A large subsoil carbon sink in the United States Corn Belt

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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-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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3 ABSTRACT

4 Current soil C inventories focus on surface layers although over half of soil C is found below 20 5 cm. Recent and ongoing changes in agricultural management, crop productivity, and climate in 6 Midwest US cropland may influence subsoil C stocks. The objectives of this study were to 7 determine how surface soil and subsoil organic C stocks have changed in croplands of Iowa and 8 Illinois and to evaluate mechanisms to explain the observed subsoil organic C changes. Using 9 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 10 magnitude in the top 20 cm (0.26 Mg C ha⁻¹ yr⁻¹). Based on this analysis, we estimated a subsoil 11 C storage rate of up to 11.8 Tg C yr⁻¹ for Iowa and Illinois, which equates to 12% of annual US 12 13 greenhouse gas emissions from crop cultivation if surface C losses and non-CO₂ greenhouse 14 gases are controlled. We also measured changes in soil organic C stocks from two long-term 15 cropping systems experiments located in Iowa, which demonstrated similar rates of subsoil C 16 changes for both historical and contemporary crop rotations. Using publicly available crop yield 17 data, we determined that changes in crop productivity likely contributed minorly to observed 18 changes in subsoil organic C. The accumulation of organic C in subsoils may be attributed to 19 regional climate change, which has led to greater precipitation and wetter subsoils that inhibit 20 transformation of soil organic C to CO₂. Because farmers may respond to increasing soil 21 wetness by expanding and intensifying artificial drainage infrastructure, there is an urgent need 22 to further assess subsoil C stocks and their vulnerability to drainage system changes.

23

24 INTRODUCTION

25	Humans have transformed one third to one half of Earth's land surface through agriculture
26	(Goldewijk, Beusen, & Drecht, 2011), resulting in the loss of approximately 133 Pg C from the
27	top 2 m of soil (Sanderman, Hengl, & Fiske, 2017). Crop production typically decreases soil
28	organic C stocks through a reduction in C inputs due to the harvest of crop material, stimulation
29	of C mineralization due to tillage and artificial drainage, and through erosion or loss of soil
30	structure (Davidson & Ackerman, 1993; Guo, L.B., Gifford, 2002; Smith, 2008). Because soil
31	organic C represents an important source and sink of greenhouse gas emissions and is critical to
32	soil health, management practices that increase soil organic C have been proposed as a "win-
33	win" strategy for climate change and food security (Minasny et al., 2017; Paustian et al., 2016).
34	Soils of the Midwest US lost 30 to 50% of their native C stocks after conversion of
35	grassland to cropland in the 19th century (David, McIsaac, Darmody, & Omonode, 2009;
36	Robertson, Paul, & Harwood, 2000). Since the mid-20 th century, Midwest cropping systems
37	transitioned from diverse rotations of grain and perennial forage crops to an annual two-crop
38	rotation of maize (Zea mays L.) and soybean [Glycine max (L.) Merr.] with higher rates of N
39	fertilizer inputs and less tillage (Karlen, Dinnes, & Singer, 2010). At the same time,
40	improvements in crop genetics and management increased crop productivity and residue inputs
41	to the soil (Hicke & Lobell, 2004) and climate change increased the amount and intensity of
42	precipitation (Angel et al., 2018; Basso, Martinez-Feria, Rill, & Ritchie, 2021). Recent regional
43	assessments indicate that surface soil organic C stocks have reached a new steady-state or are
44	increasing where soil conservation practices have been adopted (United States Environmental
45	Protection Agency, 2020). However, potential responses of subsoil organic C stocks to changes
46	in crop rotation, crop productivity, and climate remain unknown.

47 Our objectives were to determine how surface soil and subsoil organic C stocks have 48 changed in croplands of Iowa and Illinois and to evaluate mechanisms to explain the observed 49 subsoil organic C changes. We extracted historic and modern soil organic C data from 39 soil 50 profiles in Iowa and Illinois cropland that were reported in published studies (David et al., 2009; 51 Veenstra & Burras, 2015). In addition, we measured changes in soil organic C stocks from two 52 well controlled, long-term cropping systems experiments located in Iowa. These long-term 53 experiments allowed us to determine the effect of cropping system conversion by comparing 54 changes in soil organic C stocks in historical diverse rotations of grain and perennial forage 55 crops with the contemporary annual two-crop rotation of maize and soybean. Furthermore, we 56 used publicly available crop yield and weather data to estimate the impacts of changing C inputs 57 and a wetter climate on subsoil organic C stocks. Finally, we estimated change in subsoil C 58 stocks for land area in Iowa and Illinois.

59 MATERIALS AND METHODS

60 **Published observational studies**

61 We compiled historical and modern soil organic C concentrations and bulk density data 62 from observational studies to determine the net effects of agricultural management trends and 63 climate changes on soil organic C stocks (i.e. the mass of C per unit area for a given soil mass 64 increment) between the 1950s and early 2000s in Iowa and Illinois. We extracted the historical 65 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. 66 67 (2009) and Veenstra & Burras (2015). We used these articles because they reported soil organic 68 C stocks in surface and subsoil layers (extending to at least 100 cm depth) in cropland of the 69 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.

73 David et al. (2009) sampled soils by depth (0-100 cm) in 2001 and 2002 at 19 sites in 74 central Illinois that were previously sampled in the early 1900s or 1957 or were paired with 75 remant prairies. We used soil organic C concentrations and bulk density data from the ten 76 locations that were initially sampled in 1957 by the USDA Soil Conservation Service and 77 resampled in 2002. The 2002 sampling was performed using soil cores of 3.2 cm diameter. We 78 extracted the 2002 data from the 2011 errata rather than the original 2009 publication (David, 79 McIsaac, Darmody, & Omonode, 2011). The sites were in either grass or annual crop production 80 in 1957 and predominantly maize-soybean rotations in 2002. All of the sites were classified as 81 poorly drained, were tile drained, and had no known history of manure application in recent 82 decades. Veenstra and Burras (2015) sampled soils by depth (0-150 cm) in 2007 at 82 sites in 83 Iowa that were previously sampled between 1943 and 1963. We used soil organic C 84 concentrations and bulk density data from the 29 sites that had these data for multiple depths 85 from both the initial and resampling events. The 2007 sampling was performed using soil cores 86 of 7 cm diameter. The site-specific data from 2007 were not presented in the original publication, 87 but were provided by the authors. The sites were in either annual crop production or perennial 88 forages at the initial sampling time in 1956-1961 and annual row crop production in 2007. Of the 89 29 sites from Veenstra and Burras (2015) that were used in our analysis, five were classified as 90 poorly drained, nine as somewhat poorly drained, eight as moderately well drained, and seven as 91 well drained. Site information is provided in Table S1.

92 The initial soil descriptions reported in the USDA Soil Conservation Service Survey 93 Descriptions were generated using excavated soil pits, with samples collected from the pits. Bulk 94 density was measured either using the core method or clod method (Soil Conservation Service, 95 1966b). When the clod method was used, we extracted data for which soil volume was 96 determined at field moisture. For all profiles used in this analysis, the initial bulk density data 97 were available for about half of all horizons in the USDA Soil Conservation Service Survey 98 Descriptions. We used a Random Forest algorithm (Liaw & Wiener, 2002) to predict missing 99 bulk density using profile ID, soil series, master horizon, depth increment midpoint, depth 100 increment length, soil organic C concentration, clay percentage, sand percentage, calcium 101 carbonate equivalent, drainage class, and bulk density method reported in the descriptions as 102 predictor variables following an approach similar to (Sequeira, Wills, Seybold, & West, 2014). 103 Two thirds of the horizons with available bulk density data were used for model calibration and 104 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 105 106 missing bulk density values for one to four depth increments. Because few (8%) of all depth 107 increments from the resampled profiles were missing bulk density data, we estimated the missing 108 data as the average of the bulk density values from increments directly above and below the 109 increments that were missing data in the same profile. Measured and estimated bulk density 110 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.

120 To scale soil organic C stocks from both sampling events at all sites to the same reference 121 masses, we used an equivalent soil mass method (Wendt & Hauser, 2013). For the initial and 122 resampling events, bulk density and depth increment length were used to calculate the soil mass 123 of each layer. The soil organic C stock for that layer was then calculated as the product of the 124 soil organic C concentration and the soil mass. The cumulative soil masses and soil organic C 125 stocks for each profile were fitted using a cubic spline function, which was used to predict the 126 soil organic C stocks at reference masses (Wendt & Hauser, 2013). Plots of cumulative soil 127 organic C with cumulative soil mass for each profile are presented in Figure S2.

128 The reference masses were selected to represent 20, 40, 60 and 80 cm deep based on the 129 relationship between cumulative depth and cumulative soil mass from all sites and sampling 130 events (Figure S3). We considered below 20 cm to be subsoil, reflecting that the transition from 131 the A to B horizon occurred between 20 and 40 cm deep for the majority (67%) of profiles in the 132 observational dataset according to the USDA Soil Conservation Service Survey Descriptions 133 (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 134 135 annualize soil organic C changes for each site.

136 Long-term experiments

137 We conducted soil sampling at two long-term experimental sites to measure the change in 138 soil organic C stocks (0-100 cm) from 2002 to 2014 and test the effect of historical changes in 139 the dominant cropping system and C inputs on change in soil organic C stocks over this time 140 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 141 142 experiments include a maize-maize-oats (Avena sativa L.)/alfalfa (Medicago sativa L.)/alfalfa 143 crop rotation that was representative of the US Corn Belt prior to the 1980s as well as the 144 contemporary maize-soybean crop rotation that has dominated the region since that time. 145 The experimental design at both locations is a split-plot randomized complete block with 146 two (Kanawha) or three (Nashua) replicate blocks. The main plot is crop rotation, with all phases 147 of each rotation represented in all years. Main plots are subdivided to accommodate four N 148 fertilization treatments (0, 90, 180, and 270 kg N ha⁻¹) applied to maize. Both experiments are 149 situated on Iowa State University Research and Demonstration Farms. Mean annual precipitation 150 is 818 and 884 mm (1985-2014) and mean annual temperature is 8.03 and 8.37°C for Kanawha 151 and Nashua, respectively. Soils at Kanawha are classified as Typic Endoaquolls (Webster series), 152 whereas soils at Nashua are predominantly Typic Hapludolls (Kenyon series), with a smaller 153 area Aquic Hapludolls (Readlyn series) according to the USDA soil taxonomic system (Soil 154 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).

166 Soil sampling took place immediately following maize harvest and before tillage in 2002 167 and 2014. For the four-year rotation, soil sampling was conducted at the end of the first maize 168 phase of the rotation. The length of time between sampling events was such that the plots 169 sampled in 2002 were in the same phase of the rotation in 2014. At both sampling times, six 4.1-170 cm diameter cores were collected per plot using a hydraulic soil probe. The cores were taken at 171 points randomly selected within a plot stratified by position: in the row, the midpoint between 172 rows, and halfway between these two positions. In 2002, the cores were taken to a depth of 100 173 cm and split into 0-5, 5-15, 15-30, 30-50, 50-75, and 75-100 cm depth increments. In 2014, the 174 cores were taken to a depth of 100 cm and split into 0-15, 15-30, 30-60, 60-90, and 90-100 cm 175 depth increments. The segments from the six cores within each plot were composited to form six 176 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.

193 **Carbon input change**

194 Average annual above- and below-ground C inputs to the soil in Iowa and Illinois were 195 estimated for years corresponding to approximate soil sampling times derived from the 196 observational studies (David et al., 2009; Veenstra & Burras, 2015) (1950, 2000). The three-year 197 average maize and soybean yields for Iowa and Illinois centered around 1950 and 2000 were 198 collected from USDA NASS (National Agricultural Statistics Service, 2020) (Table S4) and used 199 to calculate C inputs to the soil using the method described by Bolinder et al. (Bolinder, Janzen, 200 Gregorich, Angers, & VandenBygaart, 2007). In addition, crop yield records were available for 201 each rotation at both experimental sites in Iowa from 2003-2014 and we used the same approach 202 (Bolinder et al., 2007) to transform yield data into average annual C inputs. Briefly, aboveground 203 biomass was calculated using dry matter yields and the harvest index; belowground biomass was 204 calculated using a shoot:root ratio, and rhizodeposition was calculated as a factor relative to root 205 biomass. The harvested product was subtracted from total dry matter and then dry matter was

206 converted to C input assuming plant biomass contains 45% C. For 1950 estimates, we replaced 207 modern Harvest Index (proportion of total aboveground biomass in grain) values of 0.50 and 208 0.40 for maize and soybean with 0.35 and 0.30, respectively according to historic allometric 209 relationships (Allmaras, Wilkins, Burnside, & Mulla, 1998). We assumed the root:shoot ratio 210 and depth distribution of crop roots remained constant over time because previous studies on root 211 changes due to breeding in US germplasm do not indicate that root allocation has increased with 212 yields over time (Fried, Narayanan, & Fallen, 2019; Messina et al., 2020; Reyes et al., 2018; 213 Schmidt, Poret-Peterson, Lowry, & Gaudin, 2020).

214 Average annual C inputs were apportioned into 0-20, 20-40, 40-60, and 60-80 cm depth 215 increments. The 0-20 cm depth increment included aboveground inputs and belowground inputs 216 allocated to 0-20 cm based on root distributions presented in (Fan, McConkey, Wang, & Janzen, 217 2016). The 20-40, 40-60, and 60-80 cm increments included only belowground inputs allocated 218 to each depth according to Fan, McConkey, Wang & Janzen (2016). Because most observations 219 in Bolinder et al. (2007) were from samples collected to 40-50 cm, we used root distributions 220 from Fan et al. (2016) to calculate additional C inputs below 40 cm. Cumulative C inputs 221 between 1950 and 2000 were calculated assuming a linear increase in C inputs over this time, 222 based on the linear increase in yields over this time (Egli, 2008). The cumulative C input gain 223 due to yield increase was calculated as the difference between cumulative C inputs from 1950-224 2000 and 50 years of C inputs at a constant 1950 yield level.

225 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

229 weather stations that provided moisture content to 100 cm depth. Historical data on VPD were 230 obtained from the Automated Surface Observing System Network (National Weather Service, 231 2020a). Precipitation data were collected from the National Weather Service Cooperative 232 Observer Program (National Weather Service, 2020b). Both of these networks were accessed 233 through the Iowa Environmental Mesonet (Iowa State University, 2021). The weather stations 234 for precipitation and VPD were chosen based on close proximity to weather stations reporting 235 soil volumetric moisture content. Soil volumetric moisture content data were obtained from the 236 Soil Climate Analysis Network (Natural Resources Conservation Service, 2020). Soil moisture 237 data were available for five depths, but we only analyzed data collected at 10 cm, 50 cm, and 100 238 cm because they corresponded best to the depth increments used for soil organic C stock 239 changes. The 10 cm and 50 cm depths represent the midpoints of 0-20 and 40-60 cm increments 240 used for soil organic C stock changes, while the 100 cm depth is deeper than the deepest depth 241 analyzed for soil organic C stock changes. In addition, water table depths from 1988 through 242 2016 were collected from a monitoring well installed in a grassed area at the Kanawha research 243 farm. Water table depths were also simulated for the maize-soybean rotation at the Kanawha and 244 Nashua sites using the Agricultural Production Systems Simulator (Holzworth et al., 2014) for 245 1981 through 2019 as described in (Archontoulis et al., 2020). Cumulative annual precipitation, 246 VPD, soil moisture, and water table depth were summarized by water year (October 1 -247 September 30).

248 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

252 a mean annual change in soil organic C stock across sites for each mass increment. The analysis 253 of soil organic C stocks and average annual soil organic C stock changes were done separately 254 for each mass increment to avoid obscuring significant changes in specific layers by the high 255 variability of soil organic C stocks in the entire profile (Kravchenko & Robertson, 2011). In 256 addition, some sites were missing data for deeper layers and were excluded from the analysis of 257 those mass increments. A profile 95% confidence interval of annual change in soil organic C 258 stock was calculated for each mass increment to test whether the change in average annual soil 259 organic C stock was significantly different from zero. To determine how soil organic C stock 260 changes were influenced by soil drainage class, a linear fixed effect model was constructed with 261 average annual change in soil organic C stock as the response variable and drainage class as a 262 fixed effect. The drainage class for each site was derived from the USDA Soil Conservation 263 Service Survey Descriptions.

264 For the experimental dataset, a linear mixed effect model was constructed for each mass 265 increment with soil organic C stock as the response variable, the interaction of crop rotation by 266 year sampled as the fixed effect, and block within site (Kanawha or Nashua) as a random effect. 267 For the analysis of average annual change in soil organic C stock, two models with the same 268 random effect structure (block within site) were run – one with only an intercept as a fixed effect 269 to determine the mean across rotations, and the other with crop rotation as a fixed effect. Carbon 270 inputs from the experimental dataset were analyzed by depth increment using a linear mixed 271 effect model, which included a crop rotation by year interaction as the fixed effect and site as a 272 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.

282 We estimated the water-filled pore space based on soil moisture data in each year of 283 record. First, total pore space fraction was calculated using estimated soil bulk densities at depths 284 corresponding to the soil moisture data. Specifically, we used the relationship between 285 cumulative depth and cumulative soil mass across all sites and sampling events (Figure S3) to 286 estimate bulk densities for 10-cm increments centered on the depth of soil moisture 287 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 288 289 density remained constant among sites and over the duration of soil moisture measurements. The 290 water-filled pore spaces for different soil moisture levels in Figure 5C were calculated as the 291 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).

300 Estimation of regional soil organic C stock changes

301 Using the analysis of soil organic C stock change by mass increment for each drainage 302 class from the observational dataset, we estimated rates of soil organic C stock changes within 303 the entire region of Iowa and Illinois. The average annual rate of soil organic C stock change for 304 each mass increment in each drainage class was multiplied by the total land area mapped in that 305 drainage class in the two states. The aerial extent of each drainage class in each state was 306 calculated based on the 10 x 10 m resolution gSSURGO maps (Soil Survey Staff, 2020a, 2020b). 307 We estimated the uncertainty in regional estimates of soil organic C stock changes using a 308 Bayesian approach. Posterior distributions of average annual soil organic C stock changes by 309 drainage class for each mass increment were obtained by refitting the linear fixed-effect model in 310 a Bayesian framework using the stan_glm function in R package rstanarm version 2.21.1 311 (Goodrich, Gabry, Ali, & Brilleman, 2020). In this model, the response variable was average 312 annual change in soil organic C stock and the explanatory variable was drainage class; no 313 intercept was included. Each mass increment was analyzed separately. A default normal prior 314 distribution for the drainage class parameter was used. The default prior distribution is 315 considered weakly informative to provide moderate regularization and stabilize computation 316 (Gabry & Goodrich, 2020). We found that the parameter estimates of the linear fixed-effect 317 models in a Bayesian framework agreed well with the same model in a frequentist framework, 318 indicating that the default priors were relatively non-informative. Four parallel chains were fit 319 with a burn-in of 5,000 samples then retaining the next 5,000 samples to give a total of 20,000 320 posterior samples. The number of chains and proportion of burn-in samples were selected based

321 on the default settings for the rstanarm package, but we used 10,000 rather than the default 2,000 322 iterations per chain to ensure a large effective sample size and stable estimates (Gelman et al., 323 2020), consistent with (Correndo et al., 2021). Trace plots and Gelman-Rubin statistics indicated 324 no issues with convergence. The posterior distribution of average annual change in soil organic 325 C stock for each drainage class and mass increment was multiplied by the land area of the 326 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 (50th percentile) 327 328 of the posterior distributions, while the uncertainty of each estimate was obtained as the length of the 95%-credible intervals (2.5th and 97.5th percentile) from the posterior distributions. We 329 330 compared the subsoil organic C stock change against the US EPA estimate of total US 331 greenhouse gas emissions from 'Crop Cultivation', which are 360 million metric tons of CO₂ 332 equivalents or approximately 98 Tg C (United States Environmental Protection Agency, 2020).

333 **RESULTS AND DISCUSSION**

334 Changes in soil organic C stocks

335 Across the two observational studies, which included measurements of soil organic C 336 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 337 338 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 339 C increased at an average rate of 0.31 Mg C ha⁻¹ yr⁻¹ (p=0.02). In the topsoil (mass increment of 340 341 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 342 343 and early 2000s in the whole profile soil organic C stocks (Figure 2B). The proportion of wholeprofile 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).

349 Changes in cropping system management

350 The shift from maize, small grains, and alfalfa to maize and soybean between the initial 351 and resampling events of the observational studies (1950s to 2000s) may have led to changes in 352 the quantity or depth distribution of C inputs, which can impact soil organic C stocks (McDaniel, 353 Tiemann, & Grandy, 2014). Hence, we examined the rates of C inputs and soil organic C stock 354 changes for historical (maize-maize-oats/alfalfa-alfalfa; four-year rotation) versus current crop 355 rotations (maize-soybean; two-year rotation) using the long-term experiments. We found that 356 both rotations deposited similar amounts of C to the top 20 cm of the soil, but the four-year 357 rotation added more C to the 20-40, 40-60, and 60-80 cm depth increments than the two-year 358 rotation (p<0.05; Figure 3 and Table S5), suggesting that the transition from the historical four-359 year to modern two-year cropping system led to a decrease rather than increase in C inputs to the 360 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).

373 During the period of C measurements in the observational studies, annual N fertilizer 374 rates increased by about three-fold (United States Department Of Agriculture Economic 375 Research Service, 2019). Nitrogen inputs can directly and indirectly impact soil organic C 376 accumulation by increasing crop residue quantity (Brown et al., 2014) and by altering microbial 377 community composition (Brown et al., 2014) and microbial physiology (Sinsabaugh, Manzoni, 378 Moorhead, & Richter, 2013). A greater N supply in N-limited systems is expected to result in 379 more efficient use of C by microbes and less CO₂ respired per unit of C input (Sinsabaugh et al., 380 2013). However, field-based research on the conversion of crop residue C to soil organic C has 381 not demonstrated a clear effect of N fertilizer rate on C retention efficiency (Allmaras, Linden, & 382 Clapp, 2004; Gregorich, Ellert, Drury, & Liang, 1996; Liang et al., 1998). Hence, the effect of 383 increasing N fertilizer on microbial C use efficiency likely explains little of the soil organic C 384 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

390 (Baker, Ochsner, Venterea, & Griffis, 2007). Moreover, tillage practices were not changed in the 391 long-term experiments that demonstrated increasing subsoil organic C stocks. Thus, we conclude 392 that changes in crop rotation, N fertilization rates, and tillage practices between the historical and 393 modern soil sampling events explain little of the observed increase in subsoil organic C stocks.

394

Changes in C inputs due to yield increases

395 Over the timespan of the observational datasets (~1950-2000), the additional C inputs 396 attributed to crop yield increase was 6.9 Mg C ha⁻¹ for the subsoil (20-40, 40-60 and 60-80 cm 397 depths together) (Table 2), making up 44% of total change in soil organic C for the 398 corresponding mass increment of 2,600-11,400 Mg ha⁻¹, which was 15.7 Mg C ha⁻¹. However, 399 only about 10% of the increased C inputs may be converted to soil organic C (Castellano, 400 Mueller, Olk, Sawyer, & Six, 2015), such that only a small fraction of the observed subsoil 401 organic C changes could be attributed to changes in direct C inputs to those soil layers. 402 Moreover, C inputs estimated using yield data collected in the long-term experiments did not 403 show an upward trend between 2002 and 2014 (Figure 3) even though subsoil C increases were 404 observed (Figure 4B). These long-term studies may not have shown the expected upward yield 405 trend over time because they were designed to maintain consistent management and thus likely 406 did not adopt yield-enhancing technologies as rapidly as commercial farms in the region.

407 Climate change

408 Over the last several decades, precipitation has increased (p<0.001) while VPD has 409 decreased (p<0.001) in Iowa, Illinois, and their immediate vicinity (Figure 5A and B, Table S6) 410 (Basso et al., 2021; Dai, Shulski, Hubbard, & Takle, 2016; Feng & Hu, 2004). Decreasing VPD, 411 along with increasing atmospheric CO₂, contribute to decreasing crop water demand for crop 412 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 20th and early 21st centuries as the gap between
precipitation and evapotranspiration widened (Andreadis & Lettenmaier, 2006; Angel et al.,
2018; Pan et al., 2004; Sheffield & Wood, 2008).

418 A greater gap between precipitation and evapotranspiration could lead to a deepening of 419 the soil organic C distribution by increasing the quantity of dissolved organic C delivered from 420 topsoil to subsoil horizons. Kindler et al. (2011) collected soil water samples in surface and 421 subsoil layers as well as water flux data, to estimate the downward movement of dissolved 422 organic C and its retention in the subsoil for different soil types and land uses. This research 423 showed that downward movement of dissolved organic C accounts for 0.01-0.02 Mg C ha⁻¹ yr⁻¹ 424 delivered to and retained in subsoil below 35 cm for finely textured cropland soils similar to the 425 sampling locations used in our analysis (Kindler et al., 2011). Based on the observational dataset, 426 the losses and gains in soil organic C within topsoils and subsoils, respectively were $\sim 0.30 \text{ Mg C}$ ha⁻¹ vr⁻¹. Thus, increased dissolved organic C movement likely contributed only minorly to the 427 428 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).

441 The observed changes in subsoil moisture correspond to a shift in percentage water-filled 442 pore space from 67 to 80% at 50 cm, and 74% to 99% at 100 cm (Figure 5C). Microbial CO₂ 443 production reaches a maximum at 60% water-filled pore space and decreases to 40% of the 444 maximum above 80% water-filled pore space (Linn & Doran, 1984). Using the water-filled pore 445 space changes by depth (Figure 5C) and the response of microbial CO_2 production to water-filled 446 pore space (Linn & Doran, 1984), we estimate that the increase in subsoil moisture over the 447 period of soil moisture record (2003-2020) decreased the microbial decomposition rate from 448 ~70% of maximum to ~40% of maximum at 50 cm depth and from ~50% of maximum to ~40% 449 of maximum at 100 cm depth. To evaluate the effect of excessive soil moisture on soil organic C 450 stocks, James & Fenton (1993) performed a side-by-side comparison of drained and undrained 451 Iowa soils and found 13% greater soil organic C concentrations in soil layers deeper than 20 cm 452 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 453 454 determined in our observational dataset over ~50 years was 19%. The multidecadal trends in 455 precipitation and vapor pressure deficit and recent trends in subsoil moisture in the study region, 456 along with previous research about how soil moisture affects soil organic C changes together 457 suggest that increasing soil wetness could account for a substantial portion of the subsoil organic 458 C changes.

459 Implications

460 Our results, which demonstrate significant organic C gains in subsoils between the 1950s 461 and early 2000s, indicate that soil C models and inventories focused on surface soils (Ogle et al., 462 2010; United States Environmental Protection Agency, 2020) may substantially overestimate C 463 losses or underestimate C storage rates of soils in Iowa and Illinois. If the subsoil C storage rate 464 we observed from 20 to 80 cm occurred across Illinois and Iowa, we estimate that it could 465 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 466 467 Protection Agency, 2020). However, the gain in subsoil organic C was offset by the loss of 468 organic C in the surface soil. Thus, for the subsoil C gains to effectively offset greenhouse gas 469 emissions, the surface C losses must be controlled. It is important to note that our analysis of soil 470 organic stock changes relies upon the assumption that changes between the initial and 471 resampling events were linear, when in reality the rates may have been faster or slower over 472 particular periods of time. The lack of multiple measurement time points it a weakness of our 473 dataset, and it highlights the need for more frequent monitoring of whole-profile soil organic C 474 stocks. In addition, the different methods of determining bulk density between the initial and 475 resampling events for the observational studies contributes to uncertainty in our estimates of soil 476 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

482 contained uncertainty in part due to weather and soil data coming from different locations and 483 time periods. Moreover, it is possible that other complex mechanisms could explain the subsoil 484 organic C gains. For example, it is possible that root inputs became shallower in response to 485 increasing subsoil moisture (Nichols et al., 2019), leading to less rhizosphere priming and more 486 soil organic C accumulation in the subsoil (Fontaine et al., 2007). Field studies that concurrently 487 monitor soil moisture and soil organic C stock changes and/or directly test the effect of soil 488 moisture and belowground C inputs on soil organic C are needed to better understand how 489 climate change is affecting whole-profile soil organic C stocks in this region.

490 It is important to highlight that the subsoil organic C gains presented here do not 491 represent a net sequestration of atmospheric greenhouse gases. First, the increase in subsoil 492 organic C was roughly equivalent to the loss of topsoil organic C. It is possible that the gains in 493 subsoil organic C were in part a consequence of losses of soil organic C from surface layers (e.g., 494 via downward movement of organic C through tillage or bioturbation). Second, an increase in 495 subsoil moisture can also increase soil N₂O and CH₄ fluxes because both denitrification and 496 methanogenesis are favored by limited soil aeration (Jacinthe, Vidon, Fisher, Liu, & Baker, 497 2015). In a Michigan, US maize system managed at the recommended N fertilizer input, 498 Shcherbak and Robertson (2019) found that >50% of N₂O emissions originated from subsoils 499 >20 cm depth (Shcherbak & Robertson, 2019). Increasing precipitation in the Midwest US has been predicted to increase regional N₂O emissions by 1.0 Gg N₂O-N·y⁻¹, with 0.13 Gg N₂O-500 501 $N \cdot y^{-1}$ from direct cropland emissions (Griffis et al., 2017). Considering the land area of this 502 region and a 100-year time horizon, this rate of N₂O emissions increase equates to <0.1 Mg CO₂eq ha⁻¹ yr⁻¹, a small offset to the subsoil organic C storage rate estimated here that is equal to 503 504 ~1.14 Mg CO₂-eq ha⁻¹ yr⁻¹. Like N₂O, changes in CH₄ emissions may also partially offset the soil 505 C increase, but CH₄ emissions are typically <10% of the net global warming potential of N₂O in 506 maize-based cropping systems in the Midwest US (Robertson et al., 2000) and thus would not 507 substantially offset organic C gains in subsoils.

508

Management of subsoil organic C stocks

509 About 25% of the land area in the region is classified as poorly drained (Table 1) and 510 relies on artificial drainage for agricultural production (Sugg, 2007). Our observational dataset 511 shows that the poorly drained soils had the greatest rates of soil organic C stock changes over 512 time and made up 42% of regional subsoil organic C increases (Table 1). Drainage systems in 513 the region were designed for precipitation levels of the early 1900s, which were $\sim 20\%$ below 514 current levels (Fig 4A; (Helmers, Melvin, & Lemke, 2009)). A large body of research shows that 515 insufficient soil drainage decreases potential crop yields by delaying field operations, limiting 516 root growth, and increasing the risks of plant disease and insect infestation (Castellano et al., 517 2019). Without adequate drainage, excess soil moisture is expected to cause a 7.4% decrease in 518 maize yield and cost 3 billion USD per year in lost agricultural output by mid-Century 519 (Rosenzweig, Tubiello, Goldberg, Mills, & Bloomfield, 2002). Farmers have increased the area 520 of artificial drainage by 14% between 2012 and 2017 (USDA-NASS, 2017) and are likely to 521 continue to improve contemporary drainage systems in response to climate change (Hatfield et 522 al., 2014; Morton, Hobbs, Arbuckle, & Loy, 2015).

523 Expansion and intensification of drainage systems will improve crop growth and reduce 524 N₂O and CH₄ emissions, but may reverse the observed recent subsoil C gain (Castellano et al., 525 2019). Alternative drainage system designs may offer a solution to maintain crop yields while 526 minimizing global warming potential (Castellano et al., 2019). For example, drainage systems 527 with shallower placement and narrower spacing can increase the rate of surface soil drying

528 without draining subsoils. Controlled drainage systems decrease water discharge by only

529 operating when necessary. These systems achieve the crop production benefits of conventional

530 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
- 533 crop yield, soil organic C stocks, and greenhouse gas fluxes to ensure that adaptation of crop
- 534 production to climate change does not undermine efforts to mitigate greenhouse gas emissions
- 535 (Castellano et al., 2019).
- 536

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Table 1. Estimated rates of soil organic C change \pm 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,

795 20-40, 40-60, 60-80, and 0-80 cm.

	Measured change per area (Mg C ha ⁻¹ yr ⁻¹)					
Mass	Well drained	Moderately	Somewhat	Poorly	All sites	
increment	$(n=8)^{*}$	well drained	poorly drained	drained	(n=39)	
$(Mg ha^{-1})$		(n=8)	(n=8)	(n=15)		
0-2600	-0.125 ± 0.24	-0.164 ± 0.24	-0.220 ± 0.23	-0.395 ±	-0.257 ±	
				0.17	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	$\textbf{-0.010} \pm 0.14$	0.071 ± 0.15	0.230 ± 0.11	0.123 ± 0.07	
8400-11400	$\textbf{0.128} \pm \textbf{0.09}$	0.029 ± 0.07	$\textbf{-0.010} \pm 0.08$	0.138 ± 0.06	$\boldsymbol{0.078 \pm 0.04}$	
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	Well drained	Moderately	Somewhat	Poorly	All classes [†]	
increment	(9.21 Mha)	well drained	poorly drained	drained	(27.66 Mha)	
$(Mg ha^{-1})$		(4.66 Mha)	(6.92 Mha)	(6.87 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	$\textbf{2.87} \pm \textbf{2.82}$	
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	$\textbf{2.18} \pm \textbf{1.11}$	
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

799 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

817 Table 2. Estimated annual changes in C inputs attributed to the yield increase for a maize-

818 soybean rotation from 1950 to 2000 in Iowa and Illinois. Standard errors are shown in 819 parentheses.

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Cumulative C input Cumulative C input gain Depth Carbon input $(Mg C ha^{-1} yr^{-1})^*$ $(Mg C ha^{-1})^{\dagger}$ due to yield increase (cm) $(Mg C ha^{-1})^{\ddagger}$ 1950 2000 1950-2000 1950-2000 160.0 (0.22) 0-20 2.48 (0.028) 3.80 (0.033) 33.3 (1.53) 12.7 (0.09) 20-40 0.17 (0.002) 0.32 (0.003) 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 4.38 (0.038) 183.3 (0.31) 2.80 (0.032) 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

823 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

827 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.

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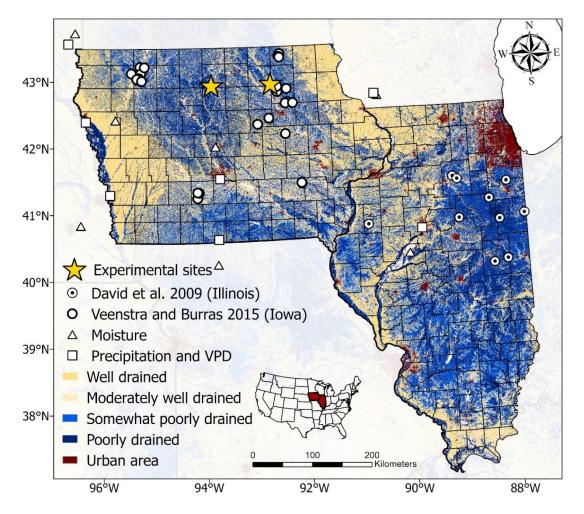




Figure 1. Soil sample locations (n=41) and weather stations (n=7 per weather variable) overlaid on a map showing USDA soil drainage classes. The region of interest encompasses Iowa and Illinois, but we included weather stations in close proximity of these states due to the limited number of weather stations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

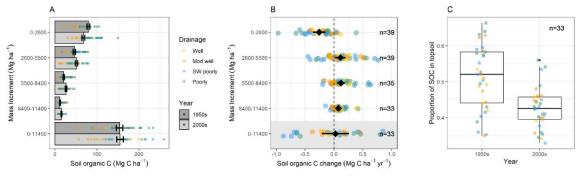
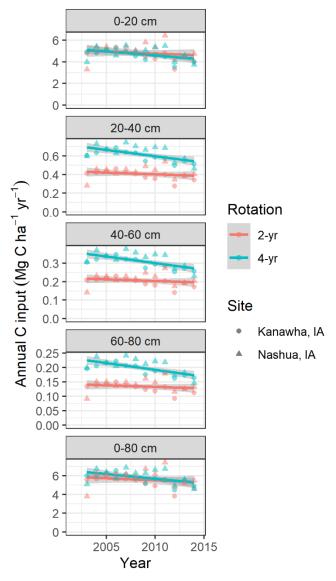
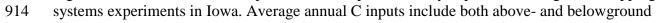




Figure 2. A) Soil organic C stocks at initial and resampling events by mass increment from the observational studies. Error bars represent \pm one standard error. B) Average annual soil organic C changes by mass increment (1950s-2000s). Error bars represent 95% confidence intervals. n represents the 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).



913 Figure 3. Historical annual C inputs for two crop rotations by depth at two long-term cropping



- 915 inputs. Shaded ribbons represent 95% confidence bands. n = 48 observations per depth 916 increment.

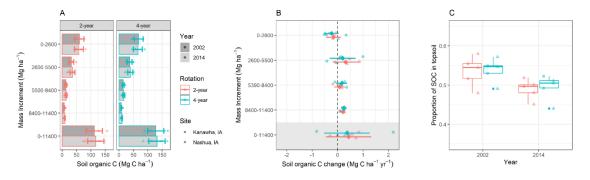
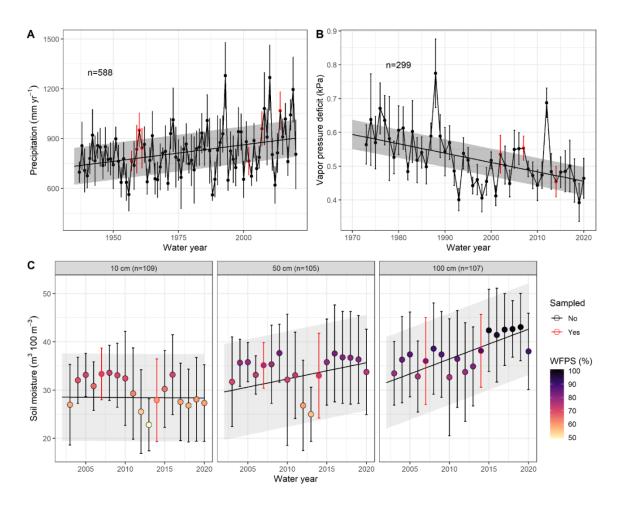




Figure 4. A) Soil organic C stocks at initial and resampling events by rotation and mass
increment from the experimental studies. Error bars represent ± one standard error. B) Average
annual soil organic C changes by rotation and mass increment (2002-2014). Error bars represent
95% confidence intervals. 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 (P), or hexplot (C)

- 937 point range (B), or boxplot (C).



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968 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

971 measurements. On plot C, the point color varies according to percentage water-filled pore space

972 (WFPS). Note different time periods due to different availabilities of long-term monitoring

973 records. Years in which soil sampling occurred are highlighted in red. Error bars represent 95%

974 confidence intervals and shaded ribbons represent 95% confidence bands.