samples (eight for East and West, 10 for Central) was then placed in a vacuum chamber for at least 12 hours in a solution of 0.01 M CaCl2 filled to the top of the first ring, allowing the solution to move upward to saturate the soils with minimal air entrapment. The top ring was removed from the cores, then the saturated cores were weighed, then transferred to a custom-built pressure cell apparatus (Ankeny et al. 1992). Measurements were made according to the protocol described by Kool et al. 2019. Briefly, the cores were drained at atmospheric pressure for 12 hours to obtain a measurement for gravity-drained values (Ψ = -2.5 cm water). Subsequent measurements were taken at matric potentials (Ψ) of -10, -25, -50, -100, -200, and -500 cm water. The samples were then oven dried at 60 deg C for at least 48 hours, then weighed. Bulk densities were estimated by dividing the oven-dried weight of soil by the ring volume (347.5 cm3; Han et al. 2016). A water balance was constructed for each core individually as quality control (supplementary material), resulting in the remove of one replicate from the no-cover treatment of the Central-silage trial, which had a visibly large hole in the center of the core upon destructive inspection, confirming it’s justified removal from the dataset.

### *Soil Texture*

The oven-dried soil was ground and passed through a 2 mm sieve. Two teaspoons of soil from each core were used for soil texture measurements. Soil texture was measured using laser diffractometry (Miller and Schaetzel 2012) with a Malvern Mastersizer 3000 and a HydroEV attachment (Malvern Panalytical Ltd, UK), producing estimates for the percentage of the soil that was sand (50-2000 micrometers), silt (6-50 micrometers), and clay (0.1-6 micrometers).

***Organic carbon***

Half of the remaining oven-dried soil cores were sent for organic matter analysis (Agsource) using the loss-on-ignition method (Nelson et al. 1983). The loss in sample weight as a percentage of the total dry sample weight upon ignition was multiplied by 0.89 as an estimate of the percentage of sample weight that was organic matter. While this method may not produce reliable absolute estimates of organic matter (Hoogsteen et al. 2015), our interest was in pairwise comparisons of treatments rather than in obtaining absolute estimates of organic matter.

## *Statistical analysis*

All data manipulation, figure creation, and model fitting was done using R version 4.0.3 (R Core Team, 2020) and the tidyverse meta-package (Wickham et al. 2019). Non-linear models were fit using the nlraa (Miguez 2021) package functionality, with specific equation fits from the HydroMe (Omuto et al. 2021) and soilphysics (Da Silva and De Lima 2015) packages. Linear models were fit and summarized using the lme4 (Bates et al. 2015) package, which fits mixed effects models, and the emmeans (Lenth 2021) package which estimates both marginal and conditional means and confidence intervals and performs pairwise comparisons.

We fit the Gardner (Gardner 1958) and van Genutchen (van Genuchten 1980) models to describe the relationship between soil moisture and soil water matric potential in our datasets. We found the models produced similar Akaike’s Information Criteria (AIC; Sakamoto 1986) values, with the Gardner model showing a slightly better fit. We chose to use the results from the Gardner model due to its simplicity and biologically meaningful parameters. The Gardner equation is as follows:

Where θ is the volumetric moisture content at a given soil water potential ; the remaining variables are fitted parameters. θr and θs are the residual and saturated water contents, respectively, *a* is the inverse of the air-entry potential, and *n* is an index for the pore size distribution, with higher values indicating a wider distribution of pore sizes. Residual water contents (θr) are estimated by the model, but can also be experimentally measured at -15,000 cm water (SSA 2008). The highest presssure we used in this study was -500 cm water, which could lead to less stable model fits due to lack of an anchoring value (Groenevelt and Grant 2004). To determine whether the model produced reasonable estimates without these anchoring values, we compared the model-estimated saturated water contents with the data, as well as the pore-size distribution parameter estimate against values estimated using capillary rise equations which provides point-wise estimates of pore neck diameters (CITE, Britt what would you cite?). We fit the Gardner model to each experimental unit, then analyzed the air-entry and pore-size distribution parameters as response variables, described below. For capillary rise equations, we assumed the mean pore neck diameter (in cm) of drained pores at a govem ssure is equal to 0.3 divided by the head pressure in cm water. Pores with mean neck diameters greater than 30 µm were considered macropores. The percent macropores was assessed as a response variable, described below.

Volumetric water contents at saturation were extracted directly from the data. Volumetric water contents at field capacity were estimated as the volumetric water content at a matric potential of -100 cm water (Moore 2021). We used this approximation because the true field capacity matric potential will depend on the distance to the water table. The trials sampled all had artificial tile drainage installed at ~1.2 meter depths and shallow water tables (Table 1), meaning field capacity will be at matric potentials less than the commonly assumed -330 cm water (Bonfante et al. 2020), and -100 is a better approximation for field capacity in Midwestern soils with shallow water tables (Moore 2021).

The effects of trial, CC treatment, and their interaction on response variables (soil texture, organic matter, water contents at saturation and field capacity, air-entry pressures, pore-size distribution index, percent macropores) were assessed using mixed-effect models. Trial, CC treatment, and their interaction were included as fixed effects. Percent sand was investigated as a covariate in appropriate models. Models without a sand covariate had random intercept effects for replicates nested within location (East, Central, West), and models that included a sand covariate had fixed effects only. Models were compared using AIC. When inclusion of the sand covariate changed interpretations, both results are reported.

Results and Discussion

## *Soil texture, organic matter, and bulk density*

All plots had textures within ranges classified as silty-clay-loams. Texture varied most strongly by trial, with the East-grain trial having the lowest amount of sand and highest silt component. In the two production field trials, the sample’s texture also varied by CC treatment. The CC plots had a significantly higher sand component, and significantly lower clay component than the no-cover plots in the West-grain and East-grain trials (**Table 1**, **Fig. 2**). While the plots in the production fields were randomly assigned a CC treatment, the East-grain site’s treatments were regularly alternating strips with blocks laid out laterally, and the West-grain sites were close to regular alternations likewise laid out laterally. In fields with a uniform texture gradient perpendicular to the blocking with only two treatments, this regularly alternating pattern could result in one treatment having significantly different textures compared to the other. The Central site had several treatments in small plots and the blocks were located in quadrants within the field, which may have better randomized spatial patterns in the soil.

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| **Figure 2.** Soil texture components varied by trial and cover crop treatment, with the cover cropped plots having significantly more sand (bolded orange color) and significantly less clay at the West-grain and East-grain trials (both commercial production fields). |

Based on these results, sand was investigated as a covariate in statistical models for response variables thought to be affected by soil texture.

Organic matter values ranged from 1.8 to 4.6%. It is feasible that soils with more sand may not accumulate organic matter as easily compared to soils with less sand (and therefore more clay), due to the high surface area and ionic charges associated with clays. The organic matter models were very sensitive to inclusion of sand as a covariate. The two trials with significantly different sand components in the cover crop and winter fallow treatments had lower organic matter in the cover crop treatments without a sand-correction, but higher organic matter with a sand correction. We therefore choose not to report the results from the organic matter analyses, but provide the results in supplementary material.

Bulk densities varied from 1.2 g cm-3 to 1.7 g cm-3. The large size of sand particles reduces packing efficiencies compared with clay, meaning a sandy soil may have lower bulk densities simply due to packing arrangements. However, regardless of the statistical model fit, all estimated CC effects were less than measurement precision of the core method for measuring bulk density (±0.12 g cm-3, Han et al. 2016), rendering their interpretation questionable. A recent summary of research on the effects of CCs on bulk densities likewise demsonstates few studies where an overwintering rye CC grown in the US reduced bulk densities more than typical measurement errors (Haruna et al. 2020).

## Soil hydrological properties

Due to the high amount of variability associated with soils, we assigned significance at p-values less than 0.10. With or without a sand correction, no trial exhibited a significant increase in water held at saturation with the use of cover cropping (**Figure 3**). Water held at saturation is largely dependent on bulk density, so these findings are consistent with the lack of meaningful effect of CCs on bulk densities in our study.

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| **Figure 3**. Soil volumetric water contents at saturation (top) and field capacity (-100 cm water, bottom) with 10+ years of winter rye cover cropping (green) or winter fallow (brown) in a maize-soybean rotation at four trials. Points are estimated means, line ranges are the 95% confidence intervals around the mean. Bold points include an adjustment for the percent sand in the sample, stars indicate signficant differences at p<0.10 with the sand adjustment. Note the different y-axes scales for ease of viewing. |

Field capacities were signifiantly higher in the cover cropped plots at both the West-grain (p = 0.07) and Central-silage (p = 0.05) trials. At the West-grain trial, the soil water at field capacity was increased (after sand correction) from 33.6 to 36.0%, and at the Central-silage trial from 38.1 to 40.6%, respectively. While the West-grain results were not significant without a sand correction, the trial exhibited the same trend of increased water at field capacity with CCing regardless of whether the sand correction was included or not. These results suggest CCs may have a larger impact on water at field capacity compared to water held at saturation at the 10-18 cm soil depth increment. To our knowledge there limited studies examining the potential for CCs to reduce flood damage in the Midwest, but the few that do only account for the increased evapotransipiration with the use of CCs (Antolini et al. 2020). An increase in the soil’s ability to hold water after gravity drainage may also contribute to peak water flow regulation. By increasing water content at field capacity by 2%, CC fields could hold an additional XX L of water in each hectare (need to do those calcs), which could…(need to compare to discharge rates to see if this is actually a meaningful amount).

The Gardner equation fit converged for all experimental units (**Figure 4**), with *a* (inverse of air-entry potential) ranging from 0.001 to 0.284, and *n* (pore-size distribution index) ranging from 0.45 to 1.49 (supplemental material). While the parameters did differ by trial, cover cropping did not significantly affect either parameter in any trial.

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| **Figure 4.** The Gardner equation was fit to each experimental unit, with four or five replicates for each cover crop treatment |

Manual estimation of the pore-size distribution confirmed CCs did not increase the percentage of macropores in any trial (**Fig. 5**). In fact, in the West-grain trial the number of macropores significantly decreased 61% macropores in the control to 48% in the CC treatment. In a review of published studies, authors found the effect of CCs on macropore makeup of the soil highly variable, but with an average increase (Haruna et al. 2020). The wide variation may be partially due to varying cut-offs in pore sizes for macropore categorization, but the variability in combination with our results again demonstrates using literature averages to predict CC impacts in Midwestern systems may be overly optimistic.

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| **Figure 5.** Repliacte percentage (points) and mean percentage (bars) macropores (>30 um) for each trial; percent macropores in the cover crop treatment was significantly lower (p=0.04) at the West-grain trial compared to the control treatment. |

## *Causal model*

There are several pathways by which CCs may affect a soil’s capacity to hold water (**Fig. 6, Table 2**).

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| **Figure 6**. Pathways by which cover crops may affect the pore size distributions, the amount of water stored at field capacity, and the amount of water at saturation in no-till systems. The effects of soil erosion are not included. |

The causal model was built using literature relevant to Midwestern systems, and when applicable was limited to evidence drawn from research conducted with a cereal rye cover crop (Table 2).

Table 2.

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| Casual Arrow | Causal relationship | Citation(s) |
| 1 | CC below-ground biomass affects soil biology | Leslie et al. 2017, Villamil et al. 2020 |
| 2 | CC above-ground biomass affects soil organic matter | Austin et al. 2017 |
| 3 | CC below-ground biomass affects soil organic matter, more strongly than above-ground biomass | Austin et al. 2017 |
| 4 | Soil biology affects soil organic matter cycling | Cotrufo et al. 2013 |
| 5 | CC below-ground biomass (root exudates) affect soil aggregation | Austin et al. 2017 |
| 6 | Soil biology affects soil aggregation | Cotrufo et al. 2013 |
| 7 | Soil organic matter affects soil aggregation | Kay et al. 1997, Abiven et al. 2009, Boyle et al. 2013 |
| 8 | Root channels affect soil porosity | Williams and Weil 2004, Ogilvie et al. 2021 |
| 9 | Soil biology (e.g. worm activity) affects soil porosity | Edwards et al. (1988) |
| 10 | Soil organic matter affects bulk density, which affects soil porosity | Ruehlmann and Korschens (2009) |
| 11 | Soil aggregation affects pore sizes | Boyle et al. 2013 |
| 12 | Soil water at saturation is affected by soil porosity |  |
| 13 | Pore size distributions affect capillary forces, which affect soil water at field capacity |  |
| 14 | Soil porosity affects space available for water which affects soil water at field capacity |  |

Causal diagrams such as **Fig. 6** are the basis for constructing structural equation models, which can greatly enhance researcher’s ability to address complex research questions in agriculture (Smith et al. 2014, Wade et al. 2020). For example, in our proposed causal diagram, below-ground biomass measurements are necessary for estimating direct effects of cover cropping on pore size distributions, field capacity, and saturation. This is further supported by recent studies and reviews that identify CC roots as being a crucial component to understanding CC effects on soil properties (Williams and Weil 2004, Haruna et al. 2020, Ogilvie et al. 2021). While there is limited data available on both above- and below-ground biomass of CCs, data collected over the period of five years in Iowa show no relationship between above- and below-ground rye biomass, with root-to-shoot ratios varying from 0.16-1.94 at similar aboveground biomass productions (Martinez-Feria et al. 2016). Therefore, above-ground biomass production cannot be used as a proxy for below-ground production with much confidence, and studies that pair above- and below-ground CC biomass with soil measurements would be advantageous in enhancing our understanding of CC effects on soil.

Conclusions

Context-specific effects of growing over-wintering CCs need to be explicitly investigated, as the constraints of the Midwestern maize-soybean system may render the effects smaller in magnitude compared to averages reported by global meta-analyses. In our study we found CCs have a more pronounced effect on water held at field capacity compared to water contents at saturation, suggesting changes in pore networks and sizes are stronger compared to decreases in bulk density. However, we found CCs increased water held at field capacity in only two of the four trials, further demonstrating the need to understand causal mechanisms in order to better predict where and when CCs will provide the most benefit.

Acknowledgments

Please list any acknowledgments here.

Supplemental Material

S1. Detailed management of trials

SX. Statistical results

SX. Soil water retention curve data

SX. Bulk density data

SX. Organic matter and texture data

SX.

Optional Sections

All data and code are available on a github repository at XX. Additionally, the data is available as a downloadable csv file in supplementary material.

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