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# A large subsoil carbon sink in the United States Corn Belt

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November 30, 2022

### Abstract

Current soil C inventories focus on surface layers although over half of soil C is found below 20 cm. Recent and ongoing changes in agricultural management, crop productivity, and climate in Midwest US cropland may influence subsoil C stocks. The objectives of this study were to determine how surface soil and subsoil organic C stocks have changed in croplands of Iowa and Illinois and to evaluate mechanisms to explain the observed subsoil organic C changes. Using resampling studies from Iowa and Illinois, we found that subsoil (20-80 cm) organic C increased at a rate of 0.31 Mg C ha-1 yr-1 between the 1950s and early 2000s despite C losses of similar magnitude in the top 20 cm (0.26 Mg C ha-1 yr-1). Based on this analysis, we estimated a subsoil C storage rate of up to 11.8 Tg C yr-1 for Iowa and Illinois, which equates to 12% of annual US greenhouse gas emissions from crop cultivation if surface C losses and non-CO2 greenhouse gases are controlled. We also measured changes in soil organic C stocks from two long-term cropping systems experiments located in Iowa, which demonstrated similar rates of subsoil C changes for both historical and contemporary crop rotations. Using publicly available crop yield data, we determined that changes in crop productivity likely contributed minorly to observed changes in subsoil organic C. The accumulation of organic C in subsoils may be attributed to regional climate change, which has led to greater precipitation and wetter subsoils that inhibit transformation of soil organic C to CO2. Because farmers may respond to increasing soil wetness by expanding and intensifying artificial drainage infrastructure, there is an urgent need to further assess subsoil C stocks and their vulnerability to drainage system changes.

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## ABSTRACT

Current soil C inventories focus on surface layers although over half of soil C is found below 20 cm. Recent and ongoing changes in agricultural management, crop productivity, and climate in Midwest US cropland may influence subsoil C stocks. The objectives of this study were to determine how surface soil and subsoil organic C stocks have changed in croplands of Iowa and Illinois and to evaluate mechanisms to explain the observed subsoil organic C changes. Using resampling studies from Iowa and Illinois, we found that subsoil (20-80 cm) organic C increased at a rate of 0.31 Mg C $ha^{-1}$ $yr^{-1}$ between the 1950s and early 2000s despite C losses of similar magnitude in the top 20 cm (0.26 Mg C $ha^{-1}$ $yr^{-1}$). Based on this analysis, we estimated a subsoil C storage rate of up to 11.8 Tg C $yr^{-1}$ for Iowa and Illinois, which equates to 12% of annual US greenhouse gas emissions from crop cultivation if surface C losses and non-$CO_2$ greenhouse gases are controlled. We also measured changes in soil organic C stocks from two long-term cropping systems experiments located in Iowa, which demonstrated similar rates of subsoil C changes for both historical and contemporary crop rotations. Using publicly available crop yield data, we determined that changes in crop productivity likely contributed minorly to observed changes in subsoil organic C. The accumulation of organic C in subsoils may be attributed to regional climate change, which has led to greater precipitation and wetter subsoils that inhibit transformation of soil organic C to $CO_2$.  Because farmers may respond to increasing soil wetness by expanding and intensifying artificial drainage infrastructure, there is an urgent need to further assess subsoil C stocks and their vulnerability to drainage system changes.


## INTRODUCTION

Humans have transformed one third to one half of Earth's land surface through agriculture (Goldewijk, Beusen, & Drecht, 2011), resulting in the loss of approximately 133 Pg C from the top 2 m of soil (Sanderman, Hengl, & Fiske, 2017). Crop production typically decreases soil organic C stocks through a reduction in C inputs due to the harvest of crop material, stimulation of C mineralization due to tillage and artificial drainage, and through erosion or loss of soil structure (Davidson & Ackerman, 1993; Guo, L.B., Gifford, 2002; Smith, 2008). Because soil organic C represents an important source and sink of greenhouse gas emissions and is critical to soil health, management practices that increase soil organic C have been proposed as a "win-win" strategy for climate change and food security (Minasny et al., 2017; Paustian et al., 2016).

Soils of the Midwest US lost 30 to 50% of their native C stocks after conversion of grassland to cropland in the 19$^{th}$ century (David, McIsaac, Darmody, & Omonode, 2009; Robertson, Paul, & Harwood, 2000). Since the mid-20$^{th}$ century, Midwest cropping systems transitioned from diverse rotations of grain and perennial forage crops to an annual two-crop rotation of maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] with higher rates of N fertilizer inputs and less tillage (Karlen, Dinnes, & Singer, 2010). At the same time, improvements in crop genetics and management increased crop productivity and residue inputs to the soil (Hicke & Lobell, 2004) and climate change increased the amount and intensity of precipitation (Angel et al., 2018; Basso, Martinez-Feria, Rill, & Ritchie, 2021). Recent regional assessments indicate that surface soil organic C stocks have reached a new steady-state or are increasing where soil conservation practices have been adopted (United States Environmental Protection Agency, 2020). However, potential responses of subsoil organic C stocks to changes in crop rotation, crop productivity, and climate remain unknown.

Our objectives were to determine how surface soil and subsoil organic C stocks have changed in croplands of Iowa and Illinois and to evaluate mechanisms to explain the observed subsoil organic C changes. We extracted historic and modern soil organic C data from 39 soil profiles in Iowa and Illinois cropland that were reported in published studies (David et al., 2009; Veenstra & Burras, 2015). In addition, we measured changes in soil organic C stocks from two well controlled, long-term cropping systems experiments located in Iowa. These long-term experiments allowed us to determine the effect of cropping system conversion by comparing changes in soil organic C stocks in historical diverse rotations of grain and perennial forage crops with the contemporary annual two-crop rotation of maize and soybean. Furthermore, we used publicly available crop yield and weather data to estimate the impacts of changing C inputs and a wetter climate on subsoil organic C stocks. Finally, we estimated change in subsoil C stocks for land area in Iowa and Illinois.

## MATERIALS AND METHODS

### Published observational studies

We compiled historical and modern soil organic C concentrations and bulk density data from observational studies to determine the net effects of agricultural management trends and climate changes on soil organic C stocks (i.e. the mass of C per unit area for a given soil mass increment) between the 1950s and early 2000s in Iowa and Illinois. We extracted the historical data from USDA Soil Conservation Service Survey Descriptions (Soil Conservation Service, 1966a, 1968, 1978) and the modern data from two peer-reviewed journal articles – David et al. (2009) and Veenstra & Burras (2015). We used these articles because they reported soil organic C stocks in surface and subsoil layers (extending to at least 100 cm depth) in cropland of the Midwest US (Figure 1). Both articles also relied on the data published by the USDA Soil

Conservation Service to calculate changes in soil organic C stocks over time, but we used the original USDA data rather than what was presented in the articles to better control for missing data.

David et al. (2009) sampled soils by depth (0-100 cm) in 2001 and 2002 at 19 sites in central Illinois that were previously sampled in the early 1900s or 1957 or were paired with remant prairies. We used soil organic C concentrations and bulk density data from the ten locations that were initially sampled in 1957 by the USDA Soil Conservation Service and resampled in 2002. The 2002 sampling was performd using soil cores of 3.2 cm diameter. We extracted the 2002 data from the 2011 errata rather than the original 2009 publication (David, McIsaac, Darmody, & Omonode, 2011). The sites were in either grass or annual crop production in 1957 and predominantly maize-soybean rotations in 2002. All of the sites were classified as poorly drained, were tile drained, and had no known history of manure application in recent decades. Veenstra and Burras (2015) sampled soils by depth (0-150 cm) in 2007 at 82 sites in Iowa that were previously sampled between 1943 and 1963. We used soil organic C concentrations and bulk density data from the 29 sites that had these data for multiple depths from both the initial and resampling events. The 2007 sampling was performed using soil cores of 7 cm diameter. The site-specific data from 2007 were not presented in the original publication, but were provided by the authors. The sites were in either annual crop production or perennial forages at the initial sampling time in 1956-1961 and annual row crop production in 2007. Of the 29 sites from Veenstra and Burras (2015) that were used in our analysis, five were classified as poorly drained, nine as somewhat poorly drained, eight as moderately well drained, and seven as well drained. Site information is provided in Table S1.

The initial soil descriptions reported in the USDA Soil Conservation Service Survey Descriptions were generated using excavated soil pits, with samples collected from the pits. Bulk density was measured either using the core method or clod method (Soil Conservation Service, 1966b). When the clod method was used, we extracted data for which soil volume was determined at field moisture. For all profiles used in this analysis, the initial bulk density data were available for about half of all horizons in the USDA Soil Conservation Service Survey Descriptions. We used a Random Forest algorithm (Liaw & Wiener, 2002) to predict missing bulk density using profile ID, soil series, master horizon, depth increment midpoint, depth increment length, soil organic C concentration, clay percentage, sand percentage, calcium carbonate equivalent, drainage class, and bulk density method reported in the descriptions as predictor variables following an approach similar to (Sequeira, Wills, Seybold, & West, 2014). Two thirds of the horizons with available bulk density data were used for model calibration and one third were used for model validation (Figure S1). The root mean squared error calculated using the validation data was 0.079 g $cm^{-3}$. In addition, 17 of the 39 resampled profiles were missing bulk density values for one to four depth increments. Because few (8%) of all depth increments from the resampled profiles were missing bulk density data, we estimated the missing data as the average of the bulk density values from increments directly above and below the increments that were missing data in the same profile. Measured and estimated bulk density values are presented in Table S2.

Soil organic C concentrations reported in the USDA Soil Conservation Service Survey Descriptions were determined using the Walkley-Black (1934) method (Soil Conservation Service, 1966b), while the resampled C concentrations from the David et al. (2009) and Veenstra and Burras (2015) studies were determined using dry combustion. David et al. (2009) removed inorganic C using acid fumigation while Veenstra and Burras (2015) excluded samples that contained inorganic C from their dataset of soil organic C changes. Both studies addressed possible bias caused by differences in analytical methods between the initial and resampled soils by performing an adjustment to Walkley-Black C results if needed. Measured and corrected soil organic C concentrations are presented in Table S2.

To scale soil organic C stocks from both sampling events at all sites to the same reference masses, we used an equivalent soil mass method (Wendt & Hauser, 2013). For the initial and resampling events, bulk density and depth increment length were used to calculate the soil mass of each layer. The soil organic C stock for that layer was then calculated as the product of the soil organic C concentration and the soil mass. The cumulative soil masses and soil organic C stocks for each profile were fitted using a cubic spline function, which was used to predict the soil organic C stocks at reference masses (Wendt & Hauser, 2013). Plots of cumulative soil organic C with cumulative soil mass for each profile are presented in Figure S2.

The reference masses were selected to represent 20, 40, 60 and 80 cm deep based on the relationship between cumulative depth and cumulative soil mass from all sites and sampling events (Figure S3). We considered below 20 cm to be subsoil, reflecting that the transition from the A to B horizon occurred between 20 and 40 cm deep for the majority (67%) of profiles in the observational dataset according to the USDA Soil Conservation Service Survey Descriptions (Table S2). The annual changes in soil organic C stocks were then calculated by site for these reference masses, using the exact number of years between the initial and resampling events to annualize soil organic C changes for each site.

**Long-term experiments**

We conducted soil sampling at two long-term experimental sites to measure the change in soil organic C stocks (0-100 cm) from 2002 to 2014 and test the effect of historical changes in the dominant cropping system and C inputs on change in soil organic C stocks over this time period. The Kanawha study was established in 1954 in northern Iowa (42°94' N, 93°17'W), and the Nashua study was established in 1979 in northeast Iowa (42°95' N, 92°54' W). Both experiments include a maize-maize-oats (*Avena sativa* L.)/alfalfa (*Medicago sativa* L.)/alfalfa crop rotation that was representative of the US Corn Belt prior to the 1980s as well as the contemporary maize-soybean crop rotation that has dominated the region since that time.

The experimental design at both locations is a split-plot randomized complete block with two (Kanawha) or three (Nashua) replicate blocks. The main plot is crop rotation, with all phases of each rotation represented in all years. Main plots are subdivided to accommodate four N fertilization treatments (0, 90, 180, and 270 kg N $ha^{-1}$) applied to maize. Both experiments are situated on Iowa State University Research and Demonstration Farms. Mean annual precipitation is 818 and 884 mm (1985-2014) and mean annual temperature is 8.03 and 8.37°C for Kanawha and Nashua, respectively. Soils at Kanawha are classified as Typic Endoaquolls (Webster series), whereas soils at Nashua are predominantly Typic Hapludolls (Kenyon series), with a smaller area Aquic Hapludolls (Readlyn series) according to the USDA soil taxonomic system (Soil Survey Staff, 2018).

Soil samples were collected from two crop rotations within the long-term experiments: a two-year maize-soybean rotation and a four-year maize-maize-oats/alfalfa-alfalfa rotation. In the 4-yr rotation, oats are used as a nurse crop to establish the alfalfa in the third year of the rotation. At both locations, maize, soybean, and oats are harvested for grain, and alfalfa hay is harvested by three or four cuttings the year after establishment. We sampled the plots receiving 180 kg N ha$^{-1}$ yr$^{-1}$ during each maize phase. This N rate treatment has been in place since experimental establishment at Nashua and since 1984 at Kanawha (between 1954 and 1984, this treatment received 136 kg N ha$^{-1}$ yr$^{-1}$ to maize). At both sites, the cropping systems are rain-fed and drained with subsoil perforated pipes. Additional information about cropping systems, soil management, N fertilization, and site characteristics have been published previously (Poffenbarger et al., 2020; Russell, Laird, Parkin, & Mallarino, 2005).

Soil sampling took place immediately following maize harvest and before tillage in 2002 and 2014. For the four-year rotation, soil sampling was conducted at the end of the first maize phase of the rotation. The length of time between sampling events was such that the plots sampled in 2002 were in the same phase of the rotation in 2014. At both sampling times, six 4.1-cm diameter cores were collected per plot using a hydraulic soil probe. The cores were taken at points randomly selected within a plot stratified by position: in the row, the midpoint between rows, and halfway between these two positions. In 2002, the cores were taken to a depth of 100 cm and split into 0-5, 5-15, 15-30, 30-50, 50-75, and 75-100 cm depth increments. In 2014, the cores were taken to a depth of 100 cm and split into 0-15, 15-30, 30-60, 60-90, and 90-100 cm depth increments. The segments from the six cores within each plot were composited to form six or five samples per plot.

The samples were air-dried, roots were removed, rock masses and volumes were determined, and soil was passed through a 2-mm sieve. A portion of each air-dried sample was finely ground for determination of C and N concentrations by dry combustion. The analysis of 2002 samples was originally performed using a Carlo-Erba NA 1500 NSC elemental analyzer (Buchler Instruments, Paterson, NJ), but we re-analyzed the archived samples from 2002 along with 2014 samples using a Vario Max CN analyzer, (Elementar Americas, Mt. Laurel, NJ).

Carbonates were determined in the 2002 samples using the modified pressure calcimeter method (Sherrod, Dunn, Peterson, & Kolberg, 2002), and in the 2014 samples by analyzing samples with and without acid fumigation pre-treatment (Harris, Horwa, & Kessel, 2001). The concentration of soil inorganic C was subtracted from total C to calculate the soil organic C concentration of each sample.

Soil organic C changes between 2002 and 2014 at Kanawha and Nashua were determined on an equivalent soil mass basis as described for the observational studies, using the same reference masses. The bulk density and soil organic C concentrations from the experimental dataset are presented in Table S3, and plots of cumulative soil organic C stock with cumulative soil mass for each profile are presented in Figure S4.

**Carbon input change**

Average annual above- and below-ground C inputs to the soil in Iowa and Illinois were estimated for years corresponding to approximate soil sampling times derived from the observational studies (David et al., 2009; Veenstra & Burras, 2015) (1950, 2000). The three-year average maize and soybean yields for Iowa and Illinois centered around 1950 and 2000 were collected from USDA NASS (National Agricultural Statistics Service, 2020) (Table S4) and used to calculate C inputs to the soil using the method described by Bolinder et al. (Bolinder, Janzen, Gregorich, Angers, & VandenBygaart, 2007). In addition, crop yield records were available for each rotation at both experimental sites in Iowa from 2003-2014 and we used the same approach (Bolinder et al., 2007) to transform yield data into average annual C inputs. Briefly, aboveground biomass was calculated using dry matter yields and the harvest index; belowground biomass was calculated using a shoot:root ratio, and rhizodeposition was calculated as a factor relative to root biomass. The harvested product was subtracted from total dry matter and then dry matter was converted to C input assuming plant biomass contains 45% C. For 1950 estimates, we replaced modern Harvest Index (proportion of total aboveground biomass in grain) values of 0.50 and 0.40 for maize and soybean with 0.35 and 0.30, respectively according to historic allometric relationships (Allmaras, Wilkins, Burnside, & Mulla, 1998). We assumed the root:shoot ratio and depth distribution of crop roots remained constant over time because previous studies on root changes due to breeding in US germplasm do not indicate that root allocation has increased with yields over time (Fried, Narayanan, & Fallen, 2019; Messina et al., 2020; Reyes et al., 2018; Schmidt, Poret-Peterson, Lowry, & Gaudin, 2020).

Average annual C inputs were apportioned into 0-20, 20-40, 40-60, and 60-80 cm depth increments. The 0-20 cm depth increment included aboveground inputs and belowground inputs allocated to 0-20 cm based on root distributions presented in (Fan, McConkey, Wang, & Janzen, 2016). The 20-40, 40-60, and 60-80 cm increments included only belowground inputs allocated to each depth according to Fan, McConkey, Wang & Janzen (2016). Because most observations in Bolinder et al. (2007) were from samples collected to 40-50 cm, we used root distributions from Fan et al. (2016) to calculate additional C inputs below 40 cm. Cumulative C inputs between 1950 and 2000 were calculated assuming a linear increase in C inputs over this time, based on the linear increase in yields over this time (Egli, 2008). The cumulative C input gain due to yield increase was calculated as the difference between cumulative C inputs from 1950-2000 and 50 years of C inputs at a constant 1950 yield level.

**Weather**

Historical precipitation, vapor pressure deficit (VPD), and soil water content data were compiled from weather stations located within or immediately surrounding Iowa and Illinois (Figure 1). We used weather stations outside of Iowa and Illinois due to the limited number of weather stations that provided moisture content to 100 cm depth. Historical data on VPD were obtained from the Automated Surface Observing System Network (National Weather Service, 2020a). Precipitation data were collected from the National Weather Service Cooperative Observer Program (National Weather Service, 2020b). Both of these networks were accessed through the Iowa Environmental Mesonet (Iowa State University, 2021). The weather stations for precipitation and VPD were chosen based on close proximity to weather stations reporting soil volumetric moisture content. Soil volumetric moisture content data were obtained from the Soil Climate Analysis Network (Natural Resources Conservation Service, 2020). Soil moisture data were available for five depths, but we only analyzed data collected at 10 cm, 50 cm, and 100 cm because they corresponded best to the depth increments used for soil organic C stock changes. The 10 cm and 50 cm depths represent the midpoints of 0-20 and 40-60 cm increments used for soil organic C stock changes, while the 100 cm depth is deeper than the deepest depth analyzed for soil organic C stock changes. In addition, water table depths from 1988 through 2016 were collected from a monitoring well installed in a grassed area at the Kanawha research farm. Water table depths were also simulated for the maize-soybean rotation at the Kanawha and Nashua sites using the Agricultural Production Systems Simulator (Holzworth et al., 2014) for 1981 through 2019 as described in (Archontoulis et al., 2020). Cumulative annual precipitation, VPD, soil moisture, and water table depth were summarized by water year (October 1 – September 30).

**Calculations and statistical analysis**

For the observational dataset, a linear mixed effect model was constructed for each mass increment with soil organic C stock as the response variable, year sampled as the fixed effect, and site as a random effect (Bates, Mächler, Bolker, & Walker, 2015). In addition, we estimated a mean annual change in soil organic C stock across sites for each mass increment. The analysis of soil organic C stocks and average annual soil organic C stock changes were done separately for each mass increment to avoid obscuring significant changes in specific layers by the high variability of soil organic C stocks in the entire profile (Kravchenko & Robertson, 2011). In addition, some sites were missing data for deeper layers and were excluded from the analysis of those mass increments. A profile 95% confidence interval of annual change in soil organic C stock was calculated for each mass increment to test whether the change in average annual soil organic C stock was significantly different from zero. To determine how soil organic C stock changes were influenced by soil drainage class, a linear fixed effect model was constructed with average annual change in soil organic C stock as the response variable and drainage class as a fixed effect. The drainage class for each site was derived from the USDA Soil Conservation Service Survey Descriptions.

For the experimental dataset, a linear mixed effect model was constructed for each mass increment with soil organic C stock as the response variable, the interaction of crop rotation by year sampled as the fixed effect, and block within site (Kanawha or Nashua) as a random effect. For the analysis of average annual change in soil organic C stock, two models with the same random effect structure (block within site) were run – one with only an intercept as a fixed effect to determine the mean across rotations, and the other with crop rotation as a fixed effect. Carbon inputs from the experimental dataset were analyzed by depth increment using a linear mixed effect model, which included a crop rotation by year interaction as the fixed effect and site as a random effect.

For precipitation, VPD, and soil moisture, data from each weather station were first plotted individually (Figures S5-S7). The data from all weather stations were truncated to a common initial year in which data were available for the majority of monitored locations. A linear mixed-effect model was constructed to analyze the pooled precipitation and VPD data from all weather stations, with water year as a fixed effect and weather station as a random effect. The same approach was used for the analysis of soil moisture, except that the data were subset by depth prior to fitting the regression models. For water table depth, Kanawha and Nashua simulated data were analyzed separately, with water year as a fixed effect in the regression models.

We estimated the water-filled pore space based on soil moisture data in each year of record. First, total pore space fraction was calculated using estimated soil bulk densities at depths corresponding to the soil moisture data. Specifically, we used the relationship between cumulative depth and cumulative soil mass across all sites and sampling events (Figure S3) to estimate bulk densities for 10-cm increments centered on the depth of soil moisture measurements (5-15, 45-55, and 95-105 cm). Pore space fraction was calculated as: 1 – (bulk density/particle density), assuming a particle density of 2.65 Mg $m^{-3}$. We assumed that bulk density remained constant among sites and over the duration of soil moisture measurements. The water-filled pore spaces for different soil moisture levels in Figure 5C were calculated as the volumetric soil moisture fraction divided by total pore space fraction.

Normal distribution of residuals and homogeneity of variances were verified for statistical models by examining normal probability plots and residuals vs. fitted values. For all models, analysis of variance was used to determine the significance of fixed effects. The emmeans() function was used to calculate confidence intervals and make comparisons among levels of the fixed factors (Lenth, 2021). In all mixed-effects models, degrees of freedom were estimated using the Satterthwaite method. Effects were considered significant at the p=0.05

level. Statistics were performed using R, version 4.1.0 (R Core Team, 2021). Plotting was performed using 'ggplot2' (Wickham, 2016).

**Estimation of regional soil organic C stock changes**

Using the analysis of soil organic C stock change by mass increment for each drainage class from the observational dataset, we estimated rates of soil organic C stock changes within the entire region of Iowa and Illinois. The average annual rate of soil organic C stock change for each mass increment in each drainage class was multiplied by the total land area mapped in that drainage class in the two states. The aerial extent of each drainage class in each state was calculated based on the 10 x 10 m resolution gSSURGO maps (Soil Survey Staff, 2020a, 2020b).

We estimated the uncertainty in regional estimates of soil organic C stock changes using a Bayesian approach. Posterior distributions of average annual soil organic C stock changes by drainage class for each mass increment were obtained by refitting the linear fixed-effect model in a Bayesian framework using the stan_glm function in R package rstanarm version 2.21.1 (Goodrich, Gabry, Ali, & Brilleman, 2020). In this model, the response variable was average annual change in soil organic C stock and the explanatory variable was drainage class; no intercept was included. Each mass increment was analyzed separately. A default normal prior distribution for the drainage class parameter was used. The default prior distribution is considered weakly informative to provide moderate regularization and stabilize computation (Gabry & Goodrich, 2020). We found that the parameter estimates of the linear fixed-effect models in a Bayesian framework agreed well with the same model in a frequentist framework, indicating that the default priors were relatively non-informative. Four parallel chains were fit with a burn-in of 5,000 samples then retaining the next 5,000 samples to give a total of 20,000 posterior samples. The number of chains and proportion of burn-in samples were selected based on the default settings for the rstanarm package, but we used 10,000 rather than the default 2,000 iterations per chain to ensure a large effective sample size and stable estimates (Gelman et al., 2020), consistent with (Correndo et al., 2021). Trace plots and Gelman-Rubin statistics indicated no issues with convergence. The posterior distribution of average annual change in soil organic C stock for each drainage class and mass increment was multiplied by the land area of the drainage class and then summed across drainage classes and mass increments to determine the regional soil organic C stock change. The estimates were retrieved as the median ($50^{th}$ percentile) of the posterior distributions, while the uncertainty of each estimate was obtained as the length of the 95%-credible intervals ($2.5^{th}$ and $97.5^{th}$ percentile) from the posterior distributions. We compared the subsoil organic C stock change against the US EPA estimate of total US greenhouse gas emissions from 'Crop Cultivation', which are 360 million metric tons of $CO_2$ equivalents or approximately 98 Tg C (United States Environmental Protection Agency, 2020).

## RESULTS AND DISCUSSION

### Changes in soil organic C stocks

Across the two observational studies, which included measurements of soil organic C stocks from two sampling events at 39 sites, we observed a significant decrease in soil organic C in the 0-2,600 Mg $ha^{-1}$ increment and significant increases in the 2,600-5,500; 5,500-8,400; and 8,400-11,400 Mg $ha^{-1}$ increments ($p<0.05$; Figures 2A and 2B). Summed across the subsoil layers (mass increments of 2,600-11,400 Mg $ha^{-1}$, equal to approximately 20-80 cm) soil organic C increased at an average rate of 0.31 Mg C $ha^{-1}$ $yr^{-1}$ ($p=0.02$). In the topsoil (mass increment of 0-2,600 Mg $ha^{-1}$, equal to approximately 0-20 cm), soil organic C decreased at an average rate of 0.26 Mg C $ha^{-1}$ $yr^{-1}$ ($p<0.001$; Figure 2B). There was no significant change between the 1950s and early 2000s in the whole profile soil organic C stocks (Figure 2B). The proportion of whole-profile soil organic C found in the topsoil (0-2,600 Mg ha$^{-1}$) decreased from 0.51 in the 1950s to 0.43 in the 2000s ($p=0.002$; Figure 2C). When soil organic C stock changes were evaluated by drainage class, the poorly drained soils showed the greatest soil organic C losses in the surface layer and gains at depth, while soil organic C stock changes in the other drainage classes were largely not significant (Table 1).

**Changes in cropping system management**

The shift from maize, small grains, and alfalfa to maize and soybean between the initial and resampling events of the observational studies (1950s to 2000s) may have led to changes in the quantity or depth distribution of C inputs, which can impact soil organic C stocks (McDaniel, Tiemann, & Grandy, 2014). Hence, we examined the rates of C inputs and soil organic C stock changes for historical (maize-maize-oats/alfalfa-alfalfa; four-year rotation) versus current crop rotations (maize-soybean; two-year rotation) using the long-term experiments. We found that both rotations deposited similar amounts of C to the top 20 cm of the soil, but the four-year rotation added more C to the 20-40, 40-60, and 60-80 cm depth increments than the two-year rotation ($p<0.05$; Figure 3 and Table S5), suggesting that the transition from the historical four-year to modern two-year cropping system led to a decrease rather than increase in C inputs to the subsoil.

Despite differences in C inputs to the subsoil between rotations, crop rotation had no effect on the topsoil organic loss and subsoil organic C gain at the two long-term experiments between 2002 and 2014 (Figure 4A and B). Both rotations demonstrated a similar rate of surface soil organic C loss that averaged 0.20 Mg C ha$^{-1}$ yr$^{-1}$ between 0 and 2,600 Mg ha$^{-1}$ of soil ($p=0.04$), and subsoil gain averaging 0.22 Mg C ha$^{-1}$ yr$^{-1}$ between 8,400 and 11,400 Mg ha$^{-1}$ ($p<0.001$; Figure 4B). In addition, the proportion of soil organic C found in the topsoil decreased from 0.54 in 2002 to 0.49 in 2014 (p=0.007) with no evidence for differences between rotations (p=0.949; Figure 4C). Overall, the vertical pattern of soil organic C stock changes at the experimental sites aligned with the observational dataset. We did not find evidence for greater subsoil organic C gains in the modern two-year rotation, which corroborates other research showing that the maize-soybean does not increase subsoil C relative to a four-year rotation (Gregorich, Drury, & Baldock, 2001; Sanford et al., 2012).

During the period of C measurements in the observational studies, annual N fertilizer rates increased by about three-fold (United States Department Of Agriculture Economic Research Service, 2019). Nitrogen inputs can directly and indirectly impact soil organic C accumulation by increasing crop residue quantity (Brown et al., 2014) and by altering microbial community composition (Brown et al., 2014) and microbial physiology (Sinsabaugh, Manzoni, Moorhead, & Richter, 2013). A greater N supply in N-limited systems is expected to result in more efficient use of C by microbes and less $CO_2$ respired per unit of C input (Sinsabaugh et al., 2013). However, field-based research on the conversion of crop residue C to soil organic C has not demonstrated a clear effect of N fertilizer rate on C retention efficiency (Allmaras, Linden, & Clapp, 2004; Gregorich, Ellert, Drury, & Liang, 1996; Liang et al., 1998). Hence, the effect of increasing N fertilizer on microbial C use efficiency likely explains little of the soil organic C increase at depth.

In addition, during the period of C measurements in the observational studies, conservation tillage also increased in the region (Karlen et al., 2010), which can increase soil organic C stocks by decreasing the rate of C mineralization (West & Post, 2002). However, changes in soil organic C upon conversion to conservation tillage are typically observed in only surface soil layers and result in a shallower – rather than deeper – distribution of soil organic C

(Baker, Ochsner, Venterea, & Griffis, 2007). Moreover, tillage practices were not changed in the long-term experiments that demonstrated increasing subsoil organic C stocks. Thus, we conclude that changes in crop rotation, N fertilization rates, and tillage practices between the historical and modern soil sampling events explain little of the observed increase in subsoil organic C stocks.

**Changes in C inputs due to yield increases**

Over the timespan of the observational datasets (~1950-2000), the additional C inputs attributed to crop yield increase was 6.9 Mg C ha$^{-1}$ for the subsoil (20-40, 40-60 and 60-80 cm depths together) (Table 2), making up 44% of total change in soil organic C for the corresponding mass increment of 2,600-11,400 Mg ha$^{-1,}$ which was 15.7 Mg C ha$^{-1}$. However, only about 10% of the increased C inputs may be converted to soil organic C (Castellano, Mueller, Olk, Sawyer, & Six, 2015), such that only a small fraction of the observed subsoil organic C changes could be attributed to changes in direct C inputs to those soil layers. Moreover, C inputs estimated using yield data collected in the long-term experiments did not show an upward trend between 2002 and 2014 (Figure 3) even though subsoil C increases were observed (Figure 4B). These long-term studies may not have shown the expected upward yield trend over time because they were designed to maintain consistent management and thus likely did not adopt yield-enhancing technologies as rapidly as commercial farms in the region.

**Climate change**

Over the last several decades, precipitation has increased ($p<0.001$) while VPD has decreased ($p<0.001$) in Iowa, Illinois, and their immediate vicinity (Figure 5A and B, Table S6) (Basso et al., 2021; Dai, Shulski, Hubbard, & Takle, 2016; Feng & Hu, 2004). Decreasing VPD, along with increasing atmospheric $CO_2$, contribute to decreasing crop water demand for crop production in this region (Basso et al., 2021; Urban, Sheffield, & Lobell, 2017). Moreover, improvements in crop water-use efficiency have enabled genetic gains in yield potential without corresponding increases in soil water extraction (Reyes et al., 2018). These trends suggest that the region became wetter in the latter 20$^{th}$ and early 21$^{st}$ centuries as the gap between precipitation and evapotranspiration widened (Andreadis & Lettenmaier, 2006; Angel et al., 2018; Pan et al., 2004; Sheffield & Wood, 2008).

A greater gap between precipitation and evapotranspiration could lead to a deepening of the soil organic C distribution by increasing the quantity of dissolved organic C delivered from topsoil to subsoil horizons. Kindler et al. (2011) collected soil water samples in surface and subsoil layers as well as water flux data, to estimate the downward movement of dissolved organic C and its retention in the subsoil for different soil types and land uses. This research showed that downward movement of dissolved organic C accounts for 0.01-0.02 Mg C $ha^{-1}$ $yr^{-1}$ delivered to and retained in subsoil below 35 cm for finely textured cropland soils similar to the sampling locations used in our analysis (Kindler et al., 2011). Based on the observational dataset, the losses and gains in soil organic C within topsoils and subsoils, respectively were ~0.30 Mg C $ha^{-1}$ $yr^{-1}$. Thus, increased dissolved organic C movement likely contributed only minorly to the observed soil organic C stock changes.

Our analysis of publicly-available weather and soil moisture records demonstrates that positive trends in precipitation and negative trends in VPD in the region correspond to increasing soil moisture at 50 and 100 cm deep between 2003 and 2020 ($p<0.001$) (Figure 5C and Table S7). Simulation modeling of water table depth at the two experimental sampling locations (Figure S8) provides weak evidence of the water table becoming more shallow at those particular sites ($p=0.49$ and 0.41 for Kanawha and Nashua, respectively). These findings suggest that water use by crops and water removal by artificial drainage systems have not kept pace with increasing rainfall. The increasing trends in deep soil moisture observed in the regional weather data correspond with subsoil moisture model predictions for the latter 20th century, which can be attributed to winter wetting (Berg, Sheffield, & Milly, 2016). This trend of increasing soil moisture in the subsoil is projected to continue in the future even as surface soils dry due to increased evaporative demand (Berg et al., 2016).

The observed changes in subsoil moisture correspond to a shift in percentage water-filled pore space from 67 to 80% at 50 cm, and 74% to 99% at 100 cm (Figure 5C). Microbial $CO_2$ production reaches a maximum at 60% water-filled pore space and decreases to 40% of the maximum above 80% water-filled pore space (Linn & Doran, 1984). Using the water-filled pore space changes by depth (Figure 5C) and the response of microbial $CO_2$ production to water-filled pore space (Linn & Doran, 1984), we estimate that the increase in subsoil moisture over the period of soil moisture record (2003-2020) decreased the microbial decomposition rate from ~70% of maximum to ~40% of maximum at 50 cm depth and from ~50% of maximum to ~40% of maximum at 100 cm depth. To evaluate the effect of excessive soil moisture on soil organic C stocks, James & Fenton (1993) performed a side-by-side comparison of drained and undrained Iowa soils and found 13% greater soil organic C concentrations in soil layers deeper than 20 cm without artificial drainage than with drainage 80 years after artificial drainage installation. By comparison, the increase in soil organic C stock in the 2,600-11,400 Mg $ha^{-1}$ mass increment determined in our observational dataset over ~50 years was 19%. The multidecadal trends in precipitation and vapor pressure deficit and recent trends in subsoil moisture in the study region, along with previous research about how soil moisture affects soil organic C changes together suggest that increasing soil wetness could account for a substantial portion of the subsoil organic C changes.

**Implications**

Our results, which demonstrate significant organic C gains in subsoils between the 1950s and early 2000s, indicate that soil C models and inventories focused on surface soils (Ogle et al., 2010; United States Environmental Protection Agency, 2020) may substantially overestimate C losses or underestimate C storage rates of soils in Iowa and Illinois. If the subsoil C storage rate we observed from 20 to 80 cm occurred across Illinois and Iowa, we estimate that it could account for 8.3 ± 3.6 Tg C per year with 95% confidence (Table 1), which is equivalent to 5-12% of annual US greenhouse gas emissions from crop cultivation (United States Environmental Protection Agency, 2020). However, the gain in subsoil organic C was offset by the loss of organic C in the surface soil. Thus, for the subsoil C gains to effectively offset greenhouse gas emissions, the surface C losses must be controlled. It is important to note that our analysis of soil organic stock changes relies upon the assumption that changes between the initial and resampling events were linear, when in reality the rates may have been faster or slower over particular periods of time. The lack of multiple measurement time points it a weakness of our dataset, and it highlights the need for more frequent monitoring of whole-profile soil organic C stocks. In addition, the different methods of determining bulk density between the initial and resampling events for the observational studies contributes to uncertainty in our estimates of soil organic C stock changes (VanRemortel & Shields, 1993).

After evaluating possible causes, we conclude that changes in cropping system management, crop productivity, and dissolved organic C leaching likely contribute minorly to the observed changes in subsoil organic C, and that decreased subsoil aeration due to climate change could explain much of the soil organic C stock changes at depth. We caution that our analysis of causative factors was based on conceptual and literature-based estimates that contained uncertainty in part due to weather and soil data coming from different locations and time periods. Moreover, it is possible that other complex mechanisms could explain the subsoil organic C gains. For example, it is possible that root inputs became shallower in response to increasing subsoil moisture (Nichols et al., 2019), leading to less rhizosphere priming and more soil organic C accumulation in the subsoil (Fontaine et al., 2007). Field studies that concurrently monitor soil moisture and soil organic C stock changes and/or directly test the effect of soil moisture and belowground C inputs on soil organic C are needed to better understand how climate change is affecting whole-profile soil organic C stocks in this region.

It is important to highlight that the subsoil organic C gains presented here do not represent a net sequestration of atmospheric greenhouse gases. First, the increase in subsoil organic C was roughly equivalent to the loss of topsoil organic C. It is possible that the gains in subsoil organic C were in part a consequence of losses of soil organic C from surface layers (e.g., via downward movement of organic C through tillage or bioturbation). Second, an increase in subsoil moisture can also increase soil $N_2O$ and $CH_4$ fluxes because both denitrification and methanogenesis are favored by limited soil aeration (Jacinthe, Vidon, Fisher, Liu, & Baker, 2015). In a Michigan, US maize system managed at the recommended N fertilizer input, Shcherbak and Robertson (2019) found that >50% of $N_2O$ emissions originated from subsoils >20 cm depth (Shcherbak & Robertson, 2019). Increasing precipitation in the Midwest US has been predicted to increase regional $N_2O$ emissions by 1.0 Gg $N_2O$-N·$y^{-1}$, with 0.13 Gg $N_2O$-N·$y^{-1}$ from direct cropland emissions (Griffis et al., 2017). Considering the land area of this region and a 100-year time horizon, this rate of $N_2O$ emissions increase equates to <0.1 Mg $CO_2$-eq $ha^{-1}$ $yr^{-1}$, a small offset to the subsoil organic C storage rate estimated here that is equal to ~1.14 Mg $CO_2$-eq $ha^{-1}$ $yr^{-1}$. Like $N_2O$, changes in $CH_4$ emissions may also partially offset the soil

C increase, but $CH_4$ emissions are typically <10% of the net global warming potential of $N_2O$ in maize-based cropping systems in the Midwest US (Robertson et al., 2000) and thus would not substantially offset organic C gains in subsoils.

**Management of subsoil organic C stocks**

About 25% of the land area in the region is classified as poorly drained (Table 1) and relies on artificial drainage for agricultural production (Sugg, 2007). Our observational dataset shows that the poorly drained soils had the greatest rates of soil organic C stock changes over time and made up 42% of regional subsoil organic C increases (Table 1). Drainage systems in the region were designed for precipitation levels of the early 1900s, which were ~20% below current levels (Fig 4A; (Helmers, Melvin, & Lemke, 2009)). A large body of research shows that insufficient soil drainage decreases potential crop yields by delaying field operations, limiting root growth, and increasing the risks of plant disease and insect infestation (Castellano et al., 2019). Without adequate drainage, excess soil moisture is expected to cause a 7.4% decrease in maize yield and cost 3 billion USD per year in lost agricultural output by mid-Century (Rosenzweig, Tubiello, Goldberg, Mills, & Bloomfield, 2002). Farmers have increased the area of artificial drainage by 14% between 2012 and 2017 (USDA-NASS, 2017) and are likely to continue to improve contemporary drainage systems in response to climate change (Hatfield et al., 2014; Morton, Hobbs, Arbuckle, & Loy, 2015).

Expansion and intensification of drainage systems will improve crop growth and reduce $N_2O$ and $CH_4$ emissions, but may reverse the observed recent subsoil C gain (Castellano et al., 2019). Alternative drainage system designs may offer a solution to maintain crop yields while minimizing global warming potential (Castellano et al., 2019). For example, drainage systems with shallower placement and narrower spacing can increase the rate of surface soil drying without draining subsoils. Controlled drainage systems decrease water discharge by only operating when necessary. These systems achieve the crop production benefits of conventional drainage systems while decreasing discharge, which improves water quality (Schott et al., 2017) and may promote subsoil C retention or accrual. There is an urgent need to assess the effects of these alternative drainage systems and other innovations, such as submergent-tolerant crops, on crop yield, soil organic C stocks, and greenhouse gas fluxes to ensure that adaptation of crop production to climate change does not undermine efforts to mitigate greenhouse gas emissions (Castellano et al., 2019).

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Table 1**.** Estimated rates of soil organic C change ± the 95% confidence interval for Iowa and Illinois by soil mass increment. Rates of change are based on the observational data presented in this study (1950s – 2000s) and extrapolated to all land area within Iowa and Illinois, excluding drainage classes that were not represented in the data set. Mass increments correspond to 0-20, 20-40, 40-60, 60-80, and 0-80 cm.

| | Measured change per area (Mg C $ha^{-1}$ $yr^{-1}$) | | | | |
|---|---|---|---|---|---|
| Mass increment (Mg $ha^{-1}$) | Well drained (n=8)* | Moderately well drained (n=8) | Somewhat poorly drained (n=8) | Poorly drained (n=15) | All sites (n=39) |
| 0-2600 | -0.125 ± 0.24 | -0.164 ± 0.24 | -0.220 ± 0.23 | **-0.395 ± 0.17** | **-0.257 ± 0.11** |
| 2600-5500 | 0.117 ± 0.21 | 0.144 ± 0.21 | 0.025 ± 0.21 | 0.139 ± 0.15 | **0.112 ± 0.09** |
| 5500-8400 | 0.128 ± 0.15 | -0.010 ± 0.14 | 0.071 ± 0.15 | **0.230 ± 0.11** | **0.123 ± 0.07** |
| 8400-11400 | **0.128 ± 0.09** | 0.029 ± 0.07 | -0.010 ± 0.08 | **0.138 ± 0.06** | **0.078 ± 0.04** |
| 0-11400 | 0.220 ± 0.63 | -0.002 ± 0.50 | -0.101 ± 0.53 | 0.035 ± 0.39 | 0.025 ± 0.23 |

| | Regionally scaled rate of change (Tg C $yr^{-1}$) | | | | |
|---|---|---|---|---|---|
| Mass increment (Mg $ha^{-1}$) | Well drained (9.21 Mha) | Moderately well drained (4.66 Mha) | Somewhat poorly drained (6.92 Mha) | Poorly drained (6.87 Mha) | All classes† (27.66 Mha) |
| 0-2600 | -1.15 ± 2.17 | -0.76 ± 1.10 | -1.52 ± 1.61 | **-2.71 ± 1.18** | **-6.15 ± 3.22** |
| 2600-5500 | 1.08 ± 1.93 | 0.67 ± 0.97 | 0.17 ± 1.45 | 0.95 ± 1.04 | **2.87 ± 2.82** |
| 5500-8400 | 1.18 ± 1.33 | -0.05 ± 0.65 | 0.49 ± 1.00 | **1.58 ± 0.72** | **3.20 ± 1.93** |
| 8400-11400 | **1.18 ± 0.82** | 0.13 ± 0.33 | -0.08 ± 0.54 | **0.95 ± 0.38** | **2.18 ± 1.11** |
| 0-11400 | 2.03 ± 5.66 | -0.01 ± 2.25 | -0.70 ± 3.66 | 0.24 ± 2.70 | 1.56 ± 7.63 |

*Statistically significant ($p<0.05$) soil organic C stock changes are in bold.

†The regionally scaled rate of change for all drainage classes was calculated as the sum of the preceding columns. This does not include excessively drained, somewhat excessively drained, and very poorly drained soils that make up another 0.88 Mha in Iowa and Illinois

Table 2. Estimated annual changes in C inputs attributed to the yield increase for a maize-soybean rotation from 1950 to 2000 in Iowa and Illinois. Standard errors are shown in parentheses.

| Depth (cm) | Carbon input ($Mg\ C\ ha^{-1}\ yr^{-1}$)* | | Cumulative C input ($Mg\ C\ ha^{-1}$)† | Cumulative C input gain due to yield increase ($Mg\ C\ ha^{-1}$)‡ |
|---|---|---|---|---|
| | 1950 | 2000 | 1950-2000 | 1950-2000 |
| 0-20 | 2.48 (0.028) | 3.80 (0.033) | 160.0 (0.22) | 33.3 (1.53) |
| 20-40 | 0.17 (0.002) | 0.32 (0.003) | 12.7 (0.09) | 3.8 (0.15) |
| 40-60 | 0.09 (0.001) | 0.16 (0.001) | 6.4 (0.02) | 1.9 (0.06) |
| 60-80 | 0.06 (0.001) | 0.11 (0.001) | 4.2 (0.01) | 1.2 (0.04) |
| 0-80 | 2.80 (0.032) | 4.38 (0.038) | 183.3 (0.31) | 40.1 (1.76) |

*Calculated using grain yields in Table S1 according to Bolinder et al. (2007), except that Harvest Index values of 0.35 and 0.30 were used for maize and soybean, respectively, for 1950 according to Allmaras et al. (1998). Average annual C inputs for 0-20 cm include aboveground inputs and belowground inputs allocated to 0-20 cm based on root distributions presented in Fan et al. (2016). Average annual C inputs for 20-40, 40-60, and 60-80 cm include only belowground inputs multiplied by the proportion of belowground C allocated to each depth according to Fan et al. (2016).

†Cumulative C inputs were calculated assuming a linear increase in C inputs over a fifty-year period, based on the linear increase in yields over this time (Egli, 2008)

‡Calculated as the difference between cumulative C inputs over a fifty-year period and 50 years of C inputs at a constant initial yield level.

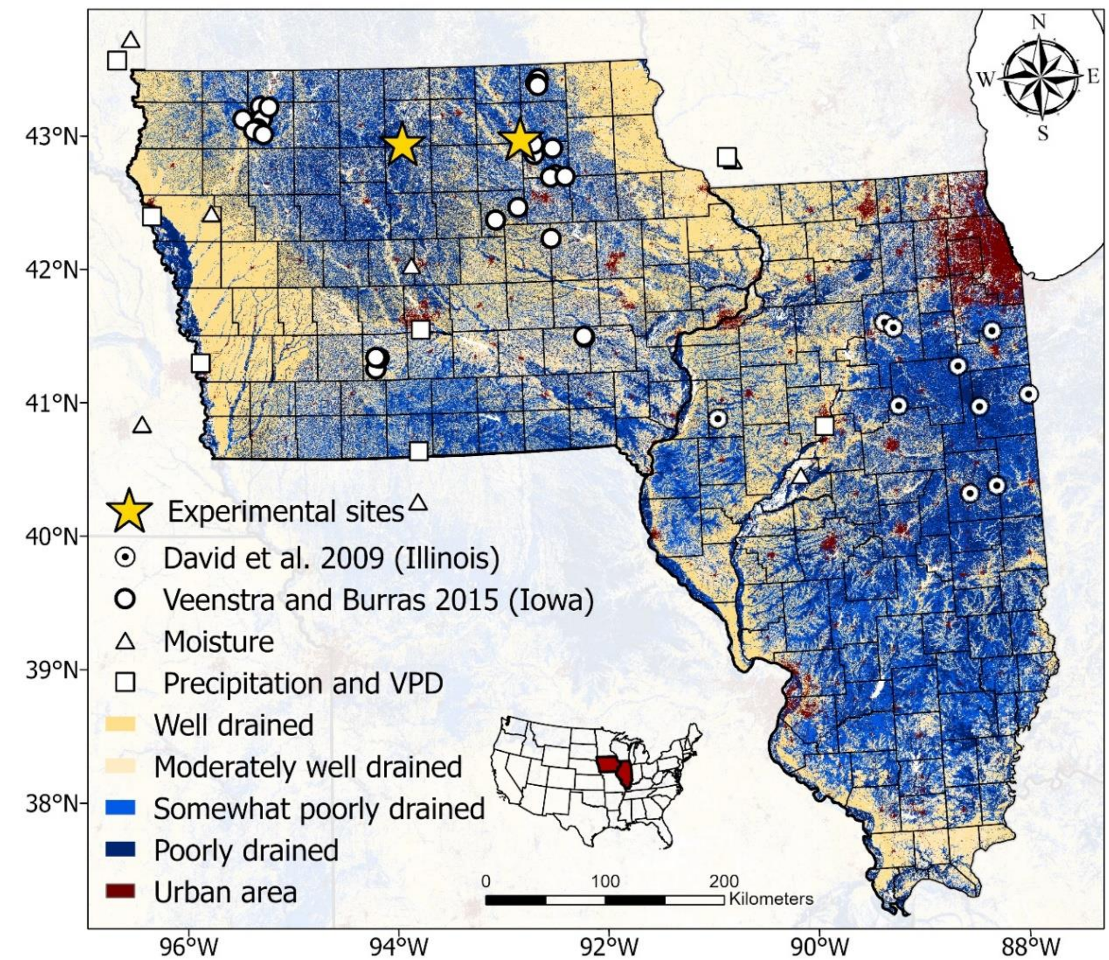


Figure 1. Soil sample locations (n=41) and weather stations (n=7 per weather variable) overlaid on a map showing USDA soil drainage classes. The region of interest encompasses Iowa and Illinois, but we included weather stations in close proximity of these states due to the limited number of weather stations with subsoil moisture monitoring

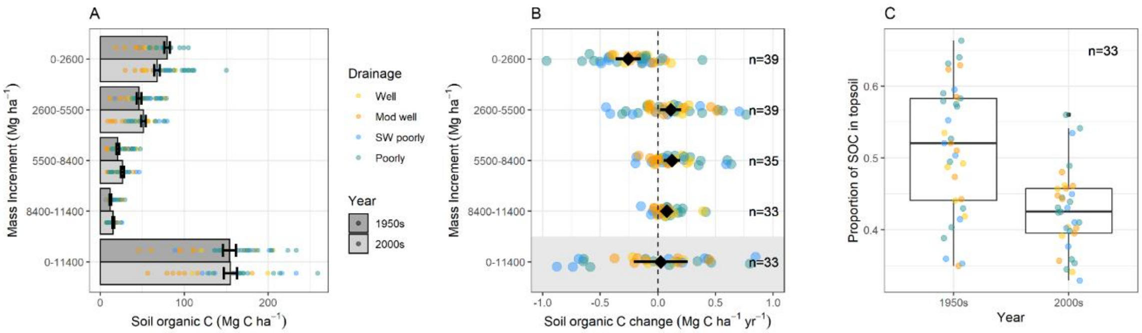


Figure 2. A) Soil organic C stocks at initial and resampling events by mass increment from the observational studies. Error bars represent ± one standard error. B) Average annual soil organic C changes by mass increment (1950s-2000s). Error bars represent 95% confidence intervals. n represents the number of sites used to calculate the SOC stock change per mass increment. C) The proportion of soil organic C (SOC) found in the topsoil increment relative to the whole soil profile for the initial and resampling events. Point colors represent soil drainage classes ("Mod well" = moderately well drained, "SW poorly" = somewhat poorly drained). The cumulative masses of 2600, 5500, 8400, and 11400 correspond to depths of 20, 40, 60 and 80 cm, respectively averaged across sites and sampling dates (Figure S3).

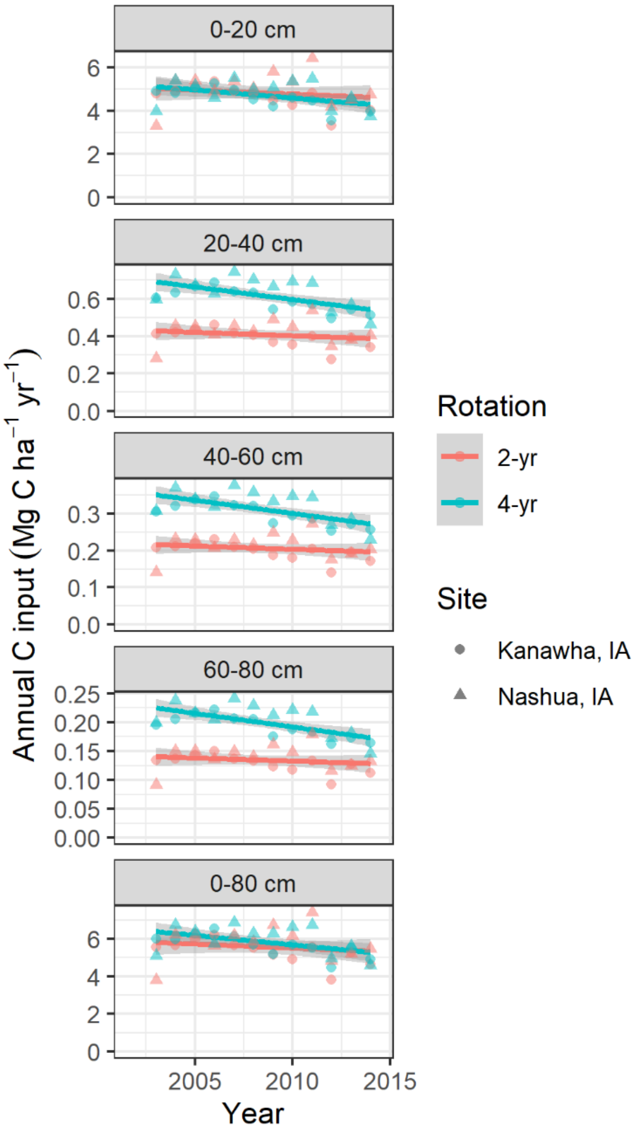


Figure 3. Historical annual C inputs for two crop rotations by depth at two long-term cropping systems experiments in Iowa. Average annual C inputs include both above- and belowground inputs. Shaded ribbons represent 95% confidence bands. n = 48 observations per depth increment.

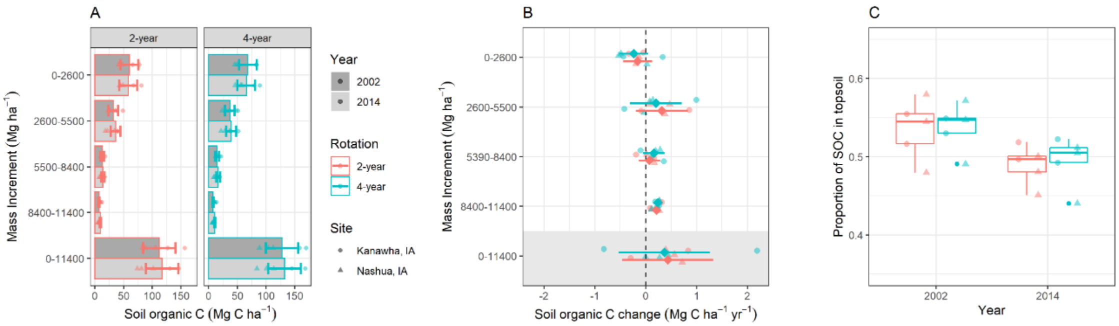


Figure 4. A) Soil organic C stocks at initial and resampling events by rotation and mass increment from the experimental studies. Error bars represent ± one standard error. B) Average annual soil organic C changes by rotation and mass increment (2002-2014). Error bars represent 95% confidence intervals. C) The proportion of soil organic C (SOC) found in the topsoil increment relative to the whole soil profile for the initial and resampling events. The cumulative masses of 2600, 5500, 8400, and 11400 correspond to depths of 20, 40, 60 and 80 cm, respectively averaged across sites and sampling dates (Figure S3). n= 5 observations per bar (A), point range (B), or boxplot (C).

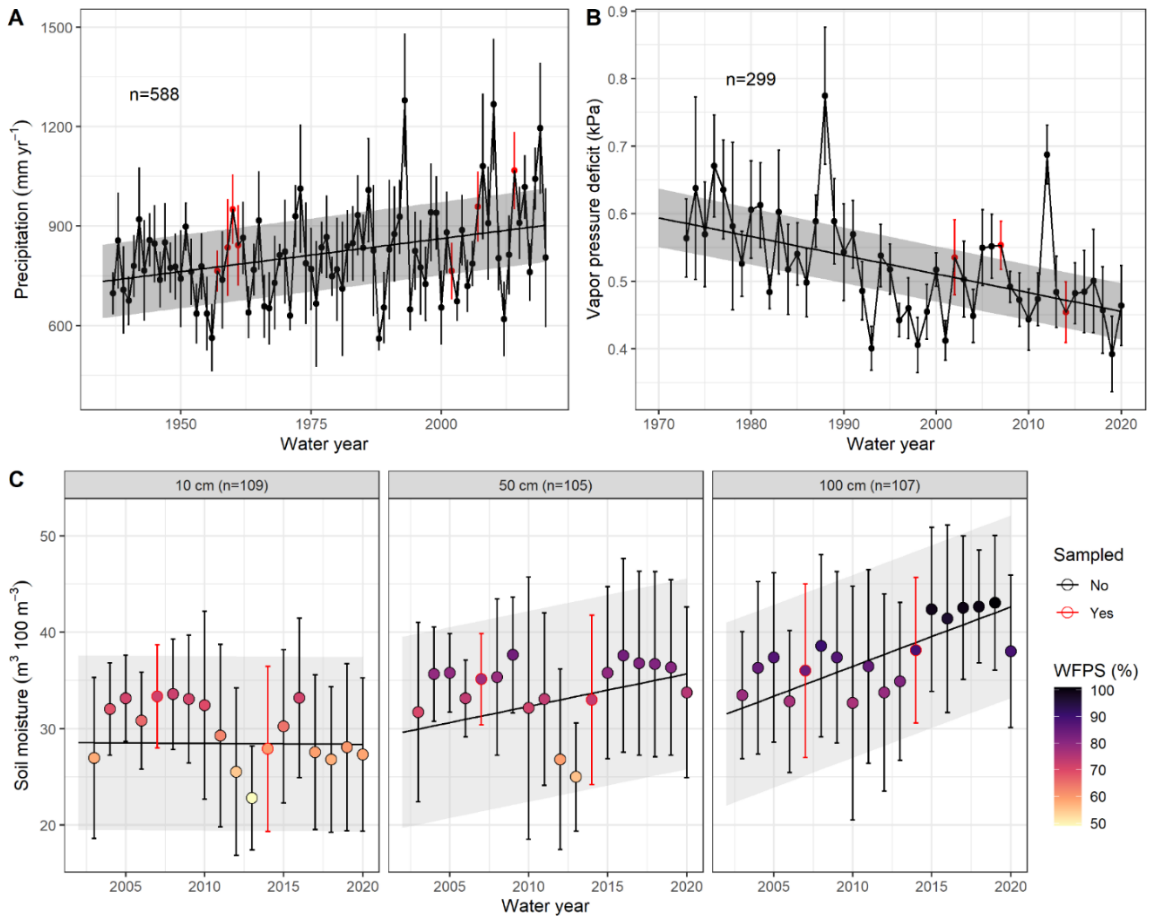


Fig 5. Historical precipitation (A), vapor pressure deficit (B), and soil moisture (C) for the study region. Each point represents the mean of seven monitoring stations distributed across Iowa, Illinois and the immediate vicinity (Figure 1), but the regression was fitted to all individual measurements. On plot C, the point color varies according to percentage water-filled pore space (WFPS). Note different time periods due to different availabilities of long-term monitoring records. Years in which soil sampling occurred are highlighted in red. Error bars represent 95% confidence intervals and shaded ribbons represent 95% confidence bands.