



## An evaluation of carbon indicators of soil health in long-term agricultural experiments

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## ABSTRACT

Soil organic carbon (SOC) is closely tied to soil health. However, additional biological indicators may also provide insight about C dynamics and microbial activity. We used SOC and the other C indicators (potential C mineralization, permanganate oxidizable C, water extractable organic C, and  $\beta$ -glucosidase enzyme activity) from the North American Project to Evaluate Soil Health Measurements to examine the continental-scale drivers of these indicators, the relationships among indicators, and the effects of soil health practices on indicator values. All indicators had greater values at cooler temperatures, and most were greater with increased precipitation and clay content. The indicators were strongly correlated with each other at the site-level, with the strongest relationship between SOC and permanganate oxidizable C. The indicator values responded positively to decreased tillage, inclusion of cover crops, application of organic nutrients, and retention of crop residue, but not the

number of harvested crops in a rotation. The effect of decreased tillage on the C indicators was generally greater at sites with higher precipitation. The magnitude and direction of the response to soil health practices was consistent across indicators within a site but measuring at least two indicators would provide additional confidence of the effects of management, especially for tillage. All C indicators responded to management, an essential criterion for evaluating soil health. Balancing the cost, sensitivity, interpretability, and availability at commercial labs, a 24-hr potential C mineralization assay could deliver the most benefit to measure in conjunction with SOC.

## 1. Introduction

A variety of terms have been used over the last century to describe soil condition in agricultural systems, e.g., soil tilth, soil quality, and most recently soil health. Although these terms as descriptions of soil condition are overlapping or sometimes used synonymously, soil health is distinct because it includes the living organisms in soils. Soil health has been defined by the U.S. Department of Agriculture Natural Resource Conservation Service as “the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans” (USDA-NRCS, 2022). Because “living” is in the definition of soil health, it seems essential to measure biological indicators of soil health, as they reflect the direct and indirect imprint of organisms on the soil. A recent review of the measurements of soil conditions found that soil organic C (SOC) or soil organic matter was the most widely measured indicator (Bünemann et al., 2018). The soil organic matter with a backbone of C forms the basis of the soil food web and biological activity and is linked to other soil functions like nutrient cycling, water cycling, and greenhouse gas emissions (Lal, 2016). As soil health is an abstraction, soil health indicators provide concrete methods to quantify soil health. These indicators must be related to soil functions but should also meet the following criteria: be responsive to management, be easy and inexpensive to collect and measure, and be interpretable by land managers (Doran and Zeiss, 2000). Interpretability has multiple components including answering the following questions: does the absolute value of the indicator depend on soil texture or climate?; does the response to management depend on soil texture or climate?; is the methodology sensitive to soil texture?; and is it easy to understand what the analysis is measuring?

Soil organic C is frequently measured to assess soil health, but it is an emergent property that represents the net balance of inputs and outputs of C to the soil over time. These inputs are largely root exudates and senescent leaves, stems, and roots, but also the deposition of materials transported by wind and water. In agricultural systems these inputs can include organic amendments (e.g., manure, compost, biosolids, biochar) to supply nutrients or organic matter. These inputs are counterbalanced by the C outputs, dominated by the mineralization of SOC to carbon dioxide by microbes. Although not an output from the soil system, microbial degradation and transformation of plant inputs creates a different suite of microbially derived organic compounds in the soil (Grandy and Neff, 2008). Outputs also include any harvested crops, residue burning and erosion. Typical soil health measurements are of the surface soils, at most 30 cm deep, meaning that transport of C deeper into the soil profile by water or pedoturbation would also result in a decrease in the measured SOC.

Historically, predicting soil C has been based on the quantification of C in operationally defined soil C pools and the rates of transformation of C in these pools (Parton et al., 1988). More recently, microbial processes have been explicitly included in the conceptualizations of C cycling (Cotrufo et al., 2013; Wieder et al., 2014). However, these approaches generally produce a tension between two soil health goals: C storage and C mineralization (Janzen, 2006). It is hard to reconcile high biological activity, which mineralizes more C, with increasing the amount of SOC. As we learn more about the interactions among the microbial communities, the chemical environment, the chemistry of organic matter, and the movement of C through the physical structure of soil, we can conceptualize how these two soil health goals can both be met (Waring

et al., 2020). It appears that microbial carbon use efficiency and the stabilization of microbial-derived C play a key role in SOC storage (Liang et al., 2017). Nonetheless, a need exists to evaluate how to select indicators of C dynamics because it is not feasible to measure all of them.

There are a variety of ways to explore the connection between C and function such as quantifying the physical, chemical, or biological fractions of carbon that comprise SOC along with the microbial activity associated with C cycling. For example, there are fractionation schemes based on the particle size and density (Grandy and Robertson, 2007; Lavalley et al., 2020). Using isotopes, it can be shown that fresh plant material changes in abundance over time in these different fractions (Haddix et al., 2020). Chemically defined pools, like the permanganate oxidizable C (POX-C; Weil et al., 2003) or the water extractable organic C (WEOC; Haney et al., 2012) have been suggested as indicators of C compounds easily catabolized by microbes. Measurements of potential C mineralization in the laboratory provide an assay of the amount of C that microbes catabolize (Haney et al., 2008). This is distinct from measurements of respiration *in situ*, which capture the metabolic activity of the whole microbial community, and potentially plant roots, under field conditions. However, field respiration is rarely suggested as a soil health measurement because of the lack of standard conditions and the complication that it can include root respiration. The activity of enzymes, such as  $\beta$ -Glucosidase (BG), can be a proxy for C cycling (Acosta-Martínez et al., 2011). The microbial biomass itself can be quantified by a variety of methods (e.g., direct counts, chloroform fumigation, or biomarkers). These assays of C fractions or microbial activity and biomass can be evaluated individually or can contribute to a soil health index (Andrews et al., 2004; Fine et al., 2017; Haney et al., 2018) (Table 1). While all these assays are linked to soil function, not all of them would meet the other three criteria of an indicator as defined by Doran and Zeiss (2000).

If the C dynamics in the soil system were fixed, that is, the inputs, outputs and microbial processes were not changing, then all the indicators would always provide a picture of the C dynamics of the system. However, seasonal changes in temperature, precipitation, plant phenology, tillage, fertilization, rotating crops, climate change, and the interactions among multiple factors, all have the potential to change the inputs, outputs, and microbial activity. For example, microbial activity and biomass change seasonally (McDaniel and Grandy, 2016); nitrogen additions over time change the amount of SOC in different fractions (Rocci et al., 2021); and tillage leads to increased CO<sub>2</sub> emissions and lower SOC (Conant et al., 2007; Reicosky et al., 1997). Untangling all the

**Table 1**

Carbon indicators included in common soil health indices: Soil Management Assessment Framework (SMAF), the Haney Soil Health Tool (Haney), and the Cornell Assessment of Soil Health (CASH).

Indicator	SMAF	Haney	CASH
Soil organic carbon	Y		Y <sup>a</sup>
Permanganate oxidizable carbon			Y
Potential carbon mineralization		Y <sup>b</sup>	Y <sup>c</sup>
$\beta$ -glucosidase enzyme activity	Y		
Water extractable organic carbon		Y	

<sup>a</sup> Loss on ignition for soil organic matter instead of dry combustion for soil organic carbon.

<sup>b</sup> 24-h C mineralization.

<sup>c</sup> 96-h C mineralization.

possible ways that these factors can interact is difficult. The amount, type, timing, and location of C additions varies, and that variability is influenced by changes in the weather and soil structure, resulting in changes in the microbial activity or community. There can also be feedbacks where adding small amounts of new organic matter can stimulate the degradation of existing SOC (Mitchell et al., 2020) or the accumulation of additional SOC (Ryals et al., 2014). One goal of a soil health indicator is to provide information on the functions and services related to soil C across the range of soil types, managements and climates.

Although it is necessary for soil health indicators to reflect the functions or services related to C, they must also be sensitive to agricultural management. If the indicators respond to change too slowly, have high spatial or temporal variability, or otherwise do not respond to management, then they cannot provide information on whether practices are altering soil health. There is a voluminous literature that describes the sensitivity of soil health indicators in surface (<30 cm) soils, especially SOC, to management. As a result, the effects of multiple practices (e.g., tillage, cover crops, organic nutrients, crop rotations) on multiple C indicators have been synthesized (Nunes et al., 2020; Jian et al., 2020; Bai et al., 2019; King and Blesh 2018; Han et al., 2016; McDaniel et al., 2014; West and Post 2002). Studies across multiple sites have shown that the indicators themselves (Culman et al., 2012) and the relative responses of indicators to management (Nunes et al., 2020) can be highly correlated. However, one limitation of using the published literature to test these relationships is that the sampling depths can vary among studies, the sample timing can vary in terms of seasonality and relative to management operations, the laboratory methods can vary for the same indicators, and the management history is often sparse.

The goal of the present study was to evaluate six potential indicators of soil health (SOC, POX-C, two potential C mineralization assays, BG, and WEOC) related to C that were analyzed with the same lab methods on soils collected with the same sampling approach at sites with long-term experimental manipulation of soil health promoting practices across the major agricultural areas of North America. Specifically, we examined the relative response of indicators to soil health practices to determine if they responded to management. We evaluated some facets of the interpretability of the indicators by testing the role of site factors in explaining variability in the absolute values of the indicators, and each indicator's response to management. Further, we explored the relationships among the six indicators and the similarity of their responses to the site factors and management. Finally, we combined the results of this quantitative analysis with a qualitative discussion of the interpretability of the indicator methods based on their strengths and weaknesses to make recommendations on choosing soil health indicators related to C.

## 2. Methods

### 2.1. Sample collection

Data used in this study came from the North American Project to Evaluate Soil Health Measurements (NAPESHM). A brief description is provided here, but more details are in Norris et al. (2020). This study collected soils at 124 long-term experimental agricultural research sites with documented management histories (Table S1). Scientists volunteered to include their sites with experimental treatments that compared the effects of six practices: tillage, cover cropping, crop rotations, nutrient amendments, irrigation, or livestock grazing intensity. The management histories and site characteristics were compiled in consultation with scientists at each site to describe the type and timing of tillage, fertilization, crop rotations, irrigation, and grazing. One site was removed from the data analysis because the management history was incomplete. At each site the treatments relevant to the six practices described above were identified and the replicates, typically one to four depending on the experimental design, of each treatment were sampled.

We collected soils from 2032 experimental units (EUs) at 124 sites, but 20 EUs lacked management data. The 2012 EUs with management data represented 688 treatments. In experiments where all phases of the crop rotation were present, only one phase of the rotation was selected for sampling, with the exception of one site, where both the corn and soybean phase were sampled. In general, we targeted the sampling time as between spring thaw and planting in northern locations and between crops at southern sites. With the exception of six sites which were sampled in fall, all other sites were sampled between February and July 2019. We attempted to sample prior to planting and any other management activities, with the exact timing dependent on crop and geography. However, in at least one treatment at nineteen sites, the tillage, fertilization, or planting had occurred within the month prior to soil collection.

Mean temperature and precipitation were calculated for each site from Daymet using the daily weather data from 2009 to 2019 (Thornton et al., 2016). The sites spanned 36° latitude and 59° longitude with mean annual temperature and precipitation ranging from 3 to 25 °C and 178 to 1773 mm yr<sup>-1</sup>. Irrigation, when present, was quantified annually based on the typical site management and added to mean annual precipitation.

Although the experimental treatments at the sites differed, the soils were always collected in the same way. In each replicate of each treatment at each site, a 15 × 15 × 15 cm hole was carefully created with a spade at six locations in a "W" pattern across the plot. If there were permanent and identifiable rows and furrows, half the samples were collected in rows and half in furrows. At each of the six holes, a soil knife was used to collect a 2.5 × 2.5 cm slice of soil from undisturbed sides of the hole down to 15 cm. The knife slices were homogenized and split to ship to the three laboratories to measure the indicators in the present study. Cornell Soil Health Laboratory and the Soil Water and Environmental Laboratory at Ohio State University received the soils on average three days after shipment. The subsamples for Ward Laboratory in Kearney, NE were sieved with an 8 mm sieve and shipped in extruded polystyrene foam coolers with ice packs to meet the requirements for other microbial community indicators not included in this study, and on average arrived two days after shipment. All samples were air dried and sieved to 2 mm prior to the analysis of the indicators included in this study.

### 2.2. Lab methods

Six indicators were evaluated from the NAPESHM database: SOC, POX-C, 96 h C mineralization (Cmin-96), 24 h C mineralization (Cmin-24), WEOC, and BG enzyme activity (Norris et al., 2020). The SOC was measured at Ohio State, POX-C and Cmin-96 were measured at Cornell, and Cmin-24, WEOC, and BG were measured at Ward Laboratories. The SOC concentration (%) was calculated as the difference between total C and inorganic C. Total C was measured by dry combustion with a CE Instruments (Lakewood, NJ) NC2100 soil analyzer (Nelson and Sommers, 1996). The presence of inorganic C was determined by fizzing with HCl and when present, inorganic C was quantified with Chittick's volumetric calcimeter method (Dreimanis, 1962). POX-C was quantified (mg C g<sup>-1</sup> soil) as the colorimetric change from the reduction of the manganese in a 0.2 M KMnO<sub>4</sub> solution with 2.5 g of air-dried soil (Moebius-Clune et al., 2016; Weil et al., 2003). In highly organic soils with POX-C > 1400 mg C g<sup>-1</sup>, the KMnO<sub>4</sub> can be completely reduced. In these cases, the assay was run with 1.25 g of air dried soil. Cmin-96 was determined by quantifying the carbon dioxide produced when 7.5 mL deionized water was added to 20 g of air-dried soil in an airtight jar with a KOH trap at room temperature for 96 h (Moebius-Clune et al., 2016). Cmin-24 was quantified using an infrared gas analyzer to measure the carbon dioxide concentration after 24 h in the headspace of an airtight jar with 40 g of air-dried soil that was allowed to rewet to approximately field capacity by capillary action (Haney et al., 2018). To facilitate the comparison of Cmin assays, both were reported with units of mg C kg<sup>-1</sup> d<sup>-1</sup>. The WEOC was quantified in mg C kg<sup>-1</sup> on a combustion total

organic C analyzer after 4 g of air-dried soil was shaken for 5 min with 40 mL of deionized water (Haney et al., 2018). The WEOC and Cmin-24 were not run for the 13 treatments at one site, representing less than 2% of treatments and 1% of the sites, because the soils were lost prior to analysis. The BG enzyme activity was quantified by incubating 1 g of air-dried soil in 4 mL of tris(hydroxymethyl)aminomethane buffer at pH 6 for 1 h at 37 °C with p-Nitrophenyl- $\beta$ -D-glucoside as the substrate. The amount of p-nitrophenol produced by the action of the enzyme on the substrate was measured ( $\text{mg pNP kg}^{-1} \text{ hr}^{-1}$ ) as the absorbance at 405 nm on a spectrophotometer (Deng and Popova, 2011). Soil pH was quantified in a 1:2 soil:water slurry with a pH electrode (Thomas, 1996).

### 2.3. Statistical approach

The data analysis was performed in RStudio Version 2021.09.1. Unless otherwise specified, all analyses were done with base R functions, and statistical significance was determined at  $p < 0.05$ . To explore the variability of the indicators, a nested model was used to partition the variance into among site, among treatments within sites, and among field replicates within treatments using the lme4 package (Bates et al., 2015). For treatments with at least three field replicates, the within treatment variability was assessed with the coefficient of variation. This analysis included 63% of the treatments and 79% of the EUs.

The similarity of the treatment averages of the C indicators were explored with a combination of correlation and regression based approaches. First, the distribution of the indicators was explored with histograms (Fig. S1). Based on the distributions, indicators were log transformed for analyses. First, the relationship among treatment means was evaluated with a correlation matrix (Oksanen et al., 2019). Second, we used a stepwise regression approach in the olsrr package (Hebbali, 2020) to determine the predictability of each C indicator by the other C indicators.

To explore the relationship between the site means of the C indicators and inherent site characteristics, we used two approaches. First, we used a multiple regression model for each of the C indicators, using the site characteristics of clay content, sand content, pH, temperature and precipitation (+irrigation) to predict the log transformed site means of the indicators. All five predictors were included in the final model regardless of their significance. Second, because of the high correlation of the C indicators, we used the rda function in the vegan package for a redundancy analysis (RDA) to explore how the suite of site variables could predict the suite of C indicators (Oksanen et al., 2019). Variables were scaled to use the correlation matrix for the ordination.

To determine the response of the indicators to management, we used a meta-analysis approach to compare treatments within sites that differed in only one of six soil health promoting practices (Table 2). Only sites with the appropriate treatment pairs were included in this analysis. The type and frequency of the tillage equipment was cataloged for each treatment, and a standard tillage intensity rating (STIR) value for each operation that disturbed the soils was assigned (USDA-ARS, 2022). Paired treatments were included if the management was the same and only tillage varied, as determined by differences in either the maximum STIR value or the sum of the STIR values for that rotation. Cover crops was a comparison between a treatment with a cover crop for at least one year of a rotation compared to a treatment with no cover crops in the rotation. A cover crop was defined as a crop or crop mix that was planted, persisted for less than one year, and was terminated by herbicides, fall frost, or tillage, but never harvested during the rotation. We did not include any treatment pairs that compared two cover crops, only the presence vs the absence of a cover crop. Organic nutrients was a comparison of treatments where organic inputs (biosolids, compost, or manure) replaced either the nitrogen, phosphorus, or both in commercial fertilizer. Crop count was a comparison between monocultures to rotations with at least two distinct cash crops of any kind. Rotation diversity was a comparison between rotations with only grains (e.g., continuous corn, wheat/sorghum) to a rotation with other types of

**Table 2**

Treatment pairs included for the response ratios in the meta-analysis of management effects. Reduced tillage compared treatments that differed in the maximum intensity of tillage. Organic nutrients were treatments where manure, compost, or biosolids were used in place of commercial fertilizer. Cover crops included any treatment where a crop was grown and not harvested at least once during the rotation. Residue retention was an increase in residue left in the field for at least one cash crop in the rotation. Crop count was the number of cash crops harvested during the rotation, i.e. excluding cover crops. Crop types for rotation diversity other than grains were legumes, canola, safflower, and cotton excluding cover crops. Some sites had multiple soil health practices.

Soil Health Practice	Treatment: Control	Paired Treatments	Number of Sites
Decreased tillage	Lower tillage intensity: higher tillage intensity	160	51
Organic nutrients	Organic nutrients: commercial fertilizer	31	12
Cover crops	Cover crops: no cover crops	21	10
Crop count	More than 1 cash crop: 1 cash crop	199	33
Rotation diversity	More than one crop type: only grains	63	24
Residue retention	Residue retained: residue removed	54	14

crops, typically legumes, but also canola, safflower, or cotton. Treatments with fallow years were excluded. Residue removal compared treatments where the crop rotation was identical, but the amount of residue removed differed for at least one year of the rotation. In some cases, more than one treatment pair was possible at each site (e.g. continuous corn with intense tillage vs minimum tillage and corn/soybean with intense tillage vs minimum tillage).

The meta-analysis methods quantified the response of all indicators to adopting six soil health management practices: decreased tillage, inclusion of cover crops, application of organic nutrients, increased crop count, increased rotation diversity, and residue retention. Controlling for site as a random factor, we tested if there was a significant difference in each soil health indicator from the adoption of each of the six soil health practices compared to the business as usual practices – a total of thirty-six separate meta-analyses. The analysis was performed with the metafor package using log response ratios as the metric on untransformed treatment means and variances (Viechtbauer, 2010).

We used the log response ratios to further explore whether the response to decreased tillage was affected by site factors, and whether the response of the indicators was similar for the significant treatment effects. We used a regression approach to examine if the site averages of the log response ratios were related to any single site property: precipitation + irrigation, temperature, sand content, clay content, and pH. The relationship among the six indicators to decreased tillage was explored with a principal components analysis using the prcomp function in the vegan package because tillage had sufficient sites for analysis (Oksanen et al., 2019). We used the site averages of the log response ratios and the correlation matrix to standardize the responses. To compare if all the C indicators responded in similar ways to management within a site, we determined if the response of each indicator to each practice was positive, neutral, or negative at each site based on a relativization of the site averages of the log response ratios. The response was based on the size of the confidence limits and delineated as negative if the log response ratio was less than  $-1.96*SE$ , neutral for a log response ratio between  $-1.96*SE$  and  $1.96*SE$ , and positive for a log response ratio greater than  $1.96*SE$ , where SE was the standard error of the log response ratio for an indicator.

### 3. Results

Based on the nested models, the variance was partitioned similarly across indicators, with the majority (63–80%) of the total variance

among sites with the remainder about evenly split among treatments and field replicates (Table S2). Within treatments, the indicators differed in the amount of variability. The mean coefficient of variation among treatments with at least three field replicates ranged from 10 to 15% for POX-C, SOC, Cmin-96, and WEOC and was ~25% for Cmin-24 and BG (Fig. 1).

The strongest correlation ( $r = 0.91$ ) was between POX-C and SOC (Table 3). For all other pairs of indicators, the correlations ranged from 0.47 to 0.71. The stepwise regression models further highlight these bivariate relationships by showing that the first predictor selected could explain almost all the variance in the full model (Table 4). Only WEOC had a second significant predictor that increased the overall adjusted  $R^2$  by more than 0.05. The POX-C or SOC was the first predictor selected for four of the six indicators, and BG and WEOC were the best predictors of each other.

The adjusted  $R^2$  for the multiple regression models to predict C indicators with inherent site characteristics ranged from 0.27 for Cmin-24 to 0.50 for POX-C (Table 5). Temperature was a significant predictor for every indicator, along with clay content for all except Cmin-96. Precipitation was significant for POX-C, SOC, and both C mineralization indicators, and pH was significant for both mineralization indicators and BG. The RDA suggests that the inherent site variables can predict some of the structure of the C indicator matrix (Fig. 2). The first and second RDA axes explained 31% and 7% of the variance, respectively. The first axis was positively associated with sand content and temperature, and the second axis was positively associated with precipitation and negatively associated with pH and clay content. There were generally two groupings of indicators: SOC, POX-C, and both C mineralization indicators were more associated with sand content and temperature, but WEOC and BG were more correlated with clay content, pH, and precipitation. These patterns largely align with the first and second RDA axes.

Based on the meta-analysis, the indicators generally responded to soil health practices other than increasing crop count and rotation diversity (Fig. 3). The indicators were significantly higher in response to

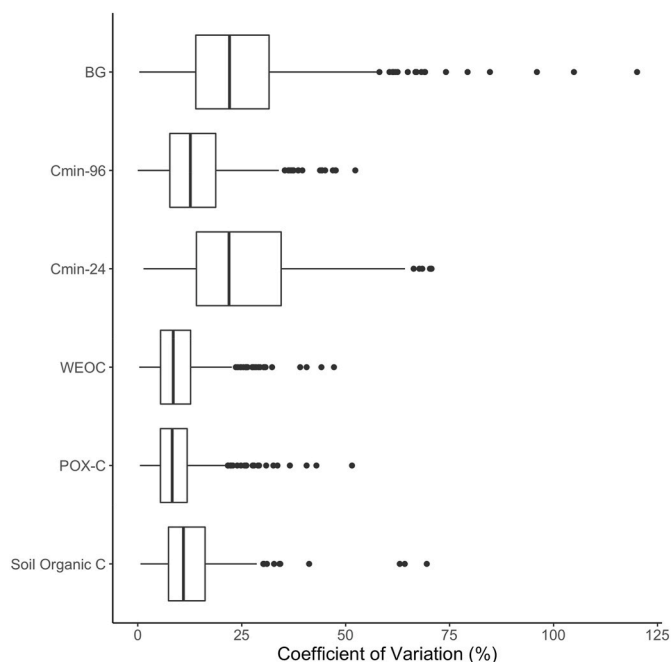


Fig. 1. Boxplots of coefficient of variation for the carbon indicators for the 432 treatments with at least three field replicates. POX-C is permanganate oxidizable carbon; WEOC is water extractable organic carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; and BG is  $\beta$ -glucosidase.

Table 3

Correlations among log transformed treatment means of C indicators. POX-C is permanganate oxidizable carbon; WEOC is water extractable organic carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; and BG is  $\beta$ -glucosidase. All are significant at  $p < 0.0001$ .

	SOC	POX-C	Cmin-24	Cmin-96	WEOC	BG
SOC	1.00	0.91	0.67	0.67	0.64	0.54
POX-C	0.91	1.00	0.67	0.65	0.55	0.47
Cmin-24	0.67	0.67	1.00	0.62	0.54	0.47
Cmin-96	0.67	0.65	0.62	1.00	0.60	0.58
WEOC	0.64	0.55	0.54	0.60	1.00	0.71
BG	0.54	0.47	0.47	0.58	0.71	1.00

Table 4

Adjusted  $R^2$  for stepwise regression models for all carbon indicators. The first predictor selected and its  $R^2$  are shown along with the  $R^2$  for all the predictors included in the final model. All indicators had at least two other indicators as significant predictors. SOC is soil organic carbon; POX-C is permanganate oxidizable carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; WEOC is water extractable organic carbon; and BG is  $\beta$ -glucosidase.

Carbon Indicator	First Predictor	First Predictor $R^2$	Final adjusted $R^2$
SOC	POX-C	0.80	0.84
POX-C	SOC	0.80	0.83
Cmin-24	POX-C	0.44	0.52
Cmin-96	SOC	0.47	0.58
WEOC <sup>a</sup>	BG	0.50	0.62
BG	WEOC	0.50	0.55

<sup>a</sup> Inclusion of second predictor (SOC) increased adjusted  $R^2$  by more than 5%.

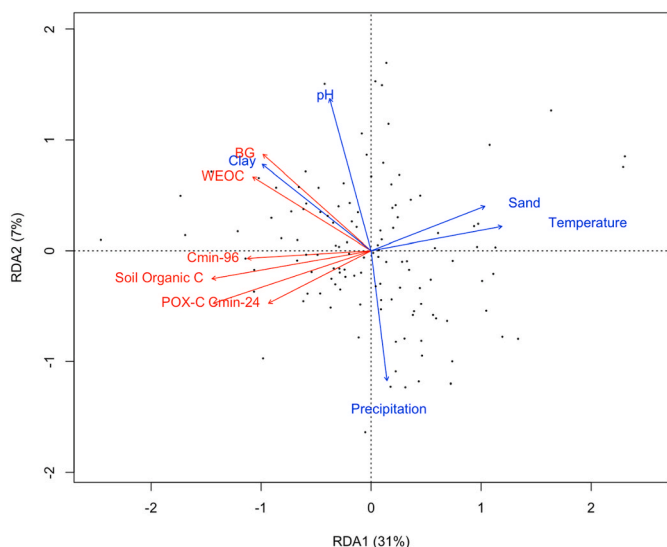
Table 5

Adjusted  $R^2$  for multiple regression models for site means of carbon indicators. Significant positive (+) and negative (-) predictors are shown as are non-significant predictors (NS) in the full model. Precipitation includes irrigation at sites with irrigation. All C indicators were log transformed. SOC is soil organic carbon; POX-C is permanganate oxidizable carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; WEOC is water extractable organic carbon; and BG is  $\beta$ -glucosidase. NS indicates the predictor was not significant in the full multiple regression model.

Carbon Indicator	Sand	Clay	pH	Temperature	Precipitation	Adjusted $R^2$
SOC	NS	+	NS	-	+	0.48
POX-C	NS	+	NS	-	+	0.50
Cmin-24	NS	+	+	-	+	0.27
Cmin-96	NS	NS	+	-	+	0.36
WEOC	NS	+	NS	-	NS	0.35
BG	NS	+	+	-	NS	0.36

the addition of organic nutrients, decreased tillage, residue retention (not significant for Cmin-96), and inclusion of cover crops. The positive effects of management on the indicators ranged from 12 to 33% for organic nutrients, 5–27% with decreased tillage, 10–33% with residue retention, and 11–42% with cover crops. In general, the indicators did not change in response to increasing the number of cash crops or the diversity of the rotation, that is having another crop type (legume, brassica, or other) in addition to at least one grain crop. However, there was a significant decrease for POX-C, and a marginally significant response ( $p < 0.1$ ) for SOC to rotation diversity.

The only significant predictor of the change in the magnitude of indicator responses (i.e. log response ratio) to decreased tillage was mean annual precipitation plus irrigation (Fig. 4). There was a significant positive relationship between precipitation plus irrigation and the site-averaged response ratios for decreased tillage with POX-C, BG, and the mineralization measurements, with the  $r^2$  ranging from 0.07 for BG to 0.15 for Cmin-96. That is, although the change in the value of the C indicators was significant in response to decreased tillage across all sites,



**Fig. 2.** Redundancy analysis predicting carbon indicators with site properties. Blue arrows indicate site variables; red arrows indicate carbon indicators; and black circles are sites. POX-C is permanganate oxidizable carbon; WEOC is water extractable organic carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; and BG is  $\beta$ -glucosidase.

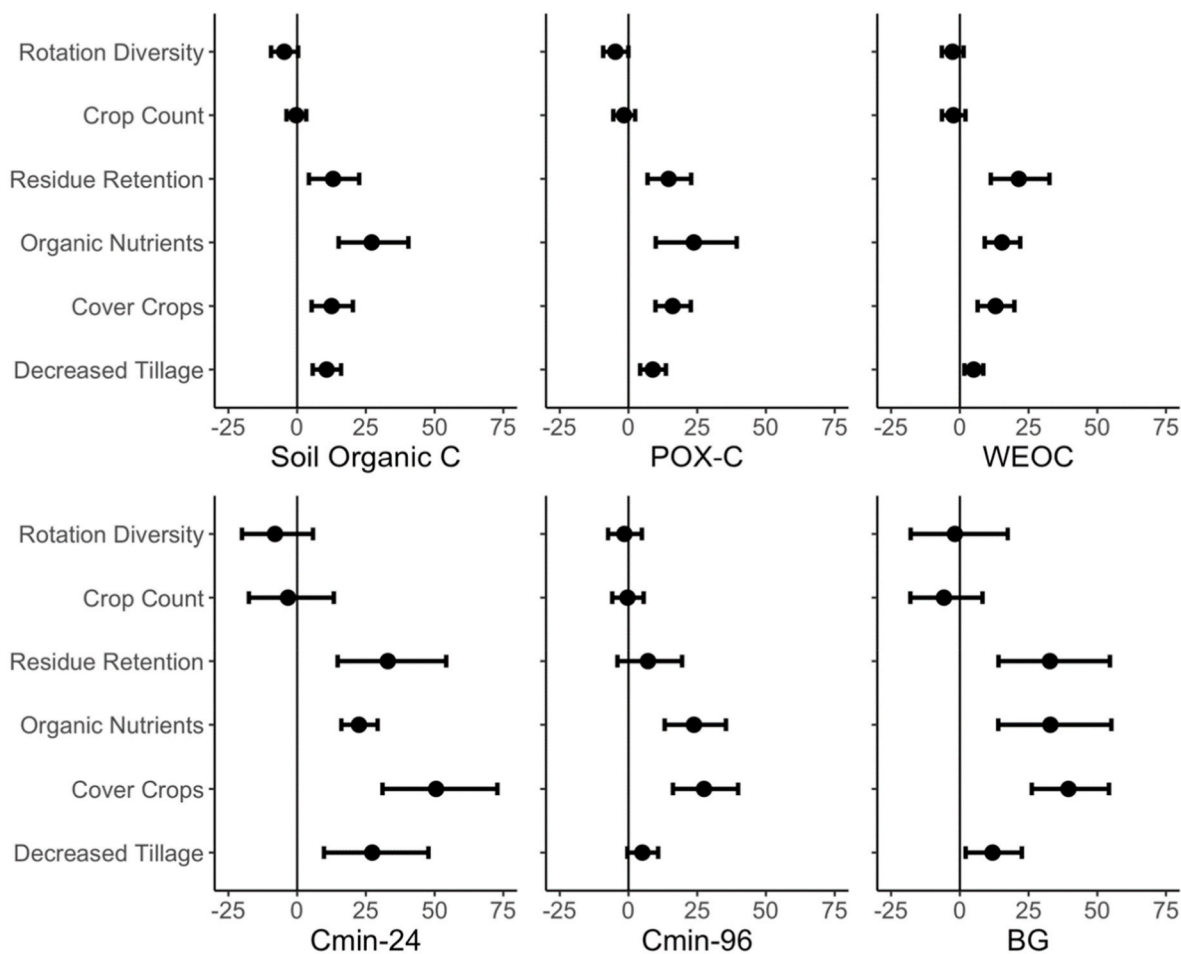
the magnitude of the effect increased as water inputs increased. For the four significant indicators, the best-fit regression line crossed the x-axis between 350 and 700 mm of water inputs, suggesting that the more arid sites showed a negative response to decreased tillage.

The paired treatments had similar responses across indicators in terms of their response ratios even though the response of the indicators did not agree for each treatment pair. The similar values on the first PC for the response ratios for decreased tillage emphasizes that the indicators responded to management in a similar way (Fig. 5). The visualization of the indicator responses also shows that the indicators generally responded in the same way (positive, neutral or negative) across sites (Fig. 6). For cover crops, all but two of the sites responded positively for the majority of the indicators. The response to organic nutrients and residue retention was similar to cover crops, although there were more neutral responses. None of the sites ever had a negative response for a majority of the indicators for these practices. The response to decreased tillage was less consistent; although more than half the sites had a majority of positive indicator responses, there were five sites with a majority of negative indicator responses. Further, there were many sites with a mixture of positive, neutral, and negative indicator responses.

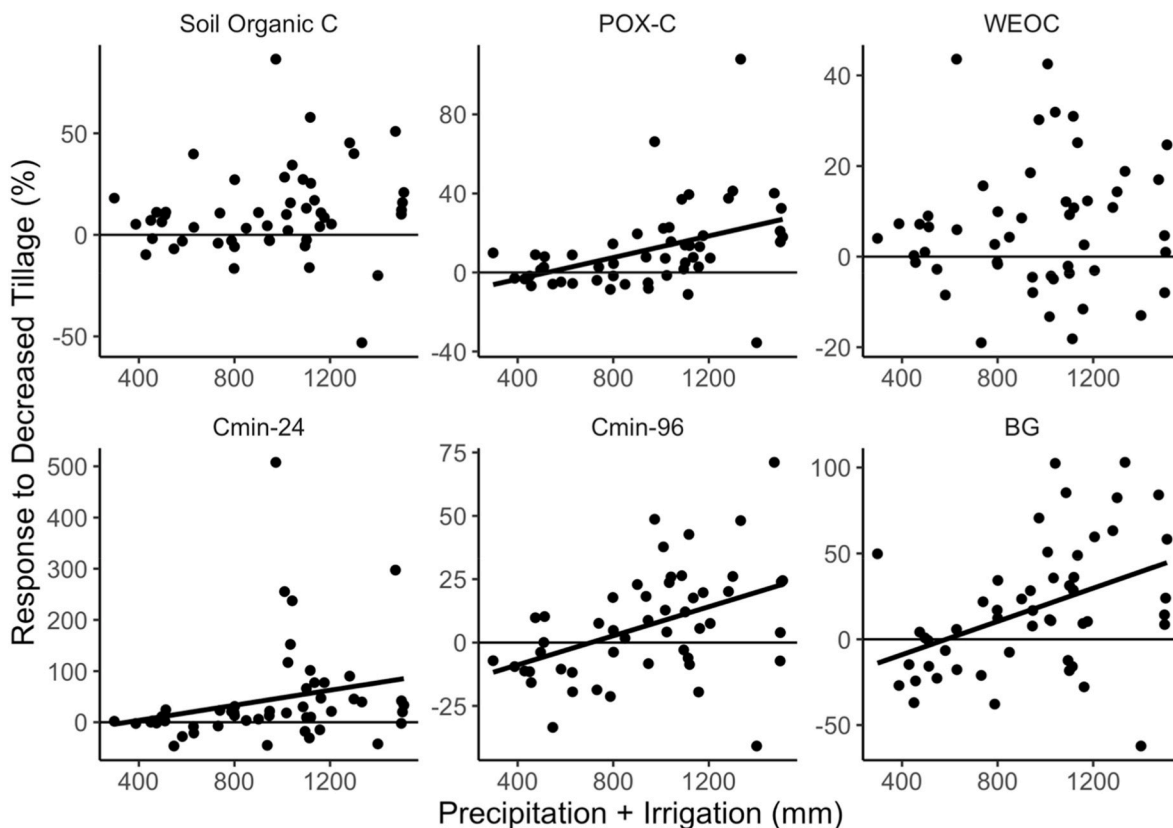
#### 4. Discussion

##### 4.1. Role of site

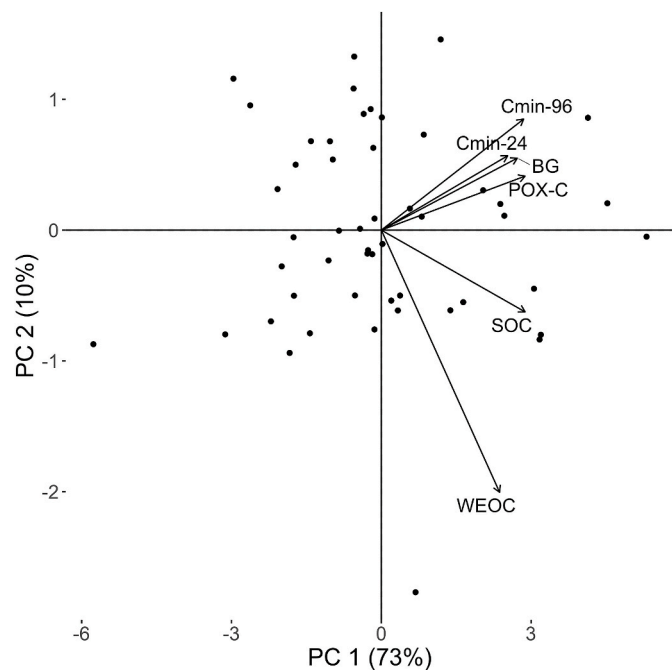
Based on the multiple regression analysis, we found that between one



**Fig. 3.** Percent difference in soil health practices by indicator for each management. The number of treatment pairs in the analysis are in Table 2. Black symbols are means and whiskers represent 95% confidence limits. POX-C is permanganate oxidizable carbon; WEOC is water extractable organic carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; and BG is  $\beta$ -glucosidase.



**Fig. 4.** Relationship between the percent difference in site averaged carbon (C) indicators in response to decreased tillage and precipitation (including irrigation). The overall effect was significant for all indicators except 96-h C mineralization. Solid lines indicate the linear best fit regression line for significant relationships between indicators and precipitation ( $p < 0.05$ ). POX-C is permanganate oxidizable carbon ( $r^2 = 0.16$ ); WEOC is water extractable organic carbon; Cmin-24 is 24-h potential carbon mineralization ( $r^2 = 0.10$ ); Cmin-96 is 96-h potential carbon mineralization ( $r^2 = 0.17$ ); and BG is  $\beta$ -glucosidase ( $r^2 = 0.14$ ).

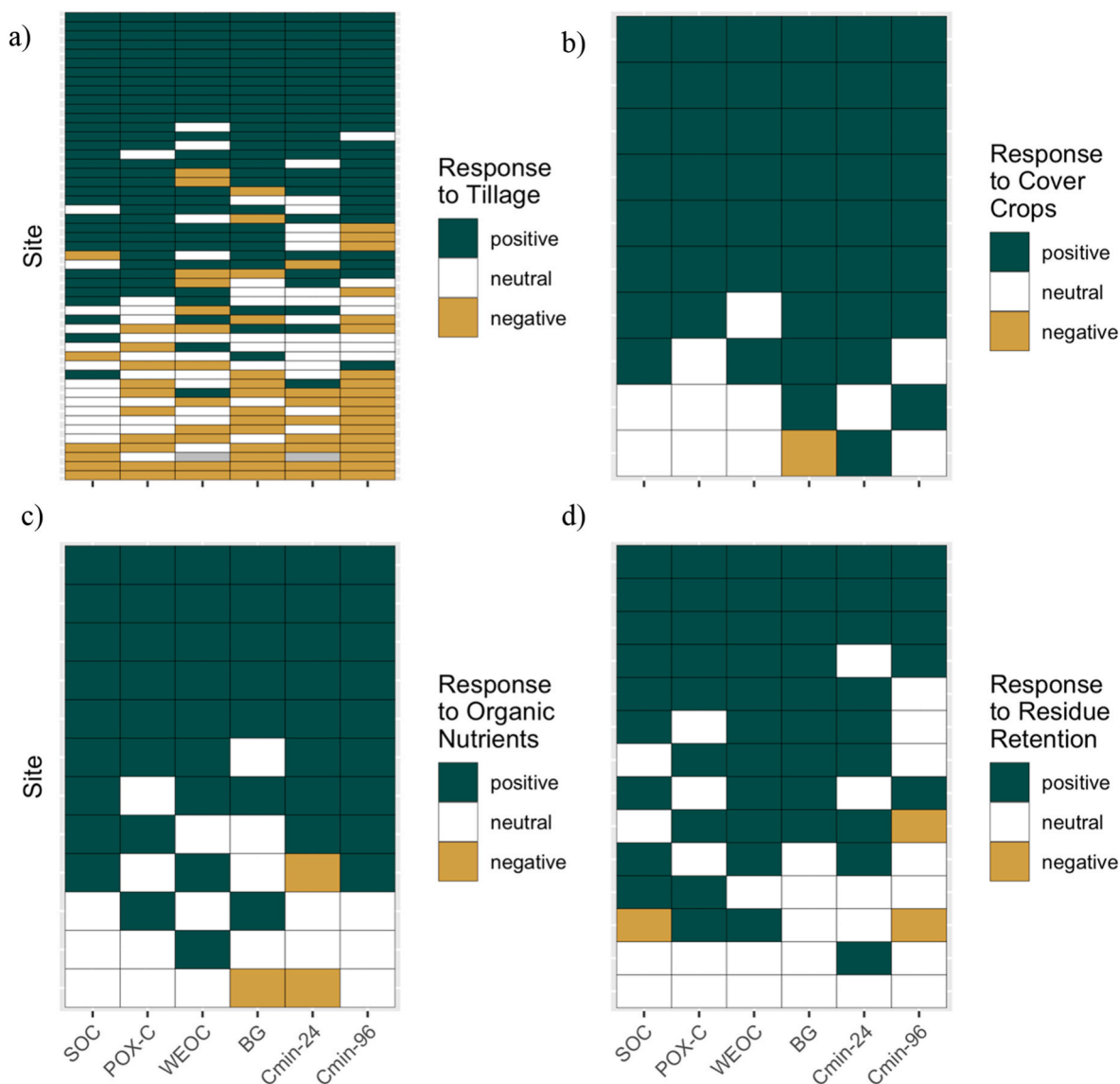


**Fig. 5.** Principal components analysis of the relationship among response ratios for carbon indicators to tillage. Black circles represent site averages of treatment pairs. SOC is soil organic carbon; POX-C is permanganate oxidizable carbon; WEOC is water extractable organic carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; and BG is  $\beta$ -glucosidase.

quarter and one half of the variance in the site means of the C indicators could be explained by the soil texture, climate, and pH, with POX-C and SOC the most predictable indicators (Table 5). This suggests that it is important to consider the site characteristics when interpreting the absolute values of the indicators. These site effects are the reason that many soil health assessment tools take soil texture and/or climate into account (Nunes et al., 2021). Although the treatments within sites could include annual or perennial crops as well as unmanaged perennials, the predictability was similar if the regression analysis was limited to the annual crops. It is not surprising that the site-level variables could predict one half of the SOC variability as there are decades of research at various spatial scales to support this finding. At small spatial scales, such as a catena (Schimel et al., 1985) or agricultural field (Jaynes et al., 1995), clay is linked to SOC. In Mollisols of the U.S. Great Plains, regression models could predict about half of the variance in SOC pools to 20 cm, with climate being more important than texture (Burke et al., 1989). Although Burke et al. (1989) included polynomial terms and interactions as predictors, in general they found similar relationships to the present study: SOC was greater at cooler temperatures, greater precipitation, and finer-textured soils. Similarly, simulation models can predict the observed SOC content in the U.S. Great Plains based on climate and texture (Parton et al., 1988). At the continental to global scale, climate is important, but geochemical factors not measured in this study, like iron and aluminum-oxyhydroxides or total iron and aluminum, are an underappreciated control on SOC (Doetterl et al., 2015; Rasmussen et al., 2018).

The indicators other than SOC responded in similar ways to site characteristics, but the predictability was lower. There is limited information in the literature on the regional or continental scale controls on these indicators. A global synthesis found that precipitation, but not





**Fig. 6.** Site-average response of six indicators to (a) decreased tillage, (b) inclusion of cover crops, (c) use of organic nutrients and (d) residue retention. Positive indicates an indicator value  $> 1.96 \cdot SE$  from the meta-analysis of response ratios; negative indicates a response ratio  $< -1.96 \cdot SE$ ; and neutral indicates a response ratio between  $-1.96 \cdot SE$  and  $1.96 \cdot SE$  where SE is the standard error. Sites are sorted from the highest (six) to lowest (zero) number of positive and lowest to highest number of negative indicators. Gray indicates no data at that site. POX-C is permanganate oxidizable carbon; WEOC is water extractable organic carbon; Cmin-24 is 24-h potential carbon mineralization; Cmin-96 is 96-h potential carbon mineralization; and BG is  $\beta$ -glucosidase.

temperature, was related to BG activity, but soil texture was not included in the analysis (Sinsabaugh et al., 2008). The relationship between clay content and BG activity has not been explored at large spatial scales. Clay can inhibit the activity of extracellular enzymes, like BG, because the enzymes become well protected in organo-mineral complexes where they are not accessible (Marx et al., 2005; Olagoke et al., 2019). Because of the potential for stabilization of extracellular enzymes on clay minerals, it has been suggested that microbial communities will respond by producing more enzymes in the presence of higher clay content leading to greater enzymatic activity in lab assays (Olagoke et al., 2019). While C mineralization assays and POX-C have been measured widely (Hurisso et al., 2016), there has not been a synthesis on the relationships between these assays and climate. In the United States, Cmin-96 was not found to differ among texture groups, similar to our findings that sand and clay content were not predictive of Cmin-96; in contrast, POX-C was 25% higher in finely textured soils compared to coarse textured soils (Fine et al., 2017). Based on our regressions and the limited information from the literature, it appears that the C

mineralization assays are less dependent on site characteristics.

#### 4.2. Relationships among indicators

There were also strong relationships among the C indicators at the treatment level. The simplest demonstration of this is the moderate to strong correlations (Table 3). Similar to the predictability with site-level characteristics, SOC and POX-C were the most predictable with other indicators at the treatment level. The multiple regression models predicted at least half of the variance in every indicator using just the other indicators (Table 4). There was little evidence from the stepwise regressions to suggest that more than one indicator was needed to predict any of the other indicators. The RDA, which predicts the matrix of indicators from the matrix of site-level variables, reinforced the results from the other approaches (Fig. 3). First, the indicators were mostly aligned along the first RDA axis. Second, this axis was mostly strongly associated with temperature, and the indicators were negatively related to temperature. Third, the second RDA axis was associated with clay

content, and BG and WEOC were the indicators most strongly aligned with this axis suggesting that they may be more sensitive to soil texture than the other indicators.

Many other studies have looked at relationships between indicators at particular locations. For example, Culman et al. (2012) reported that at twelve sites across the U.S., the  $r^2$  values for the relationship between POX-C and SOC was 0.60–0.95, with the exception of one site which was 0.01. Within sites, Hurisso et al. (2016) reported  $r^2$  values from 0.01 to 0.81 for POX-C and 0.01 and 0.91 for SOC with mineralizable C. However, this cross-site analysis of these variables highlights that the relationship between POX-C and SOC is as strong or stronger across sites compared to within sites. In summary, the C indicators are greater in colder and wetter sites with more clay, especially BG and WEOC, and the variables are moderately to strongly related to each other across sites.

Is it surprising that the indicators of various aspects of C dynamics are related? One explanation is that there is more of everything in some soils: more SOC in the soil and more C inputs that are being cycled. That explanation seems unlikely given that plant productivity generally increases with warmer temperatures (Michaletz et al., 2018), but SOC decreased with warmer temperatures in the NAPESHM dataset. Another possibility is that these biological indicators are not directly tied to rates of C cycling in the field. For example, soil respiration measured *in situ* is lower in no-till (Abdalla et al., 2016), but we found that potential C mineralization is greater. Because of the sample homogenization, destruction of aggregates from sieving, and uniform incubation temperatures, it is not surprising that lab measurements differ from field measurements. Regardless of the explanation, the indicators of C pools and cycling changed in similar ways across sites.

#### 4.3. Response to management

One generally accepted criterion for a soil health indicator is that it should be sensitive to management (Doran and Zeiss, 2000; Stott, 2019). Although the majority of the variance in the whole dataset was among sites, there were still predictable differences within sites in response to management. Further, there were only minor effects of the site variables on the responses to management with the driest sites predicted to show a negative response to decreased tillage for POX-C, Cmin-96, and BG. This means that while site characteristics are a major determinant of the absolute value of the indicators, they are a minor contributor to the response to management, or the *change* in indicator with change in management. Only water inputs (precipitation plus irrigation) had a significant effect on the response to decreased tillage for Cmin-24, POX-C, Cmin-96, and BG. The response for these indicators was predicted to be positive at all but at the driest sites. The C indicators detected positive effects of decreased tillage (except for Cmin-96), inclusion of cover crops, application of organic nutrients, and removal of residue (except for Cmin-96). We found almost no evidence that increasing the number of annual crop species or including crops other than grains affected the C indicators. If anything, increasing diversity resulted in lower values for the C indicators. The POX-C was significantly lower with rotation diversity while SOC was marginally lower; that is, a rotation that included a crop other than a grain had less SOC than a rotation with continuous grains.

Corn-soybean comprised one-quarter of the comparisons in the NAPESHM dataset, but a wide variety of others (e.g., wheat-pea, corn-cotton, corn-peanut) were also included. Corn-soybean (West and Post, 2002) and more generally grain-legume rotations (King and Blesh, 2018) have been found to have lower SOC than continuous corn or grain only rotations respectively. The difference in residue amount and composition likely both play a role. Soybeans return less C as residue to soils than corn (Poffenbarger et al., 2017). Further, N-rich soybean litter stimulates microbial biomass leading to greater decomposition of both SOC and N-poor corn litter as well as higher N mineralization rates, possibly explaining the soybean N credit applied to fertilization rates in the corn year of corn-soybean rotations (Hall et al., 2019). There are

many reasons to increase crop diversity, such as increasing yield, increasing drought resistance, or decreasing pests, even if there are no effects on SOC or other C indicators (Karlen et al., 1994; Sanford et al., 2021). While not all rotation effects may be mediated by the soil, there may be metrics of disease pressure that could be soil health indicators. Adding perennials or cover crops into a rotation have been found to result in greater values of C indicators, but it is not possible to separate the effects of diversity from the effects of the greater living roots and cover in many experiments (King and Blesh, 2018; McDaniel et al., 2014; Tiemann et al., 2015).

The effects of management have been most extensively studied for SOC (Table 6). Our findings are similar to recently published meta-analyses on the effects of tillage (Bai et al., 2019; Cooper et al., 2016; Francaviglia et al., 2017; Jian et al., 2020a; Luo et al., 2010; Ogle et al., 2019), organic nutrients (Liu et al., 2020), cover crops (Bai et al., 2019; Jian et al., 2020a; McClelland et al., 2021; Poeplau and Don, 2015), and residue (Lehtinen et al., 2014; Liu et al., 2014; Poeplau et al., 2017; Xu et al., 2019; Zhao et al., 2020) on soil C stocks. In general, these studies found the largest increases in soil C stocks near the surface. While this manuscript has focused on the increase of SOC concentrations in response to management, bulk density did show a significant negative response to decreased tillage, organic nutrients, and residue retention in NAPESHM dataset (Bagnall et al., 2022). Because the change in SOC concentration was so much larger than the change in bulk density in the NAPESHM dataset, the effect size of the meta-analysis of C stocks would differ by less than 1%. While the indicators other than SOC have been less studied, there is evidence that the effects of cover crops, organic nutrients, and residue retention on these indicators were also positive (Jian et al., 2020b; Kim et al., 2020; Li et al., 2019; Liu et al., 2014, 2020; Poeplau et al., 2017; Zhao et al., 2020).

The responses of C indicators to management are typically looked at in isolation. Studies may look at multiple indicators, but still evaluate them individually. In the NAPESHM dataset, it was not just the average responses of the C indicators that were similar. The PCA of the response ratios for decreased tillage (Fig. 5) highlights that indicators respond in similar ways to management in the NAPESHM data, similar to the findings of Nunes et al. (2020). Similarly, the comparison of response ratios across indicators highlights that for the majority of the sites most indicators responded in the same way to soil health practices (Fig. 6). For cover crops, organic nutrients, and residue retention, the response was overwhelming positive and consistent across sites (Fig. 6b–d). There were only one or two of the sites included in the meta-analysis for these practices with any negative responses. In contrast, the response to decreased tillage was more likely to be negative and was variable across sites and indicators (Fig. 6a). Only twenty-one out of the fifty-one sites had all positive/neutral responses and nine sites had all negative/neutral responses across the six indicators. Thus, a majority of sites had a consistent response to decreased tillage although that response was sometimes negative. Although there were twenty-one sites with a mixture of positive and negative responses of indicators, only three sites had at least two positive and two negative indicators. It is perhaps not surprising that tillage has more mixed results as tillage periodically disrupts the soil structure. There are short-term changes in microbial activity, nutrient cycling, and bulk density from soil disturbance. Therefore, the time since tillage may lead to more inconsistent responses (Jackson et al., 2003; Reicosky et al., 1997). Tillage also redistributes residue deeper into the soil where it would not be detected in surface soil measurements and where it could decompose more slowly (Angers and Eriksen-Hamel, 2008). While we attempted to standardize the sampling timing as much as possible, the time since tillage did vary among sites. The increase in SOC concentration associated with reduced tillage was 14% at sites without recent tillage but was only 3.5% when the tillage occurred in the previous 3 months (Fig. S2).

**Table 6**

Significance and direction of response ratios by indicator for soil health practices in meta-analysis studies. The numbers refer to the meta-analysis in the present study (reference 1) or to meta-analyses in the published literature (reference 2-18). The references in the table reported significant and positive responses of the practices on the indicators except for those noted with ns for non-significant responses, or neg for significant negative responses. POX-C is permanganate oxidizable carbon; Cmin is potential carbon mineralization; WEOC is water extractable organic carbon; and BG is  $\beta$ -glucosidase.

Carbon Indicator	Tillage	Cover Crops	Organic Amendments	Residue Retention	Crop Count	Rotation Diversity
Soil Organic C	1,2,3,4,12,14	1,2,5,6,13,15	1,11	1,8,9,16,17,18	1 <sup>ns</sup>	1 <sup>*neg</sup>
POX-C	1	1	1	1	1 <sup>ns</sup>	1 <sup>neg</sup>
Cmin	1	1,6,7	1	1	1 <sup>ns</sup>	1 <sup>ns</sup>
WEOC	1,9	1	1,9	1,10,16,18	1 <sup>ns</sup>	1 <sup>ns</sup>
BG	1	1,6,7	1,11	1	1 <sup>ns</sup>	1 <sup>ns</sup>

\*Significant at  $p < 0.10$ .

1- Present Study.

2- Bai et al., (2019)..

3- Cooper et al., (2016)..

4- Francaviglia et al., (2017)..

5- Jian et al., (2020a)..

6- Jian et al., (2020b)..

7- Kim et al., (2020)..

8- Lehtinen et al., (2014)..

9- Li et al., (2019)..

10- Liu et al., (2014)..

11- Liu et al., (2020)..

12- Luo et al., (2010)..

13- McClelland et al., (2021)..

14- Ogle et al., (2019)..

15- Poeplau and Don (2015)..

16- Poeplau et al., (2017)..

17- Xu et al., (2019)..

18- Zhao et al., (2020)..

#### 4.4. Interpretability of indicators

Indicators of soil health need to be interpretable in order to be useful. In general, it is assumed that a greater value of the C indicators is better (Andrews et al., 2004; Fine et al., 2017). However, it is also important to think about the underlying variability and not just the mean value of the indicator. There are three possible sources of variability, excluding human error, that complicate interpreting the measurements: spatial, temporal, and analytical conditions. The present study focused on continental-scale spatial variability. In the NAPESHM dataset, the coefficient of variation of treatments for the indicators of activity (Cmin and BG) was, on average, about double the coefficient of variation for indicators of amounts of C (SOC, POX-C, and WEOC). Greater variability means that more samples would be needed to be confident of a given difference among treatments or over time.

Although this study did not address temporal variability, it is well known that more labile pools and process rates will vary throughout the season. For example, seasonal differences have been reported for POX-C (Culman et al., 2013; Diederich et al., 2019; Omer et al., 2018). Even SOC has been found to vary throughout the year in cropping systems (Diederich et al., 2019; Omer et al., 2018; Ryan et al., 2009). These seasonal patterns are associated with the changing balance of plant inputs and microbial activity that mineralize C because of seasonal differences in weather and the crop phenology. Although the absolute value of indicators varies seasonally, there is evidence that the relative difference among treatments is maintained throughout the season (Culman et al., 2013). Given the diversity of cropping systems and climates in North America, it is impossible to define a single time of year where soil conditions and the plant cover are equivalent in every agroecosystem. This project aimed to sample soon before planting, which varied by several months, largely from south to north. However, around planting is often a hectic time, especially in systems where planting happens as soon as the fields are trafficable in the spring. Given that most indicators will vary seasonally at a site, it is strongly recommended to measure soil health at the same time of year and to take soil samples at a time that is not too burdensome for the land managers.

Perhaps sampling a few weeks after planting the summer crop would provide the best way to standardize the timing at a comparatively less busy time of year.

The interpretability of C indicator data can be affected by the analytical conditions and sample handling and preparation. It is generally assumed that SOC is a key metric for soil health because it is linked to many soil functions like nutrient cycling and water storage. It has been postulated that a disadvantage to using SOC as a soil health indicator is the relatively slow response to changes in management (Stott, 2019). However, there are empirical studies finding detectable differences in SOC concentrations in surface soils within 2 years of converting a site to no-till (Franzluebbers and Stuedemann, 2008; McCarty et al., 1998). The SOC response to changing tillage was found to increase over time, but even short (<5 year) experiments on average showed significant changes in concentration (Bai et al., 2019). In contrast, there are also studies that show that changes in C indicators can take decades to be quantifiable (De et al., 2020). Given the long-term experiments in the present study it wasn't possible to investigate the response time of the indicators. Further, is not well established how the time for C indicators to respond to management may depend on climate, soil texture, cropping system, and soil health before the management change. One advantage of SOC concentration measurements is that there are no disagreements about the analytical conditions because there is no rewetting of the soil, pH buffer needed, or extraction involved. One disadvantage of the relatively shallow measurements of SOC concentration for soil health is that SOC concentration change will not directly indicate change in C storage, one of the key functions associated with C in soils. Both a greater depth of soil sampling and bulk density measurements would be needed to quantify SOC stock.

Both POX-C and WEOC are often suggested to be more responsive indicators than SOC, but these chemically-defined fractions are more difficult to standardize and interpret. The analytical variability is greater for POX-C than typical nutrient extractions but is similar to C mineralization (Hurisso, 2016). More problematic, recent research has shown that soil mass and SOC content of the soil affects the POX-C measurement because of the kinetics of the reaction with permanganate

(Pulleman et al., 2021; Wade et al., 2020). However, soils with <10% SOC and a soil mass of 2.5 g, as the present study used, should provide consistent results especially when analytical replicates are performed (Wade et al., 2020). A problem that POX-C and WEOC share is that while they both represent small fractions of SOC (3.4% for POX-C and 0.9% for WEOC for the NAPESHM dataset, Fig. S3), the chemical composition of this C is unknown. The most cited methods paper referred to POX-C as active C (Weil et al., 2003), but subsequent research has shown that the oxidized compounds are not necessarily more “active.” For example, disaccharides like sucrose, were oxidized much more slowly than reducing sugars like the monosaccharides that comprise sucrose, even though both should be readily available to microbes (Tirol-Padre and Ladha, 2004).

Although decades of work on characterizing the chemistry, sources, and fluxes of dissolved organic matter in soils exist in a wide variety of ecosystems (McDowell, 2003), soluble C has been less well characterized in the context of soil health. Similar to POX-C, the WEOC represents a mixture of compounds that likely vary in their energetics. Further, the amount of WEOC extracted depends on the soil:extract ratio and the clay content (Lim et al., 2015). One potential easy metric of quality is the ratio of WEOC to water extractable organic N because they are often measured simultaneously. Although the decomposition of different types of plant residue (e.g. corn vs soybean) is strongly related to plant C:N ratios (Parton et al., 2007), WEOC:WEOON was not strongly associated with C mineralization in the NAPESHM dataset ( $r < 0.1$ ). Nor was WEOC:WEOON strongly associated with any of the other indicators. Further, it is not likely that the entire WEOC pool is a highly labile substrate for the microbial community. There is evidence that C mineralization and WEOC are strongly correlated; however, the CO<sub>2</sub> produced in Cmin assays cannot just be mineralized WEOC because WEOC can be smaller than Cmin (Davidson et al., 1987). As a comparison, in the NAPESHM dataset, the C produced by the 24- and 96-h C mineralization assays as CO<sub>2</sub> was on average 48% and 106% of WEOC. Finally, there are lingering questions about the effects of air drying samples on these indicators because air drying is known to change the amount and composition of WEOC (Davidson et al., 1987; Kaiser et al., 2015).

A more direct assay of C readily available to microbes is potential C mineralization. With this biological approach, the microbes determine how much C is available instead of defining the fractions chemically. Although the NAPESHM data and previous studies have found strong correlations between C mineralization and WEOC (Davidson et al., 1987; Haney et al., 2012), there are questions about how to interpret these Cmin assays because of the standardized conditions. While the multiple C mineralization methods have been found to be highly correlated, the length of the incubation, the temperature, the moisture content, and the wetting process differ between these standardized methods, and all can affect the rates (Franzuebbers and Veum, 2020). The *in situ* antecedent soil moisture can affect the amount of C mineralization in two ways that both favor drier soils: (a) because microbial processes continue during air drying and wetter soils take longer to dry, wetter soils may respire more during the drying process (Belanger et al., 2021); and (b) drier soils have been found to have larger pulses of CO<sub>2</sub> produced in response to rewetting (Manzoni et al., 2020). Thus, wetter soils may have lost more easily respired C prior to the start of the incubation and they may have a lesser response to rewetting. The response to rewetting is typically a peak of mineralization on the first day, then a decline to a basal respiration rate after one to several more days (Fierer and Schimel, 2003; Steenwerth et al., 2005). This can be seen in the NAPESHM data as the Cmin-24 is twice as high as Cmin-96 when both are reported on a daily basis. Although C mineralization rates change dramatically over the hours to days of typical incubations, the relative rates between samples have been found to be consistent throughout (Creamer et al., 2014; Franzuebbers et al., 2000). There has been more focus on the effects of air drying than temperature of the incubation, but the sites in the NAPESHM dataset ranged from 3 to 25 °C in mean annual

temperature. Microbial communities clearly change throughout the year and respond differently to incubation temperatures (Lipson et al., 2002). It is not known how much the potential C mineralization represents the available C versus the biomass of the microbial community that survives sieving and air drying drives the respiration at a standardized incubation temperature and moisture.

The BG assay has similar issues to other biological indicators. It may have a site-specific optimum temperature and pH, so using a standard temperature and buffer may make inter-site comparisons difficult (Bell et al., 2013). Enzyme activity can be sensitive to interferences with dissolved organic matter, substrate concentrations, and the method to terminate the reaction (Margenot et al., 2018). When compared across studies and soil types, air drying can lead to different results compared to fresh soils (Lorenz and Dick, 2011).

One other indicator related to C that is potentially useful is loss on ignition (%). It was quantified on the NAPESHM dataset at Cornell at 500 °C (LOI<sub>500</sub>) because it is part of the CASH test. In addition, it was measured at Ward Laboratory at 365 °C (LOI<sub>365</sub>). Because LOI is sensitive to the ignition temperature and the clay content, it cannot be universally related to SOC; while a large ranges of values have been reported, a conversion factor from SOC to LOI of 2 has been suggested (Pribyl, 2010). For the treatments means of the NAPESHM dataset, the conversion factors were 1.49 for LOI<sub>365</sub> and 1.94 for LOI<sub>500</sub>. Although the conversion factors were quite different, the correlation of LOI<sub>365</sub> and LOI<sub>500</sub> was strong ( $r = 0.90$ ) as were their correlations with SOC ( $r = 0.93$  and  $0.84$  respectively). Both LOI measurements responded to the soil health practices the same way except the rotation diversity effect at -6.5% was significantly negative for LOI at 365 °C compared to -4.7% for SOC. The biggest complication of using LOI is that the relationship with SOC depends on clay (Fig. S4). For example, based on a multiple regression model with clay, SOC, and their interaction, at the mean SOC content (1.7%), the predicted LOI<sub>365</sub> would be 3.0% at 10% clay but 4.0% at 50% clay. Because of this texture bias, LOI is less interpretable as an indicator than SOC. That being said, if SOC analysis is not available, LOI at a given temperature could be considered as long as there was careful consideration of the soil texture.

In summary, all the C indicators have complications. The indicators vary seasonally and spatially, but sampling enough soil to capture the spatial heterogeneity and sampling at the same time of year can minimize these effects. The indicators, with the exception of SOC, also have additional challenges in interpretation. Both WEOC and POX-C are straightforward methods, but they are difficult to interpret because they represent chemically-defined pools. The C mineralization assays and BG require choosing standard conditions of temperature and pH, which may change the absolute values, but not the relative differences between samples. Air drying the soils also changes the absolute values for these indicators and the effects likely depend on initial soil moisture. Part of the challenge is how universal the absolute values of these indicators need to be in order to be interpretable by land managers. These issues are minimized when measuring a single site sampled at the same time of year over multiple years or for comparing different management practices sampled at the same time.

#### 4.5. Choosing indicator(s)?

It is common to measure SOC and at least one other C indicator for soil health studies and for calculating soil health indices. It is essential to measure at least some biological indicators of soil health. There are many criteria that could be used to select soil health indicators, but as Doran and Zeiss (2000) suggested, they should be responsive to management, easy and inexpensive to collect and measure, and be interpretable by land managers. The C indicators were all predictable with the same site-level variables, were strongly correlated with each other, and responded to soil health practices. Additionally, the response to management was generally consistent across site variables. That is, unlike the predicting the values of the indicators themselves, site

characteristics had minimal predictive capacity on the response to management suggesting these practices are generally beneficial across North America. There were subtle differences among the indicators in this study, but the indicators generally performed similarly. The POX-C was most highly correlated with SOC, so it may not be necessary to measure both. More research is needed to evaluate if some of the indicators respond more quickly than SOC to changes in management. The Cmin-24 and BG were more variable within a treatment, but they also showed the greatest responses to management. The Cmin-96 was the only indicator where the response to decreased tillage and residue retention was not significant.

The interpretability differs among the indicators. Both WEOC and POX-C have been suggested to be measuring a more available pool of C, but they are defined by their response to an extractant and an oxidant, making them more difficult for producers to interpret. In contrast, Cmin provides a direct assay of the amount of C available for microbes to respire. The length of time of the Cmin assay potentially captures different phases of the microbial response, which complicates the interpretation, but the relative amount of CO<sub>2</sub> produced was similar across samples. In addition, a longer incubation means lower throughput for a lab. While POX-C methodology has largely been standardized, there are lingering questions about the kinetics of the reaction, especially in high SOC soils. Finally, the WEOC and BG assays are sensitive to clay content, as sorption of WEOC and BG on clays can occur.

To be useful to agricultural producers, these tests need to be available in commercial labs at a reasonable price, and a growing number of labs can measure most of these C indicators (Table S3). The BG enzyme activity is not currently widely available. The WEOC is cheaper than the other indicators, which are similar in price. Both POX-C (Weil et al., 2003) and Cmin-24 (Haney et al., 2008) even have “field” tests that can be done without having to send samples to a lab. New techniques for measuring SOC using visible/near infrared or mid infrared spectroscopy are improving and there is hope that *in situ* spectroscopy measurements will be widely available, affordable, and interpretable. For now, it is essential to continue measuring SOC with dry combustion. The NAPESHM dataset was not designed to answer questions about how long it takes indicators to respond or about adoption of soil health management systems. With the identical field sampling and lab methods for all sites and detailed management histories in the NAPESHM dataset, we observed strong relationships among the indicators at the continental scale and consistent responses to the adoption of soil health practices with the exception of diversifying the type and number of crops in a rotation. All C indicators measured as part of NAPESHM responded positively to soil health practices overall, providing evidence that the complex physical, chemical, and biological changes in response to site characteristics and management are captured by these indicators. While the indicators are moderately to strongly correlated, it is not recommended to substitute one indicator for another. For some soil health indices, LOI is used instead of SOC. Although LOI responded in similar ways to the site variables and management in the NAPESHM dataset, we only report on SOC as a C indicator because dry combustion is a more accurate, precise, and comparable (Nelson and Sommers, 1996).

Because the interpretation of Cmin is most straightforward, we suggest the Cmin-24 would be the best choice for its ability to indicate microbial activity, but as long as the same indicator is used over time or across management practices at the same time, all of these indicators can provide insight into C dynamics.

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#### Declaration of competing interest

The authors declare that they have no known competing financial

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#### Appendix A. Supplementary data

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