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Maximizing soil organic carbon stocks under cover cropping: insights from long-term agricultural experiments in North America

Yajun Peng [a], Elizabeth L. Rieke [b], Inderjot Chahal [a], Charlotte E. Norris [c], Ken Janovicek [d], Jeffrey P. Mitchell [e], Kraig L. Roozeboom [f], Zachary D. Hayden [g], Jeffrey S. Strock [h], Stephen Machado [i], Virginia R. Sykes [j], Bill Deen [d], Oscar Bañuelos Tavarez [k], Audrey V. Gamble [l], Kate M. Scow [m], Daniel C. Brainard [g], Neville Millar [n], Gregg A. Johnson [o], Robert R. Schindelbeck [p], Kirsten S.M. Kurtz [p], Harold van Es [p], Sandeep Kumar [q], Laura L. Van Eerd [a,*]

[a] University of Guelph, Ridgetown Campus, School of Environmental Sciences, Ridgetown, ON N0P 2C0, Canada
[b] Soil Health Institute, Morrisville, NC 27560, USA
[c] Natural Resources Canada, Canadian Forest Service, Victoria, BC V8Z 1M5, Canada
[d] University of Guelph, Department of Plant Agriculture, Guelph, ON N1G 2W1, Canada
[e] University of California, Davis Department of Plant Sciences, Parlier, CA 93648, USA
[f] Kansas State University, Department of Agronomy, Manhattan, KS 66506, USA
[g] Michigan State University, Department of Horticulture, East Lansing, MI 48824, USA
[h] University of Minnesota, Department of Soil, Water and Climate, Southwest Research & Outreach Center, Lamberton, MN 56152, USA
[i] Oregon State University, Columbia Basin Agricultural Research Center, Adams, OR 97810, USA
[j] University of Tennessee, Department of Plant Sciences, Knoxville, TN 37996, USA
[k] International Maize and Wheat Improvement Center (CIMMYT), Texcoco 56237, Mexico
[l] Auburn University, Department of Crop, Soil and Environmental Sciences, AL 36849, USA
[m] University of California, Davis Department of Land, Air, and Water Resources, Davis, CA 95616, USA
[n] Michigan State University, Department of Earth and Environmental Sciences, East Lansing, MI 48824, USA
[o] University of Minnesota, Southern Research and Outreach Center, Waseca, MN 56093, USA
[p] Cornell University, Department of Soil and Crop Sciences, Ithaca, NY 14853, USA
[q] South Dakota State University, Department of Agronomy, Horticulture and Plant Science, Brookings, SD 57007, USA

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ABSTRACT

Cover crops are widely advocated for increasing soil organic carbon (SOC) levels, thereby benefiting soil health improvement and climate change mitigation. Few regional-scale studies have robustly explored SOC stocks under cover cropping, due to limited long-term experiments. We used the unique experimental data from the North American Project to Evaluate Soil Health Measurements conducted in 2019 to address this issue. This study included 19 agricultural research sites with 36 pairs of cover cropping established between 1896 and 2014. Explanatory variables related to site-specific environmental conditions and management practices were collected to identify and prioritize contributing factors that affect SOC stocks with cover crops, by coupling the Boruta algorithm and structural equation modeling. Overall, cover crops significantly ($P < 0.05$) improved several indicators of soil health, including greater SOC (concentration: +8%; stock: +7%), total nitrogen (+8%), water-stable aggregates (+15%), and potential carbon mineralization (+34%), on average, compared to no cover crop control. Likewise, on average, cover crops sequestered SOC 3.55 Mg C ha^{-1} (0–15 cm depth), with a sequestration rate of 0.24 Mg C ha^{-1} yr^{-1}. In addition, we found climate (Hargreaves climatic moisture deficit) was important in explaining the variation of SOC stocks with cover crops, followed by soil properties (e.g., soil clay content). In terms of management practices, cover crop type had a significant positive (0.36) effect on SOC stocks, with non-legumes showing a greater impact, compared to legumes and mixtures. Crop rotational diversity also had a positive (0.28) effect on SOC accumulation. Our findings suggested that integrating non-legume cover

* Corresponding author.
E-mail address: lvaneerd@uoguelph.ca (L.L. Van Eerd).

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crops into diverse crop rotation is likely to be a promising strategy to maximize SOC stocks with cover crops across North America.

1. Introduction

Soil organic carbon (SOC) in agricultural soil is an important controlling factor for soil health (Van Eerd et al., 2023). However, losses of SOC in the cultivation zone (i.e., up to 30 cm depth) have been globally observed in most croplands (Abdalla et al., 2020). Reductions in SOC are likely to pose a risk to crop productivity and atmospheric CO_2 mitigation (Friedlingstein et al., 2020). Cover crops, also named inter-crops, catch crops, service crops and green manures, are commonly grown for a purpose (i.e., a service) other than for the harvest (Van Eerd et al., 2023). Integration of cover crops into crop rotations has been proposed as a promising practice for improving SOC stocks, mainly by adding additional plant C inputs into the soil (Van Eerd et al., 2023; Kaye and Quemada, 2017) and physically protecting SOC losses from erosion (Jastrow and Miller, 1997). However, the potential sequestration of SOC with cover cropping is affected by various factors, such as climate conditions, soil properties, and agricultural management practices.

The amount of sequestered SOC with cover cropping depends on the quality and quantity of residues and their decomposition process in the soil (Ghimire et al., 2017). Overall, the biomass production of cover crops (i.e., quantity) is largely determined by the cover crop species grown and its agronomic management, climate conditions, especially temperature and precipitation (Jian et al., 2020), as well as soil conditions such as moisture and nutrient availability (e.g., nitrogen; Van Eerd, 2018). For example, a humid, temperate climate had a higher cover crop productivity (3.37 $\pm$ 2.96 Mg ha^{-1}), compared to a semiarid temperate climate (2.61 $\pm$ 2.42 Mg ha^{-1}; Ruis et al., 2019). A positive correlation between the sequestered SOC with cover cropping and annual temperature and precipitation has also been reported (Jian et al., 2020). The rate of residue decomposition is impacted by residue quality. However, the role of cover crop quality in SOC accumulation and stabilization has not yielded consistent results. Low-quality residues (high C/N ratios) are likely to promote soil organic matter (SOM) stabilization, due to having a great proportion of recalcitrant compounds (e.g., lignin and phenols) that are physiochemically decomposed slowly (Castellano et al., 2015). Some argued that high-quality residues (low C/N ratios) contribute to SOM stabilization, which is derived from microbial by-products (Cotrufo and Lavallee, 2022). Moreover, soil texture, especially the amount of soil clay content, represents another inherent factor that affects the stabilization of SOM (Six et al., 2002). Soil particle-size distribution may also impact plant productivity, due to its strong influence on water holding capacity and nutrient availability (Kern, 1995). Soil pH may also affect the decomposition of organic matters through its influence on SOC solubility as well as microbial growth, activity, and structure (Wang et al., 2017). Other soil factors such as initial SOC levels may affect the rate/amount of SOC accrual under cover cropping. For example, soils with low SOC stocks may accumulate SOC quicker or to a greater extent when cover crops are included in the system (Jian et al., 2020).

In addition, management practices affect SOC accumulation under cover cropping, including tillage and crop rotation (McClelland et al., 2021; West and Post, 2002). For example, decreasing tillage intensity mitigates soil aggregate breakdown from periodic disturbance, thereby physically protecting SOM from biodegradation (Balesdent et al., 2000). Likewise, improving crop rotational diversity not only provides diverse C inputs, but also often enhances soil properties, such as soil aggregate stability and soil microbial activity (Rieke et al., 2022a; b; Van Eerd et al., 2018), which may increase crop residues and root-exudate inputs, as well as reduce SOC losses (Chamberlain et al., 2020). Moreover, crop rotation can affect the growing season of cover crops, indirectly determining the amount of cover crop biomass returned to the soil (Van Eerd, 2018).

Recent literature reviews have quantified the SOC sequestration potential of cover cropping and identified contributing factors by using meta-analyses, at a global or regional scale (e.g., McClelland et al., 2021; Jian et al., 2020; Abdalla et al., 2020). The challenges with this methodology are the inconsistent format of SOC reports (e.g., concentration vs. stock and with vs. without standard error) and varied soil sampling depth among the studies collected, which creates uncertainty in the calculation of SOC sequestration. While valuable, the low quality and reproducibility of literature review-based meta-analysis on SOC have been reported (Beillouin et al., 2022). Moreover, these meta-analyses could not prioritize the contributing factors that affect SOC stocks with cover cropping because explanatory variables (moderators) are assessed individually. These limitations require an improved dataset to estimate the effect size of cover crops on SOC and alternative methodology to identify the order of contributing factors.

In this study, we used the unique experimental data of soil samples collected at the same time from 19 diverse long-term agricultural experiments (across a continent) from the North American Project to Evaluate Soil Health Measurements (NAPESHM). Long-term data are particularly important to understanding the maximum SOC sequestration potential of cover crops in soils. Previous studies from this project demonstrated the important role of SOC in determining soil microbial community (Rieke et al., 2022b), and other carbon-based soil health indicators (Liptzin et al., 2022). However, there is less information on how to improve the capacity of cover cropping to sequester SOC. Thus, we (1) estimated overall cover crop effects on surface soil properties using meta-analysis, including soil bulk density (BD), SOC concentrations/stocks, total nitrogen (TN), water-stable aggregates (WSA), and potential carbon mineralization (Cmin); and (2) quantified SOC sequestration potential under cover cropping. We employed novel statistical procedures to prioritize contributing factors (i.e., climate, soil texture, soil pH, tillage, rotation, cover crop type, and the duration of cover cropping) controlling SOC stocks with cover crops, by combining Boruta analysis and structural equation modeling (SEM). Understanding these key factors could help farmers adjust management practices to maximize SOC stocks by using cover crops.

2. Materials and methods

2.1. Selected research sites with cover cropping

The NAPESHM project included 19 research sites with cover cropping as an experimental factor (Norris et al., 2020). These field experiments were initiated between 1896 and 2014, therefore the duration of cover cropping ranged from 5 to 123 years when SOC was quantified in 2019 (Table 1). Out of the 19 field sites, 9 sites had more than one (i.e., 2–4) cover cropping treatments, and all sites had a no cover crop control. Thus, a total of 36 cover cropping treatments were compared to no cover crop controls (Table 1). Each treatment had 2–6 replications, except for USAL02 where only one observation from the cover cropping treatment was available (USAL02 was not included in the meta-analysis). Therefore, a total of 250 observations (experimental units) were included in the present study. In addition, five years of management practices were collected for each treatment, which provided details on the main crop (e.g., rotational diversity, tillage system) and cover cropping practices. As cover crop biomass was not sampled at each site when the soil was sampled, we collected information on cover crop type (non-legumes, legumes, and mixture) from this dataset. We did not analyze the effect of cover crop growing window nor termination method because more than 85% of cover crop treatments in this study were overwintered and spring terminated using herbicides.

Table 1
Details of cover cropping treatments (TRT), crop rotation, tillage, and duration of cover cropping at nineteen research sites with cover cropping (Site and TRT ID) included in the NAPESHM project.

Site	TRT ID	Cover cropping treatments	Crop rotation	Tillage†	Duration‡
CAON01	a	Red clover + conventional tillage	Corn-corn-soybean-winter wheat	140.3	39
	b	Red clover + conservation tillage	Corn-corn-soybean-winter wheat	91.3	
CAON04	a	Radish/cereal rye	Vegetable, grain and oilseed crops	140	12
	b	Radish	Vegetable, grain and oilseed crops	140	
	c	Cereal rye	Vegetable, grain and oilseed crops	140	
MXMO01	a	Crotalaria + minimum tillage + residue retained	Continuous corn	37.6	8
	b	Crotalaria + no-till+ residue retained	Continuous corn	7	
USAL02		Winter legume + 0 N, spring P and K	Continuous cotton	13.8	123
USCA01		Bell bean + Lana vetch + oat	Continuous winter wheat	140.9	26
USCA02		Bell bean + Lana vetch + oat	Corn-tomato	149.53	26
USCA03	a	Cover crop mixture※ + standard tillage	Garbanzo bean-sorghum	253.97	20
	b	Cover crop mixture※ + no tillage	Garbanzo bean-sorghum	3.97	
USIA02		Cereal rye	Continuous corn	4	11
USKS01	a	Summer cover crop + 0 kg N ha^{-1}§	Sorghum-soybean-winter wheat	2.55	12
	b	Summer cover crop + 130 kg N ha^{-1}§	Sorghum-soybean-winter wheat	2.55	
USMI02	a	Cereal rye/vetch or vetch + moldboard	Snap bean-squash*-sweet corn	82.4	11
	b	Cereal rye /vetch or vetch + strip-till	Snap bean-squash*-sweet corn	28.4	
	c	Cereal rye + moldboard	Snap bean-squash*-sweet corn	82.4	
	d	Cereal rye + strip-till	Snap bean-squash*-sweet corn	28.4	
USMI03	a	Red clover/cereal rye + crop rotation	Corn-soybean-winter wheat	117.3	19
	b	Red clover + crop rotation	Corn-soybean-winter wheat	117.3	
	c	Red clover + continuous corn	Continuous corn	181.3	
USMN01		Cereal rye	Corn-soybean	103	5
USMN02		Cereal rye	Corn-soybean	103	5
USMN04		Cereal rye	Corn-soybean	103	5
USNY04	a	Cereal rye + zone-till	Continuous corn	14	26
	b	Cereal rye + plow-till	Corn-soybean-winter wheat	90.1	
USOR01		Purple vetch + no-till	Continuous winter wheat	24	16
USSD01		Cereal rye + no-till	Corn-soybean	2.85	28
USTN01	a	Hairy vetch + continuous cotton	Continuous cotton	7.8	18
	b	Hairy vetch + crop rotation	Cotton-corn-cotton-soybean	7.8	
	c	Hairy vetch + continuous corn	Continuous corn	7.8	
	d	Hairy vetch + continuous soybean	Continuous soybean	7.8	
USTN02	a	Hairy vetch + continuous corn	Continuous corn	7.8	18
	b	Hairy vetch + continuous soybean	Continuous soybean	7.8	
	c	Hairy vetch + crop rotation	Corn-soybean	7.8	

†Standard tillage intensity rating (STIR) was used to indicate the tillage intensity where the higher STIR values represent the stronger soil disturbance by tillage. The STIR was calculated based on tillage type modifier, speed, tillage depth, and area disturbed (USDA-ARS 2022: https://soilhealthinstitute.org/dr-michael-cope-management-indices-that-reflect-foundational-soil-health-practices).
‡Duration represents the years since the research sites with cover cropping were established, which were calculated by the difference between the year of sample collection (2019) and the year of the research site established. ※Cover crop mixture represents cereal rye (*Secale cereal* L.) + common vetch (*Vicia sativa* L.) + triticale (x *Triticosecale* Wittmack) + radish (*Raphanus sativus* L.) + red clover (*Trifolium pratense* L.). § The sorghum (*Sorghum bicolor* L.) received either 0 or 130 kg N ha^{-1} after the termination of cover crops. *In some years, cucumber (*Cucumis sativus* L.) was planted rather than squash (*Cucurbita pepo* L.). The binomial name for corn/sweet corn (*Zea mays* L.), soybean (*Glycine max* L.), winter wheat (*Triticum aestivum* L.), crotalaria (*Crotalaria retusa* L.), bell bean (*Vicia faba* L.), Lana vetch/hairy vetch (*Vicia villosa* Roth.), oat (*Avena sativa* L.), tomato (*Solanum lycopersicum* L.), snap bean (*Phaseolus vulgaris* L.), purple vetch (*Vicia benghalensis* L.) and cotton (*Gossypium* hirsutum L.).

2.2. Soil collection and laboratory analysis

To limit the immediate effect of management practices on soil sampling, soil samples were predominantly collected in the spring of 2019, prior to fertilizer application, spring tillage and seeding. A comprehensive description of soil sample collection is available elsewhere (Rieke et al., 2022b; Liptzin et al., 2022; Norris et al., 2020). Briefly, for the measurement of soil BD and WSA, four soil cores were collected using a cylinder to a depth of 7.6 cm in each experimental unit. Two of the 4 soil cores were individually stored, while the remaining 2 soil cores were composited into a bag. All soil core samples were then sent to the Cornell Soil Health Laboratory (Ithaca, NY). Soil BD was measured by the weight of dry soils and rectified by the coarse fragments' mass percentage. For soil samples with less than 2% coarse fragments by mass (determined during particle size analysis preparations), the soil BD values were calculated as the mean bulk density of all 4 soil cores. For soil samples with more than 2% coarse fragments by mass, the soil BD values were calculated as the mean of the 2 composited soil cores, following the removal of coarse fragments, with adjustments correcting for mass and volume of the coarse fragments. Soil WSA was measured using the Cornell Sprinkle Infiltrometer (Moebius-Clune et al., 2016). Approximately 30 g of air-dried soils were placed under a rainfall simulator for 5 min. Unstable soil aggregates passed through the sieve, while the remaining soils on the sieve were used to calculate the percentage of WSA.

In each experimental unit, a composite sample (based on 18 soil samples) was collected using a knife (15 cm by 4 cm) to a depth of 15 cm, which was sent to the Soil Water and Environmental Lab at Ohio State University for the analysis of soil particle size, soil pH, total C and N, inorganic C, and Cmin. Measurements of these indicators were described in Rieke et al. (2022b). Briefly, the percentage of particle size (i.e., sand, silt, and clay) was calculated using the pipette method (Gee and Or, 2002). Soil pH was measured in a 1: 2 (soil: water) suspension using a glass electrode pH meter (Thomas, 1996). Soil total C and N concentrations were measured by dry combustion (Nelson and Sommers, 2015). Inorganic C was measured based on Chittick gasometric calcimeter (St. Louis, MO, USA; Dreimanis, 1962; Loeppert and Suarez, 1996). The difference between soil total C and inorganic C is SOC concentration (%). The Cmin was determined by a 24-hour CO_2 burst (Zibilske, 1994).

The SOC stocks (Mg C ha^{-1}) in the 0–15 cm depth were calculated by multiplying the SOC concentration (%) by the mass of soil at the fixed soil depth. The mass of soil was determined from the measured soil BD (0–7.6 cm) and the depth of the sampled layer for SOC concentration (0–15 cm). Here, we assumed soil BD in the depth of 0–15 cm is equal to 0–7.6 cm depth, which was consistent with previous research (e.g., soil BD measured in the 0–10 cm depth to calculate SOC stocks in the 0–32 cm depth; Sleutel et al., 2006). This assumption was also based on the limited cover cropping effect on soil BD observed in this study (see Section 3.1) as well as in other studies (Bagnall et al., 2023; Blanco--Canqui and Ruis, 2020), suggesting the stratification of soil BD between 0–7.6 and 7.6–15 cm depth is likely to be small. In addition, we did not observe a significant difference between observed SOC stocks using soil BD in the 0–7.6 cm and predicated SOC stocks using pedotransfer function by Abdelbaki (2018) to estimate soil BD in the 0–15 cm depth (Fig. S1). Next, we calculated the amount of SOC sequestered (Mg C ha^{-1}) for each cover cropping treatment, based on the difference in mean SOC stocks between treatments with and without cover crops grown, due to the lack of block information at each site. The SOC sequestration rate (Mg C ha^{-1} yr^{-1}) for each cover cropping treatment was calculated using SOC sequestered (Mg C ha^{-1}) divided by the years since the cover cropping experiment was established (i.e., duration; Table 1).

The Hargreaves Climate Moisture Deficit (CMD) was obtained in ClimateNA (ClimateNA_Map: https://www.climatewna.com). The monthly moisture deficit (in mm) was the difference between the reference evaporation and precipitation for a given location (Wang et al., 2016). When the monthly precipitation is higher than the reference evapotranspiration, the monthly moisture deficit is recorded as zero. The annual moisture deficit is the sum of the monthly moisture deficits for the given site. In this study, the CMD calculation was an estimate of the annual moisture deficit averaged between 1991 and 2020.

2.3. Statistical analysis

2.3.1. Calculating effect size of cover cropping on soil properties using meta-analysis

All statistical analyses were performed in RStudio (Ver 4.1.2; R Core Team, 2019). The estimated effect sizes of cover cropping on soil properties, including soil BD, SOC, TN, WSA, Cmin, and SOC stock (Table 2), were performed using the *metafor* package (a meta-analysis package; Viechtbauer, 2022). We did not examine the effect of cover crops on soil pH, as it has been regularly adjusted by lime application in some sites of this study. The effect size for each treatment was measured as

$$\mathrm{LnRR} = \ln\frac{\overline{Y}_{CC}}{\overline{Y}_{NO\_CC}}$$

where the LnRR is the natural log of response ratio. The $\overline{Y}_{CC}$ is the mean of a response variable (e.g., SOC, TN) under cover cropping treatment, whereas the $\overline{Y}_{NO\_CC}$ is the mean of the same response variable under no cover cropping control. At sites with more than one cover cropping treatments (e.g., CAON01), the aggregated LnRR values were estimated by using the *aggregate* function (Viechtbauer, 2022). In the *escalc* function, we used the "ROM" method to calculate the log transformed ratio of means (Lajeunesse, 2011; Hedges et al., 1999). The "REML" approach was employed in the *rma* function to calculate the estimated effect size and 95% confidence intervals (CI). To easily explain and compare the

Table 2
Description of the variables used for the statistical analysis. SEM represents structural equation modeling.

Parameters	Variabletypes	Factor levels	Meta-analysis	Boruta	SEM
Climate					
Hargreaves climate moisture deficit (CMD)	Continuous			√	√
Main crop system					
Tillage	Continuous			√	√
Rotation diversity	Factor	Continuous Diverse		√	√
Cover crop system					
Duration of cover cropping	Discrete			√	√
Cover crop type	Factor	Legumes Non-legumes Mixtures		√	√
Soil properties					
Bulk density (BD)	Continuous		√		
pH	Continuous			√	√
Soil organic carbon (SOC)	Continuous		√		
SOC stock	Continuous		√	√	√
Total nitrogen (TN)	Continuous		√		
Sand	Continuous				
Clay	Continuous			√	√
Silt	Continuous			√	√
Water-stable aggregates (WSA)	Continuous		√		
Potential carbon mineralization (Cmin)	Continuous		√		

LnRR values from the meta-analysis, we transformed them to percentage by the formulation $\mathrm{SOC}\,(\%) = 100 \times \left(e^{\mathrm{LnRR_{soc}}} - 1\right)$ (e.g., SOC concentration).

2.3.2. Identifying contributing factors affecting SOC stocks using Boruta analysis and SEM

To examine the extent to which environmental and management factors (Table 2) explain the variability among experiments of the magnitude of cover cropping effects on SOC stocks, we built SEM via the *lavaan* (latent variable analysis) package (Rosseel, 2023). Environmental factors included climate and soil properties, while management factors included cover crop type, duration of cover cropping (how many years of cover crops have been used; Table 1), tillage, and the number of crops in the rotation (Table 2). The SEM has been increasingly used to quantify the causal relationships between environmental variables and soil C, on a regional basis (e.g., Dai et al., 2022), as well as the cover cropping effect on crop yield (Hill et al., 2017). Prior to building the SEM model, a preprocessing stage was performed by the Boruta feature selection method to remove unimportant factors (i.e., we retained only relevant features; Kursa et al., 2020).

Boruta analysis used as a feature selection approach to reduce data redundancy has been only recently employed in agricultural research (e.g., Dai et al., 2022). We ran Boruta analysis via the *Boruta* package (Kursa and Rudnicki, 2010). The Boruta analysis is an "all relevant feature selection" wrapper algorithm around random forest (Kursa and Rudnicki, 2010). Boruta approach was preferred over other random forest feature selection methods (e.g., VSURF and varSelRF) because it can handle category variables (e.g., crop rotational diversity and cover crop type in our study) and has relatively low inherent error rates and computation times (Speiser et al., 2019). Prior to the Boruta analysis, variables used to calculate SOC stocks were excluded (i.e., soil BD and SOC concentration; Table 2). We also reduced the number of continuous variables with high collinearity by using the pairwise correlation coefficient (i.e., Pearson's correlation coefficient exceeding the threshold 0.7; Fig. S2; Dormann et al., 2013); hence, TN and soil sand content were not included in Boruta analysis (Table 2).

After unimportant factors were removed (i.e., Boruta analysis), we built the hypothesized model for SEM, based on the well-known cover cropping effects on SOC (e.g., McClelland et al., 2021; Poeplau and Don, 2015). The hypothesized model in SEM was adjusted, based on the goodness-of-fit indices, including comparative fit index (CFI > 0.95), Tucker Lewis index (TLI > 0.95), standardized root mean square residual (SRMS < 0.06), and root mean square error of approximation (RMSEA < 0.06; Hu and Bentler, 1999).

The final proposed SEM was executed using the maximum likelihood method (Rosseel, 2023). All parameters included for SEM analysis were log transformed to fit linear models. As the maximum likelihood estimator is specialized for continuous variables, we assigned ordinal values to cover crop type and crop rotational diversity. For example, crop rotational diversity had two categories: diverse rotation (i.e., more than one crop) was assigned a value of 2, and continuous cropping (i.e., one crop; Table 2) was assigned 1. Likewise, the cover crop type was assigned the following values: non-legumes (3), legumes (2), and mixtures of legumes and non-legumes (1).

3. Results

3.1. Overall effect of cover crops on soil properties

For soil BD, the average pooled effect size with 95% CI crossed zero (LnRR = 0; Fig. 1a), which indicates that the impact of cover cropping on this parameter was non-significant ($P > 0.05$). However, cover cropping had significantly ($P < 0.05$) greater surface SOC and TN concentrations, WSA, and Cmin, based on their positive pooled LnRR estimates (Fig. 1b, c, d, e). On average, in the depth of 0–15 cm, cover cropping had 8% (95% CI: 2–15%) greater SOC concentration across the dataset (Fig. 1b). The highest change in surface SOC concentration under cover cropping was detected at USAL02 (+288%; calculated individually rather than by meta-analysis), followed by USCA02 (+35%; Fig. 1b). However, the surface SOC concentration was 22% less under cover cropping at USMN01 (Fig. 1b). The greater TN (average: 8%; 95% CI: 2–14%) under cover cropping was also found (Fig. 1c). The average WSA and Cmin were also greater by 15% (95% CI: 5–26%) and 34% (95% CI: 15–55%), respectively, compared to no cover cropping controls (Fig. 1d, e).

3.2. Soil organic carbon sequestration

Cover cropping had significantly ($P < 0.05$) greater SOC stocks in the 0–15 cm depth, with an average value of 7% (95% CI: 1–14%; Fig. 1f), which equated to 1.69 Mg C ha^{-1} (95% CI: 0.24–3.38 Mg C ha^{-1}) greater SOC stocks. Similar to SOC concentration, the highest change in surface SOC stocks by cover cropping was also observed at USAL02 (+208%), followed by USCA02 (+36%; Fig. 1f).

Out of 36 pairs of cover cropping treatments, 27 treatments (75%) sequestered SOC, which ranged from 0.21 to 15.98 Mg C ha^{-1}, with a mean of 3.55 Mg C ha^{-1} (Fig. 2a). The "Old Rotation" site (USAL02) had the highest net surface SOC sequestration value (Fig. 2a), which was established in 1896 (Table 1). In contrast, 25% of cover cropping treatments ($n = 9$) had less surface SOC stocks, compared to the no cover cropping controls for the given period (Fig. 2a). For example, the USTN02c had about 20.29% less surface SOC stocks for cover cropping treatment (24.39 Mg C ha^{-1}), compared to its fallow control (30.60 Mg C ha^{-1}; Fig. 2a).

The average surface SOC sequestration rate under cover cropping was 0.24 Mg C ha^{-1} yr^{-1}, with a range of 0.02–1.10 Mg C ha^{-1} yr^{-1} (Fig. 2b). In contrast to SOC sequestration, the highest surface SOC sequestration rate was at USMN02, which was established in 2014 (Fig. 2b; Table 1). It was followed by some cover cropping treatments established in 2007 (e.g., USKS01b) and 2008 (e.g., USMI02), with about 10-yrs of cover cropping (Fig. 2b; Table 1). However, the relatively low surface SOC sequestration rate was occupied by the cover cropping treatments established in 1991 (USSD01) and 2001 (USTN01), respectively (Fig. 2b; Table 1).

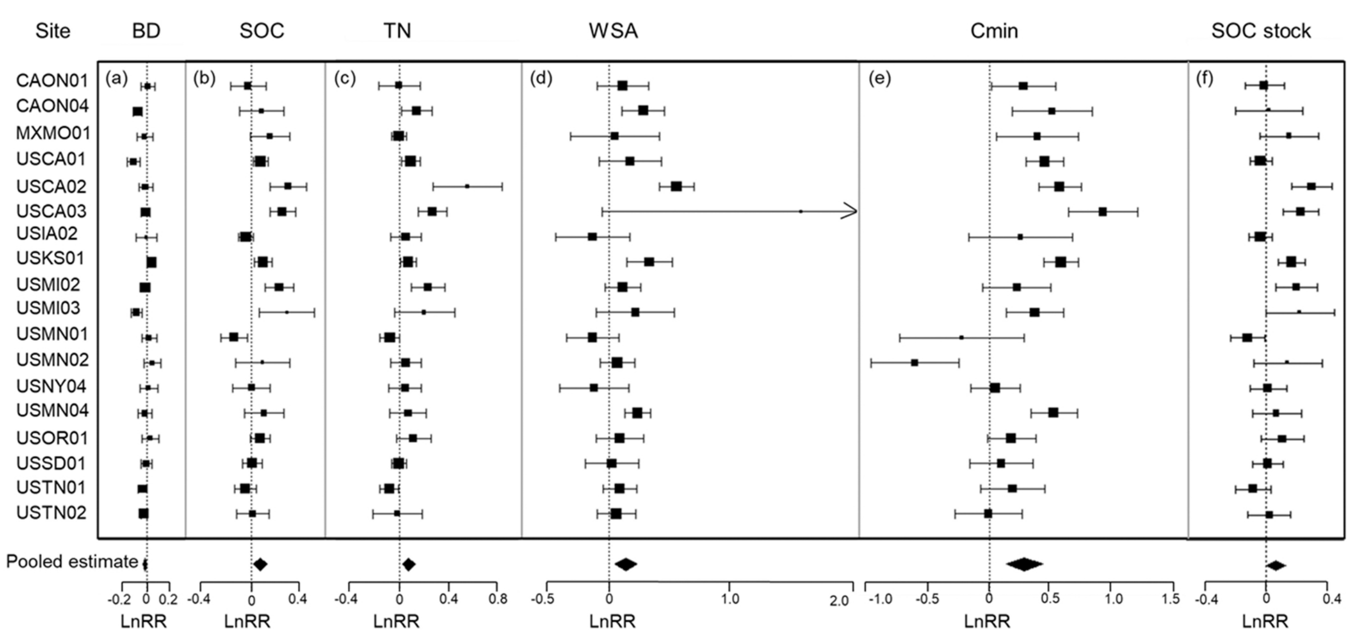

Fig. 1. The estimated effect size of cover cropping on soil properties (df = 17): (a) bulk density (BD), (b) soil organic carbon (SOC) concentration, (c) total nitrogen (TN), (d) water-stable aggregates (WSA), (e) potential carbon mineralization (Cmin), and (f) SOC stock. The estimated effect size (LnRR: the natural log of response ratio) for each site is indicated by the square, and the whisker through the square represents the 95% confidence interval (CI). A larger size of the square indicates a greater weight of the site in the pooled estimate. The pooled effect size (diamond) with 95% CI is given at the bottom of each forest plot. When the 95% CI line crossed the null value (LnRR = 0), the effect of cover cropping is insignificant. Note: Data from the USAL02 site was not included in this analysis due to a lack of replication with cover crop treatments; information on the site can be found in Table 1.

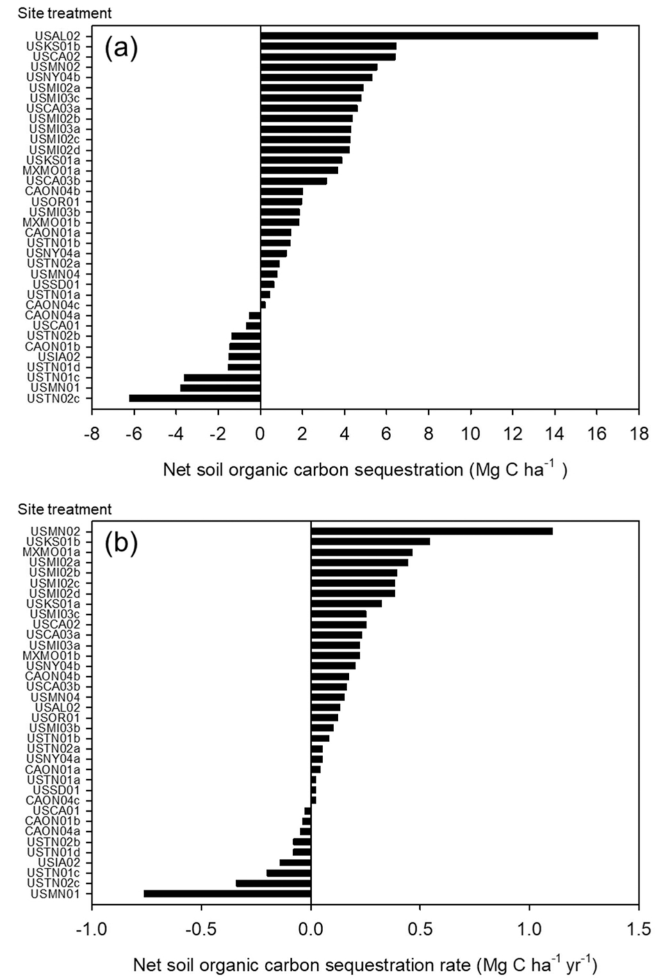

Fig. 2. Net soil organic carbon (SOC) sequestration (a) and SOC sequestration rate (b) due to cover cropping. Information on the site treatment (Site and treatment (TRT) ID) can be found in Table 1.

3.3. Factors affecting SOC stocks with cover crops

The SEM analysis was conducted to better understand the factors that influence surface SOC stocks (0–15 cm depth) with cover crops. At first, the Boruta analysis confirmed that all selected factors were important attributes (Table 2; Fig. 3), in descending order of importance values were climate (Hargreaves climatic moisture deficit), cover cropping system (i.e., cover crop type and duration of cover cropping), soil properties (i.e., clay, silt, and soil pH), and finally main crop management practices (i.e., tillage and rotation; Fig. 3). Thus, the confirmed 8 factors were included in the SEM analysis (Table 2).

According to the goodness-of-fit indicators, the proposed structural model matched the given data well (Fig. 4). The Hargreaves climatic moisture deficit (i.e., CMD) was the main driver of SOC stock variation under cover cropping. Its negative coefficient (−0.52) indicates that increasing precipitation (i.e., going from a high CMD to a low CMD) had a positive effect on SOC accumulation under cover cropping (Fig. 4). Soil clay content (0.34) and soil pH (0.38) had a significantly positive effect on SOC stocks under cover cropping, while soil silt content was a non-significant ($P = 0.378$) source of SOC stock variation with cover crops. In terms of main crop management practices, tillage did not significantly ($P = 0.225$) affect SOC stocks with cover cropping, while crop rotation had a significant ($P < 0.001$) positive (0.28) effect on SOC stocks under cover cropping (Fig. 4). In addition, we found a significant ($P < 0.001$) positive (0.36) relationship between the type of cover crops and SOC stocks, indicating that using non-legume cover crops is likely to be more effective for improving SOC stocks, compared to legumes and mixtures (Fig. 4). A significant ($P < 0.001$) positive (0.20) relationship between the duration of cover cropping and SOC stocks was also measured (Fig. 4), suggesting that longer use of cover crops is better to improve SOC stocks.

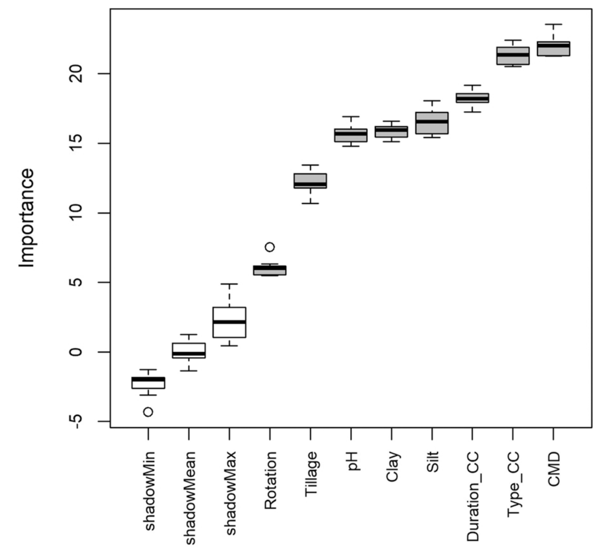

Fig. 3. Important order of factors driving the variability in soil organic carbon stocks under cover cropping treatments ($n = 132$). White boxplots correspond to the minimal, average, and maximum Z score of a shadow attribute. Grey boxplots represent Z scores of confirmed important variables. CMD: Hargreaves climatic moisture deficit; Type_CC: cover crop type; Duration_CC: duration of cover cropping.

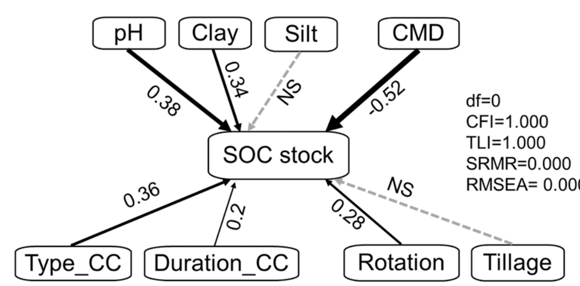

Fig. 4. The effect of selected climate, soil properties, and management practices on soil organic carbon (SOC) stock under cover cropping (CC; $n = 132$), according to structural equation modeling (SEM). The hypothesized model in SEM was the regression model (SOC stock ~ CMD + clay + silt + pH + tillage +rotation + type_CC + duration_CC; $R^2 = 0.71$). The solid arrows indicate significant effects (P (>|z|) < 0.05), while grey dash arrows indicate the non-significant (NS) path. The width of the arrows represents the strength of the effects. The value near each arrow was the standardized path coefficient for the given variable. CMD: Hargreaves climatic moisture deficit (mm); CFI: Confirmatory Factor Index; TLI: Tucker-Lewis Index; SRMR: standardized root mean square residual; RMSEA: root mean square error of approximation.

4. Discussion

4.1. Cover crop effects on soil properties

The results of this study demonstrated that in the majority of cases cover cropping had positive effects on soil physical properties; however, the influence on different soil physical indicators (i.e., soil BD and WSA) varied (Fig. 1). Our observed lack of statistically significant overall cover crop effect on soil BD is somewhat inconsistent with a recent review by Haruna et al. (2020) who reported that cover cropping decreased soil BD by 4%. Our data implied that more than 5-yrs of cover cropping was insufficient to markedly reduce soil BD, even in the surface soil (0–7.6 cm depth in this study; Table 1; Fig. 1a). While most of the cover crops used in this research have relatively fine roots system (Table 1), tap-root cover crops (e.g., daikon radish: *Raphanus sativus* var. *longipinnatus*) may have a greater capacity to reduce subsurface soil compaction than fibrous-rooted species (e.g., cereal rye: *Secale cereale* L.; Chen and Weil, 2010), which might explain the non-significant influence on soil BD. Also, the approach (core method: cylinder) used to measure soil BD might have contributed to the lack of cover cropping effect on soil BD, due to its less measurement accuracy than the indirect radiation method (Al-Shammary et al., 2018). In contrast, a positive effect on soil aggregate stability was detectable (Fig. 1d) and agreed with the study of Blanco-Canqui and Ruis (2020), with an average increase of 16%. The positive effect on soil aggregation may enhance the resistance to physical stress (e.g., erosion), hence reducing SOC losses from the soil and increasing the stabilization of SOM (Chaplot and Cooper, 2015).

Cover cropping had a positive influence on soil biological indicators (Fig. 1b, c, e). For example, a greater average value of short-term Cmin (24 h) with cover crops was found (Fig. 1e). Similar results of improved soil microbial activity driven by cover cropping have been reported (e.g., Chahal and Van Eerd, 2019; Ghimire et al., 2017). Moreover, our results showed significantly ($P < 0.05$) greater surface SOC and TN concentration under cover cropping (Fig. 1b, c). This is consistent with previous literature synthesis (e.g., Van Eerd et al., 2023; Sharma et al., 2018; Blanco-Canqui et al., 2015), on-farm studies (e.g., Farmaha et al., 2022), and agricultural research sites (e.g., Chahal and Van Eerd, 2018). The use of cover crops results in consistently greater surface SOC stocks when compared with their absence (Fig. 1 f). However, the size of its effect varies among studies. For example, a meta-analysis of 181 paired SOC observations (15% from long-term experiments) from 40 studies (spatial scale similar to this study) showed that cover crops had 12% greater SOC stocks, in the 0–30 cm soil depth (McClelland et al., 2021). This estimate was greater than our result (8%; Fig. 1f), although shallower soil depth (0–15 cm) and more long-term experiments (86%) were included in our study (Table 1). Another meta-analysis (75% of data collocated from the temperate zone) reported that the potential SOC sequestration rate with cover crops was 0.32 ± 0.08 Mg C ha^{-1} yr^{-1}, in the 0–20 cm soil depth (Poeplau and Don, 2015), which is about 33% greater than our finding (0.24 Mg C ha^{-1} yr^{-1}; Fig. 2b). All SOC sequestration estimates (net) are relative to the no cover cropping controls, therefore, they may be influenced by the rate/magnitude of SOC losses over time in the fallow controls. However, in general, site-specific factors such as cover crop type, its root system, and its agronomic management (e.g., growth window and fertilization; hence, biomass production) may explain the considerable differences in SOC sequestration under cover cropping.

4.2. Maximizing soil organic carbon stock

Adapting cover crops on croplands is an effective SOC sequestration strategy; however, its sequestration capacity depends on various factors (McClelland et al., 2021; Jian et al., 2020). Here we identified the important order of these factors, descending from climate > cover crop management (i.e., type and study duration) > soil properties > main crop management (i.e., rotational diversity and tillage), based on their importance values (Fig. 3). It is well known that cover cropping with high annual precipitation and soil clay content has greater SOC stocks (Jian et al., 2020; Six et al., 2002), which is consistent with our understanding of SOC accrual (Fig. 4). Since these factors are fixed to a particular site, we will focus on management practices that are under the farmer's decision. We found cover crop type had a greater positive relationship with surface SOC stocks, compared to the duration of cover cropping (Fig. 4), suggesting that the selection of cover crop species is the key to improving SOC stocks under cover cropping.

Cover crop type may differentially influence the pathways and magnitude of SOC sequestration, due to distinct biogeochemical plant traits (e.g., biomass C, C/N ratio, lignin content, etc.; Zhang et al., 2022). In this study, the use of non-legume cover crops, most frequently cereal rye (Table 1), showed greater SOC stocks, compared to either legumes or mixtures (Fig. 4). The amount of C input from cover crop residues entered into the soil explained most SOC variation, especially the labile SOC fraction (Duval et al., 2016). For example, Higashi et al. (2014) found that cereal rye had 56% greater SOC stocks (13.4 Mg C ha^{-1}) than hairy vetch (*Vicia villosa* Roth; 8.6 Mg C ha^{-1}), due to greater biomass produced during the 9-yrs of cover cropping. In addition, cover crop species with fine-branched roots (e.g., ryegrass (*Lolium* spp.) and cereal rye) are more effective in reducing C loss by erosion (De Baets et al., 2011) and commonly grown in North America (Morrison and Lawley, 2021; O'Connell et al., 2015). Cover crop legumes and grass mixtures (5 sites; Table 1) may have less influence on SOC stocks, compared to non-legumes and legumes (Fig. 3). Florence and McGuire (2020) reported that the best-performing mixtures did not generally perform better on cover crop production and soil biology indicators (e.g., SOC and SOM) in the extracted comparisons, compared to the best-performing monocultures. However, the lack of numeric information on cover crop biomass, as well as the proportion of legumes and non-legumes within mixtures restricted us from deeply exploring the effect of cover crop type on surface SOC stocks. Regardless, the mechanism of SOC accrual is likely to be a combined effect of adding additional plant C inputs and mitigating SOC losses (Van Eerd et al., 2023; De Baets et al., 2011); however, increased C inputs are generally regarded as the main driver of SOC accumulation with cover crops in the system (Seitz et al., 2022).

Apart from cover crop biomass production, Johnson et al. (2007) argued that residue quality plays an important role in SOC stabilization. A recent conceptual framework of microbe-derived C highlighted that high-quality residue (i.e., low C/N ratios) was more effectively converted to stable SOM than residues with high C/N ratios (Cotrufo and Lavallee, 2022; Liang and Zhu, 2021). Decomposition of high-quality residue may promote microbial growth efficiency, which results in more microbial-derived SOM (i.e., dead cells) stabilized in association with clay minerals (Cotrufo et al., 2015). For example, legumes had a greater accumulation of microbially-derived C in mineral-associated SOM than non-legumes (Zhang et al., 2022). However, the labile soil C pool (e.g., plant-derived C) should not be ignored, which could effectively capture atmospheric C through rapid biomass production despite its short retention time in soil (Lavallee et al., 2020). These latest studies highlight opportunities for SOC sequestration by cover cropping to promote long-term soil C stabilization as cover crops generally have lower C/N values (< 30) than main crop residues (e.g., wheat straw > 50; Ruark and Franzen, 2020; Huang et al., 2004). Moreover, the study of Ghimire et al. (2017) suggested a need for at least 5 Mg ha^{-1} of cover crop residue to maintain SOC, regardless of the quality of cover crops used. Thus, maximizing the biomass of cover crops returned into soils is essential to increasing SOC stocks, and appears more important than optimizing C/N ratios through the inclusion of legumes (Ardenti et al., 2023).

A positive crop rotational diversity effect on SOC accumulation under cover cropping was observed (Fig. 4), which agreed with other studies (e.g., West and Post, 2002). A meta-analysis found that adding one or more cash crops into a monoculture rotation increased SOC concentration by 3.6% (McDaniel et al., 2014). In particular, when crop rotations included cover crops, SOC increased by 8.5% (McDaniel et al., 2014). Increasing plant biodiversity (e.g., crop rotational diversity and cover crops) can enrich the variety of biomass and exudates entering the soil over time (Chamberlain et al., 2020) and improve soil microbial activity (Tosi et al., 2022); hence, increasing SOC accumulation. However, enhancing crop diversity from corn (*Zea mays* L.) to corn-soybean (*Glycine max* L.) may not lead to a greater SOC concentration, due to a lower residue C input and a higher SOC decomposition rate under soybean than corn (West and Post, 2002). Moreover, Congreves et al. (2017) reported that cropping rotation (corn-soybean) had greater SOC stocks with winter wheat (*Triticum aestivum* L.) than without. These studies indicate that the effect of cover cropping on SOC accumulation is not only dependent on the number of individual crops but also on crop species grown in the rotation.

We hypothesized a positive effect of reduced tillage on SOC stocks under cover cropping, which was not supported by our results (Fig. 4). The lack of tillage effect differs from a global analysis of 67 long-term experiments, which reported that no-till improved SOC sequestration rate by 0.57 Mg C ha^{-1} yr^{-1}, compared to conventional tillage (West and Post, 2002). The increase in SOC stocks due to decreased tillage intensity was also confirmed by previous meta-analyses (e.g., Crystal-Ornelas et al., 2021). From the full NAPESHM dataset when specifically examining each individual management practice, decreased tillage intensity and cover cropping had greater SOC concentrations (Liptzin et al., 2022). In our study, the unexpected lack of tillage effects within cover crop systems might be because cover crops helped to negate any soil and carbon losses due to erosion that might occur with tillage. Furthermore, our shallow sampling (0–15 cm) may be insufficient to assess the effect of reduced tillage under cover cropping on SOC stocks (Baker et al., 2007). More data and research are needed to fully explore the interactive effect of tillage intensity and cover cropping and the potential of cover crops to mitigate tillage effects on SOC.

4.3. Limitations and future research

This study systematically compared and quantified the effects of climate, soil properties, and crop management practices on SOC stocks under cover cropping. However, due to the limited number of paired SOC observations ($n = 18$), we were unable to use LnRR values to build models. Like meta-analyses, our approach is limited by the low number of cover crop long-term experiments and the few cover crop types under study.

In addition, the lack of cover crop characteristic data, such as biomass production and C/N ratio, restricted us from further understanding the relationship between the functional types of cover crops and SOC accumulation. Exploring the effect of the quantity (biomass) and quality (C/N ratios) of cover crops grown on SOC stocks remains a research gap. Also, how different sources of C inputs, such as biomass of shoots and roots, phyllo- (senescent leaves) and rhizo-deposition (root exudates and small fragments), from both cover crop and main crop residues affect SOC accumulation and stabilization is not well-known. Moreover, deeper SOC in response to cover cropping needs to be examined, especially in long-term experiments, to better understand mechanisms of SOC stocks accrual under cover cropping.

In terms of SOC stocks calculation, the fixed depth approach was used, instead of the equivalent soil mass basis in this study, as it is a comparable and convenient approach (Rovira et al., 2022). Estimation errors of SOC stocks by fixed depth have been noticed in hypothetical analysis (von Haden et al., 2020; Wendt and Hauser, 2013), literature reviews (Rovira et al., 2022; Xiao et al., 2020), and field research (Ellert and Bettany, 1995) if soil BD changes with depth (Wendt and Hauser, 2013) or if soil BD is different among treatments (i.e., land use: pasture vs. forest (Rovira et al., 2022) or soil management (e.g., tillage system; Xiao et al., 2020). There is limited evidence of a change in soil BD induced by cover cropping in the literature (see review by Blanco-Canqui and Ruis (2020) as well as in this study (Fig. 1a) and another NAPESHM study (Bagnall et al., 2023). Rovira et al. (2022) suggested that the fixed depth approach may be a better choice than the equivalent soil mass approach when the comparison of SOC stocks is at a regional or global scale, with different climate, geological, and soil conditions. This is the case of our study. Further research should compare the fixed depth and equivalent soil mass approaches in long-term field experiments and at deeper soil layers.

5. Conclusions

Cover cropping had significantly greater surface (0–15 cm depth) soil organic carbon (SOC), total nitrogen, water-stable aggregates, and potential carbon mineralization, whereas the effect on soil bulk density was non-significant. Here we explored the potential pathways to maximize SOC stocks under cover cropping. To the best of our knowledge, this is the first study to identify and prioritize factors affecting SOC stocks under cover cropping by combining the Boruta algorithm and SEM. This approach may be applied to explore other causal relationships, especially when the dataset includes both categorized and continuous variables.

Although cover crop management data were very limiting, results from 19 sites with long-term cover cropping across North America suggest that the potential for SOC sequestration under cover cropping is mainly determined by climate (moisture deficit), followed by soil inherent properties (esp. soil clay content). However, farmers can adjust agricultural management practices to maximize SOC stocks under cover cropping, including selecting cover crops that not only add plant C inputs but also mitigate SOC losses. We also highlight the importance of stacking multiple sustainable agricultural practices (e.g., diverse crop rotation) with cover cropping, to optimize the ability of cover cropping to sequester SOC. The extent to which cover crop functional types contributes to SOC formation and stabilization (e.g., different C pools), especially at deeper soil depth, is not fully understood.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108599.

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