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Adding anaerobic digestate to commercial farm fields increases soil organic carbon

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ABSTRACT

Integrating anaerobic digestion technology can help farms meet agronomic, environmental, and economic goals. The slurry by-product from anaerobic digestion – anaerobic digestate – can be applied to croplands as an organic amendment and fertilizer. However, digestate effects on soil organic carbon (SOC) and soil fertility are poorly understood, especially at the field scale. In this study, we analyzed data from a multi-field commercial farm in Iowa, USA, which integrates agricultural production with anaerobic digestion. The digestate produced in this system was applied to 14 crop fields over 5–12 years. To assess the digestate effects on SOC stocks and soil plant-available nutrients, we analyzed the digestate chemical composition, digestate rates, and soil test results of 421 samples taken at 0–15 cm soil depth. Most sampled points (86 %) increased SOC stock, with greater gains observed in soils with lower initial SOC levels. The average SOC accrual rate was 0.8 Mg ha^{-1} y^{-1} (confidence interval: 0.6–0.9 Mg ha^{-1} yr^{-1}), and isotope analysis (^{13}C and ^{15}N) indicated that new soil organic matter is primarily derived from digestate. Assuming simplified SOC dynamics, the SOC formation efficiency from digestate was estimated at 18 % (higher than the estimate for raw manure). Anaerobic digestate also increased soil test phosphorus (STP) and potassium, with STP values doubling over eight years, exceeding crop requirements. Integrating anaerobic digestion on farms can help reverse soil degradation and enhance agricultural sustainability, although STP should be monitored to prevent potential adverse environmental impacts.

1. Introduction

There is a pressing need to develop sustainable agricultural systems that provide food, energy, and ecosystem services to support society. Long-term and widespread soil degradation, which has resulted in 15–50 % losses in soil organic carbon (SOC) from croplands [1–3], needs to be reversed to achieve these goals [4,5]. Loss of SOC has two significant negative consequences: i) emission of carbon dioxide (CO_2) into the atmosphere [6] - the most important anthropogenic greenhouse gas - (GHG), and ii) loss of crop productivity and soil ecosystem services [7–9].

Integrating anaerobic digestion technologies with agriculture could help farmers restore soil health while meeting additional agronomic, environmental, and economic goals [10]. Anaerobic digesters are controlled environments in which organic materials, such as agricultural residues, animal manure, and food waste, are broken down by microbes into simpler organic and inorganic compounds. This results in significant methane (CH_4) production which can be captured and upgraded to renewable natural gas (RNG). When anaerobic digestion is coupled with agriculture, the resulting slurry by-product – known as anaerobic digestate – can be applied to croplands to improve SOC stocks, soil health, and soil fertility [11–13].

The anaerobic digestion process makes digestate chemically and biologically different from its agricultural feedstock inputs (animal manure and plant biomass). Thus, we expect digestate to be decomposed and cycled differently in soil compared to the original inputs (i.e., manure and plant biomass). For instance, researchers found that digestate-SOC formation efficiency (SOC_{FE}) – i.e. the proportion of digestate that is not respired after its decomposition and contributes to SOC formation – is higher than that of undigested plant biomass or

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manure [14,15]. On the other hand, digestate applications can reduce the rate of endogenous SOC mineralization [14,16], an effect known as negative priming [17]. These effects impact the net C balance and are crucial for understanding how digestate can influence SOC dynamics. However, since most of these studies were conducted under controlled conditions, the ability to scale these results to a commercial farm scale is not well knownal.

Experiments applying digestates to soils at the commercial scale are needed to understand their effects under real-world conditions. Four recent field experiments, 2–8 years in duration, on plots ranging from 6 to 1350 m^2 have shown that SOC can increase 5–20 % after digestate additions [18–21]. These results are noteworthy because SOC is typically a slow-responding variable, requiring more than five years to detect changes after land use/management change, especially in temperate regions [22,23]. In some cases, even after >20 years of manure inputs in the Midwest US, researchers have shown little to no change in SOC [24,25].

Collaborative, on-farm research between farmers and scientists is critical to address agriculture's most pressing challenges. This research occurs at scales that are meaningful for production, increasing farmer interest and motivation to adopt new management techniques, and incorporates the complex interactions between all factors embedded in real-world farm management, such as the size of farm equipment, landscape variability, and farmers' decisions [26,27]. Additionally, on-farm research can reduce crop yield variability when compared to small-plot trials, increasing the potential to detect significant differences [28]. Experiments under controlled conditions in laboratories and on smaller plot scales provide relevant information, and both types of experimentation are necessary and complementary to understand better how adding digestate to fields affects SOC.

In this study, we analyzed data from a commercial farm in Eastern Iowa, USA, that produces beef, maize (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and biogas through the anaerobic digestion of plant biomass, manure, and off-farm waste. We analyzed 14 crop fields (378 ha in total) that received digestate for 5–12 years (Fig. 1). Our goal was to assess the effect of digestates on SOC stocks and soil plant-available nutrients. We also assessed soil test potassium (STK) and phosphorus (STP) because they are key indicators of two essential plant-available macronutrients [29], and because STP is also a relevant index of potential water quality issues that may arise as trade-offs when adding animal manure [30].

2. Materials and methods

2.1. Farm system description

The facilities on the farm include two large digester tanks with a volume of 3700 m^3 each that produce approximately 11,000 m^3/day of biogas composed of 60–70 % methane. Between 2013 and 2022, the biogas was used to power an engine to generate electricity, part of which

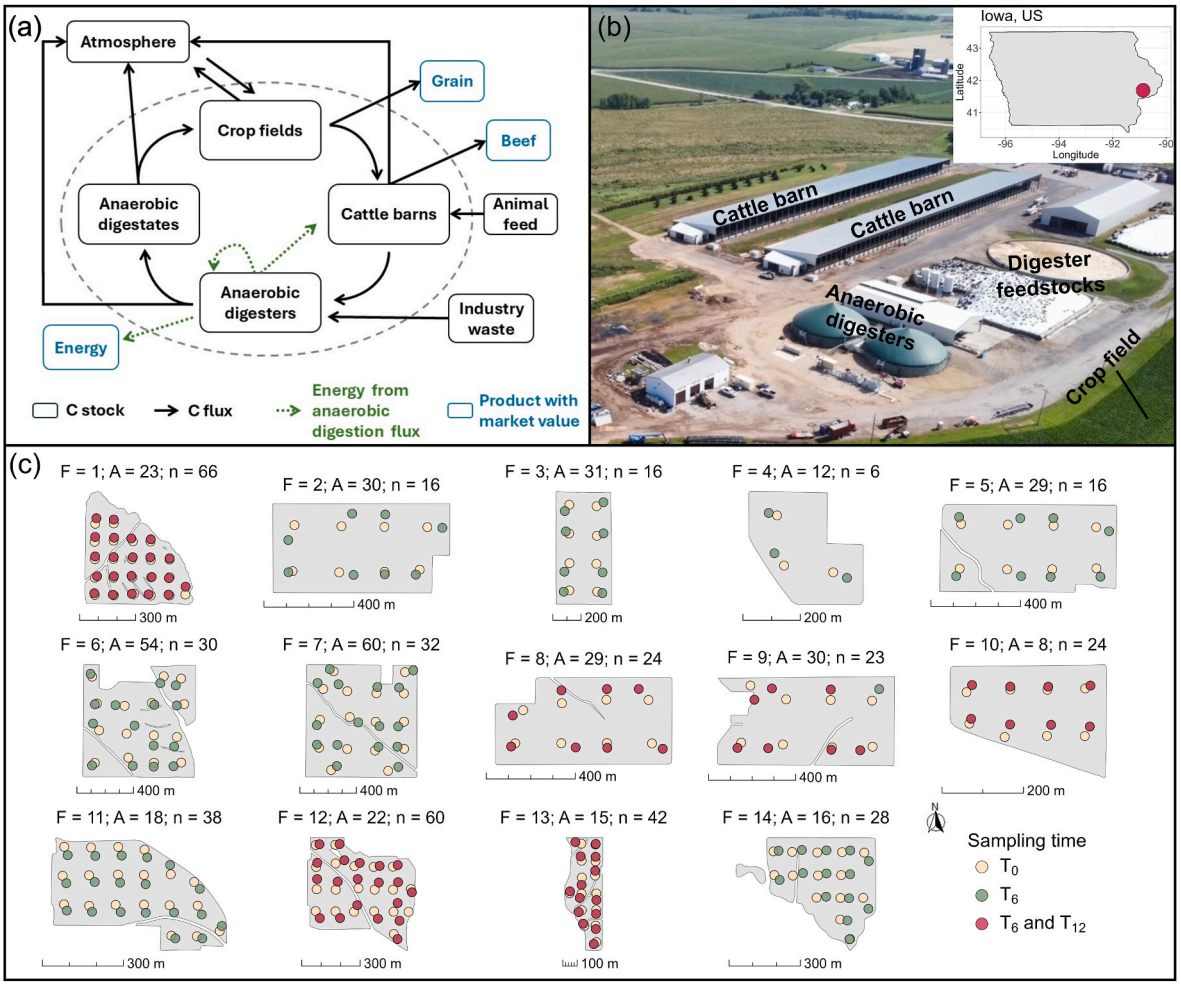

Fig. 1. **(a)** A schematic representation of carbon (C) stocks and fluxes, energy from anaerobic digestion, and products with market value of the commercial farm (gray dashed circle indicates system limits); **(b)** farm location in Iowa, US (red point) and an aerial photo of the farm showing integration of cattle barn, crop fields, and anaerobic digesters; **(c)** 14 fields with gridded soil sampling locations of the 14 analyzed fields. F: field number, A: area (ha), n: soil samples number, T_0: initial time (before the digestates application), T_6: six years after digestates application (on average), T_{12}: twelve years after digestates application (on average).

is used on-site for farming and digestion operations, while the remainder was sold to the grid (Fig. 1b). The facility was recently reconfigured to upgrade the biogas to RNG, which is injected into a pipeline. The main organic inputs to the digesters between 2013 and 2014 were manure mixed with maize (*Zea mays* L.) stover. Maize stover was harvested from some crop fields for use as bedding in the cattle barns. From there, the maize stover and manure mixture were fed into the anaerobic digester (Fig. 1b). For the 2014–2022 period, off-farm organic waste, such as food processing waste, was another relevant input to the digester, representing 40 % of total organic inputs (Supplemental, Fig. S1).

After biogas production, the resulting digestate was separated into solid and liquid phases. Approximately 10,000 Mg of solid and 75,000 m^3 of liquid digestate per year were produced and applied to the fields (Fig. 1b). Chemical analyses of digestates were performed annually at Minnesota Valley Testing Laboratories (MVTL, 2024) (Table 1). Liquid digestate was injected into the soil during fall or spring before planting maize at rates ranging from 13 to 85 m^3 ha^{-1}, and solid digestate was broadcasted at rates ranging from 6 to 144 Mg ha^{-1} the following winter or early spring in those fields where the maize stover was harvested for cattle bedding. Most of the fields (79 %) received both liquid and solid digestates, while one field received only solid digestate and two fields received only liquid digestate (Table S1).

Crop fields are in eastern Iowa (Fig. 1b), within a landscape characterized by a dendritic drainage pattern with a median slope gradient of 4 %, and have a long history of maize and soybean (*Glycine max* L. Merr.) cultivation. From 2011 to 2020, the crop sequence consisted of corn and soybeans under no-till management. The proportion of corn ranged from 50 to 63 % across most fields, except in fields 10, 11, 12, and 13, where it increased to 90 % (Table S1). This region's average annual temperature and precipitation are 11 °C and 1000 mm, respectively. Soils are deep and well-drained with silt loam and silty clay loam textures, 3.7 % of soil organic matter (SOM), and a pH near 7 in the 0–15 soil layer (Table 2).

2.2. Soil data

Soil sampling was carried out at three different times. The first sampling occurred between 2011 and 2013, before digestate application (T_0), the second was six years later (T_6), and the third was 12 years after digestate (T_{12}), on average. All fields were sampled at T_0 and T_6, and six fields (fields 1, 8, 9, 10, 12, and 13) were also sampled at T_{12} (Fig. 1c). All soil samples were collected from 0 to 15 cm depth, but in T_{12}, we also sampled at the 15–30 cm depth. All soil samples were georeferenced, thus each location was revisited at different times. The total number of soil samples was 421, and the sampling density ranged between 0.3 and 1 sample ha^{-1} (Fig. 1c).

Soil tests at T_0 and T_6 were conducted in commercial labs and included pH, SOM determined by the loss-on-ignition (LOI) method (2 h at 360 °C) [31], STP determined by the Mehlich III method (Mehlich, 1984), and STK determined by ammonium acetate extraction (Hanlon and Johnson, 1984). At T_{12}, soil analyses were conducted at Iowa State University, USA, and we measured SOC, soil nitrogen (N), soil particle size distribution, and bulk density. Total C and N were measured using a Vario Max (Elementar; Langenselbold, Germany) equipped with a thermal conductivity detector for CO_2 and N_2 that measures C and N after combustion at 900 °C. Soil samples were fumigated with hydrochloric acid [32] before analyzing C and N to remove potential inorganic C. Soil particle size distribution was measured using laser diffractometry [33]. Soil samples for bulk density were collected using a 3 cm diameter soil corer. An aliquot was taken from each soil sample and oven-dried at 105 °C to determine soil moisture, and water weight was subtracted from the total soil mass to calculate the total dry weight soil. Then, bulk density was calculated as the dry weight divided by the soil volume.

At T_0 and T_6, SOC was estimated by multiplying SOM by 0.45 [34]. Conversion factors for estimating SOC from SOM are not universal constants, as they can vary with factors like vegetation and soil type [35]. A conversion factor of 0.58 SOM was historically utilized [36], but recent studies have shown this value to be too high, leading to significant overestimates of SOC stocks in topsoils [34,35]. We selected the 0.45 factor for two main reasons. First, we aimed to take a conservative approach in our estimates, and this value falls between the global estimate [35] and a value reported specifically for Iowa's topsoils [37]. Second, a recent study [34] reported that the SOC proportion in SOM ranged from 0.45 to 0.52. We adopted the lower end of this range because the conversion factor tends to be lower in finer soil textures [34, 35], and the studied fields have silt loam and silty clay loam soils (Table 2). Finally, we conducted a sensitivity analysis (Table 3, Fig. S2) using three different conversion factors to evaluate their influence on SOC change estimates over time (fitted models are described in Section 2.3). The estimated SOC change rate ranged from 0.6 to 0.9 Mg ha^{-1} yr^{-1} as the conversion factor varied from 0.5 to 0.41. This range falls within the 95 % confidence interval (CI_{95}) of the estimations obtained with the 0.45 conversion factor, confirming that it represents a conservative choice (Table 3).

Soil organic carbon stocks were estimated using the following equation:

$$SOC = C \times Depth \times BD \quad (1)$$

where SOC stock is the organic carbon stock (Mg ha^{-1}), C is the carbon concentration (%), BD is the bulk density (Mg m^{-3}), and Depth is soil depth (cm). An average BD of 1.1 Mg m^{-3} was assumed in T_0 and T_6, where BD was not measured (Table 2). A unique value of BD for each field was utilized assuming this soil property was stable across time.

At T_{12}, we also measured the natural abundance of $\delta^{13}C$ and $\delta^{15}N$ in three fields (fields 1, 8, and 9) using a Thermo Finnigan Delta Plus XL mass spectrometer (ThermoFisher Scientific; Waltham, Massachusetts) in continuous flow mode connected to an Elemental Analyzer (Costech Analytical Tech Inc. Valencia, California) at the Stable Isotope Lab, Iowa State University, USA. We selected those fields because they have similar crop rotation (Table S1) but received contrasting cumulative digestate rates: low (Field 1, 9.9 Mg C ha^{-1}), medium (Field 8, 24.2 Mg C ha^{-1}), and high rate (Field 9, 35.8 Mg C ha^{-1}).

2.3. Carbon inputs from digestate and C-loading estimations

Total carbon input into the soil from digestate was calculated as the sum of C-input from solid and liquid digestates. The digestates C-inputs were estimated using the digestates rates and C concentrations (Table 1),

Table 1
Chemical properties of liquid and solid anaerobic digestate from 2011 to 2022[a].

Chemical property	Liquid Digestate (n = 12)		Solid Digestate (n = 10)	
	Mean	CV[b]	Mean	CV
Dry matter, %	7.7	42	29.7	9
Carbon (C), %	2.0	–	11.9	–
Total nitrogen (N), %	0.6	34	0.8	16
Ammonium-N, %	0.4	54	0.3	43
Organic-N (N-Org), %	0.2	30	0.4	11
C:N ratio	3	–	15	–
C:N-org ratio	8	–	27	–
P, %	0.2	45	0.3	25
K, %	0.3	17	0.3	20
S, mg kg^{-1}	657	47	1411	12
Ca, mg kg^{-1}	1369	59	2845	12
Mg, mg kg^{-1}	701	60	1118	17
Na, mg kg^{-1}	695	41	650	22
Cu, mg kg^{-1}	4.99	26	6.4	14
Fe, mg kg^{-1}	771	117	794	24
Mn, mg kg^{-1}	35	93	35	10
Zn, mg kg^{-1}	30	25	39	15

[a] Concentration is expressed on a fresh weight basis.
[b] CV: Coefficient of variation, in percentage (%). CV was not calculated for carbon (−) because it was measured in only one year (2022).

Table 2
Mean (± standard deviation) of soil properties across 14 crop fields at 0–15 cm soil depth[a].

Field	pH	SOM	SOC	STP	STK	CEC	Sand	Silt	Clay	BD
		%	Mg ha^{-1}	mg kg^{-1}	mg kg^{-1}	meq kg^{-1}	%	%	%	Mg m^{-3}
1	7.3 ± 0.5	2.8 ± 0.7	22 ± 5	31 ± 19	109 ± 24	1.5 ± 0.2	19 ± 2	55 ± 2	26 ± 3	1.2
2	6.6 ± 0.3	4.0 ± 0.2	30 ± 2	14 ± 6	123 ± 24	–	–	–	–	–
3	6.2 ± 0.3	4.0 ± 0.2	30 ± 2	19 ± 5	152 ± 26	–	–	–	–	–
4	6.6 ± 0.1	4.4 ± 0.2	33 ± 1	21 ± 13	159 ± 35	–	–	–	–	–
5	6.8 ± 0.3	3.8 ± 0.4	28 ± 3	24 ± 7	155 ± 48	–	–	–	–	–
6	7.0 ± 0.4	3.6 ± 0.4	27 ± 3	17 ± 9	121 ± 21	–	–	–	–	–
7	6.4 ± 0.3	4.2 ± 0.5	32 ± 4	23 ± 12	202 ± 88	–	–	–	–	–
8	6.9 ± 0.5	3.8 ± 0.5	27 ± 4	28 ± 17	137 ± 83	–	19 ± 3	53 ± 1	28 ± 2	1.0
9	7.2 ± 0.4	5.0 ± 0.7	41 ± 6	52 ± 27	202 ± 71	–	17 ± 2	52 ± 2	31 ± 1	1.2
10	6.8 ± 0.1	3.5 ± 0.2	27 ± 2	56 ± 17	146 ± 19	1.6 ± 0.1	18 ± 1	65 ± 1	17 ± 1	1.1
11	7.3 ± 0.3	3.4 ± 0.3	26 ± 3	38 ± 20	140 ± 32	1.4 ± 0.3	–	–	–	–
12	7.0 ± 0.3	3.1 ± 0.3	23 ± 2	95 ± 64	147 ± 35	1.5 ± 0.1	22 ± 4	62 ± 3	16 ± 1	1.1
13	6.9 ± 0.3	3.3 ± 0.3	24 ± 2	59 ± 38	146 ± 25	1.7 ± 0.1	21 ± 2	63 ± 2	16 ± 1	1.1
14	6.6 ± 0.6	3.4 ± 0.6	26 ± 4	24 ± 14	150 ± 66	1.5 ± 0.2	–	–	–	–
All	6.8 ± 0.3	3.7 ± 0.6	28 ± 5	36 ± 23	149 ± 26	6.6 ± 0.8	19 ± 2	58 ± 6	22 ± 7	1.1

‡Soil Parameter Abbreviations: SOM: soil organic matter, SOC: soil organic carbon, STP: soil test phosphorus, STK: soil test potassium, CEC: cation exchange capacity, BD: bulk density, ±: standard deviation, -: no data.
[a] The values correspond to the initial time (T_0) except for particle size distribution and bulk density measured at the final time (T_3).

Table 3
Sensitivity analysis of soil organic matter (SOM) to soil organic carbon (SOC) conversion factors on fixed-effect parameters and R^2 of the linear mixed model of SOC (Mg ha^{-1}) across time (years).

SOM to SOC conversion factor	Intercept		Slope		R^2
	Estimate	[a]CI_{95}	Estimate	CI_{95}	
	Mg ha^{-1}		Mg ha^{-1} yr^{-1}		
0.5 [35]	31.8	29.3–34.3	0.6	0.5–0.7	0.30
0.45 [34]	28.3	26.0–30.6	0.8	0.6–0.9	0.41
0.41[b] [37]	25.5	23.3–27.6	0.9	0.8–1.0	0.49

[a] The 95 % confidence interval.
[b] Correspond to the slope of the fitted regression model: SOC ~ 0.41 SOM – 0.04.

as follows:

$$C - input_{LD} = Rate_{LD} \times \frac{C_{LD}}{100} \quad (2)$$

$$C - input_{SD} = Rate_{SD} \times \frac{C_{SD}}{100} \quad (3)$$

$$Total\ C - input = C - input_{LD} + C - input_{SD} \quad (4)$$

where C-input$_{LD}$ is the C-input from the liquid digestate, Rate$_{LD}$ is the liquid digestate rate, C_{LD} is the carbon concentration (%) in the liquid digestate, C-input$_{SD}$ is the C-input from the solid digestate, Rate$_{SD}$ is the solid digestate rate, and C_{SD} is the carbon concentration (%) in the solid digestate.

Chemical and physical associations between organic matter and soil minerals are the most effective mechanisms for stabilizing SOC [38], particularly in high clay soils such as those found in Iowa. However, the soil's capacity for SOC storage in this form is limited because minerals have a finite potential to bond with organic compounds [39]. Once this potential is reached, the soil becomes C-saturated. The mineral-associated organic carbon mass ratio per silt and clay mass (i.e., g C per kg silt + clay) informs the C-loading of the silt + clay fraction [40]. Here, we used the ratio of SOC to the mass of silt + clay to estimate the C-loading. While this indicator includes particulate organic carbon (POC) — the C fraction not associated with minerals and thus not subject to saturation — we still employ it as a proxy for C-loading because: i) our goal is to explore the relationship between C-loading and SOC changes and not to evaluate the C-saturation limit, and ii) the proportion of POC is typically small in Iowa croplands (<10 %; [41]) and we assume that the potential noise introduced due to POC changes is minimal.

2.4. Data analysis

Linear mixed-effects models were fitted using the *nlme* package [42] in R software [43]. SOC stock was modeled as a function of time, digestate-C, and initial C-loading with random intercepts for each field nested within sampling points (Fig. 2c). An exponential spatial correlation structure was applied to account for spatial autocorrelation in the coordinates of each sample location. Soil test phosphorus and STK were modeled as a function of time with the same model structure. The conditional coefficient of determination (R^2_c) [44] was calculated using a slightly modified version [45]. Change in SOC was modeled as a function of initial SOC stocks with random intercepts for each field. The initial SOC stocks categories were constructed according to the 33 % and 66 % percentiles: low minimum to 33 % percentile; medium, 33 % percentile to 66 % percentile; and high, 66 % percentile to the maximum. The $\delta^{13}C$ and $\delta^{15}N$ were compared across digestate rates using the *emmeans* package [46]. The 95 % confidence intervals (CI_{95}) were calculated for the initial SOC stocks categories and the slopes of the fitted models.

3. Results

3.1. SOC changes and their explanatory factors

The average SOC stock increased by 0.8 Mg ha^{-1} yr^{-1} (CI_{95} = 0.6–0.9 Mg ha^{-1} yr^{-1}) (Fig. 2a). At the initial time (T_0), the fields had an average SOC stock of 28 Mg ha^{-1} with a range of 19 Mg ha^{-1} (Table 1). At the last sampling time (T_6 or T_{12}, Fig. 1c), the average SOC stock of fields increased to 35 Mg ha^{-1} with a range of 14 Mg ha^{-1}. The lower limit of the range increased more (21–28 Mg ha^{-1}) than in the upper limit (41–42 Mg ha^{-1}).

Increases in SOC stocks were positively associated with digestate-C inputs (Fig. 2b) and negatively associated with initial C-loading (Fig. 2c). The fitted model for digestate -C predicts that each Mg ha^{-1} of digestate-C applied to the soil increases SOC stock by 0.18 Mg ha^{-1} (Fig. 2b). No SOC increases above 30 g C silt + clay kg^{-1} were predicted based on data from six fields (n = 79; Fig. 2c). It should be noted that this C-loading measure includes bulk SOC, not the mineral-associated C commonly used in C-loading or C saturation analyses and inferences.

Digestate increased SOC stocks of most of the sampled soils (86 %; Fig. 3). SOC stock changes decreased as initial SOC stock increased, with mean changes of 10 Mg ha^{-1} (CI_{95} = 7–13 Mg ha^{-1}), 7 Mg ha^{-1} (CI_{95} =

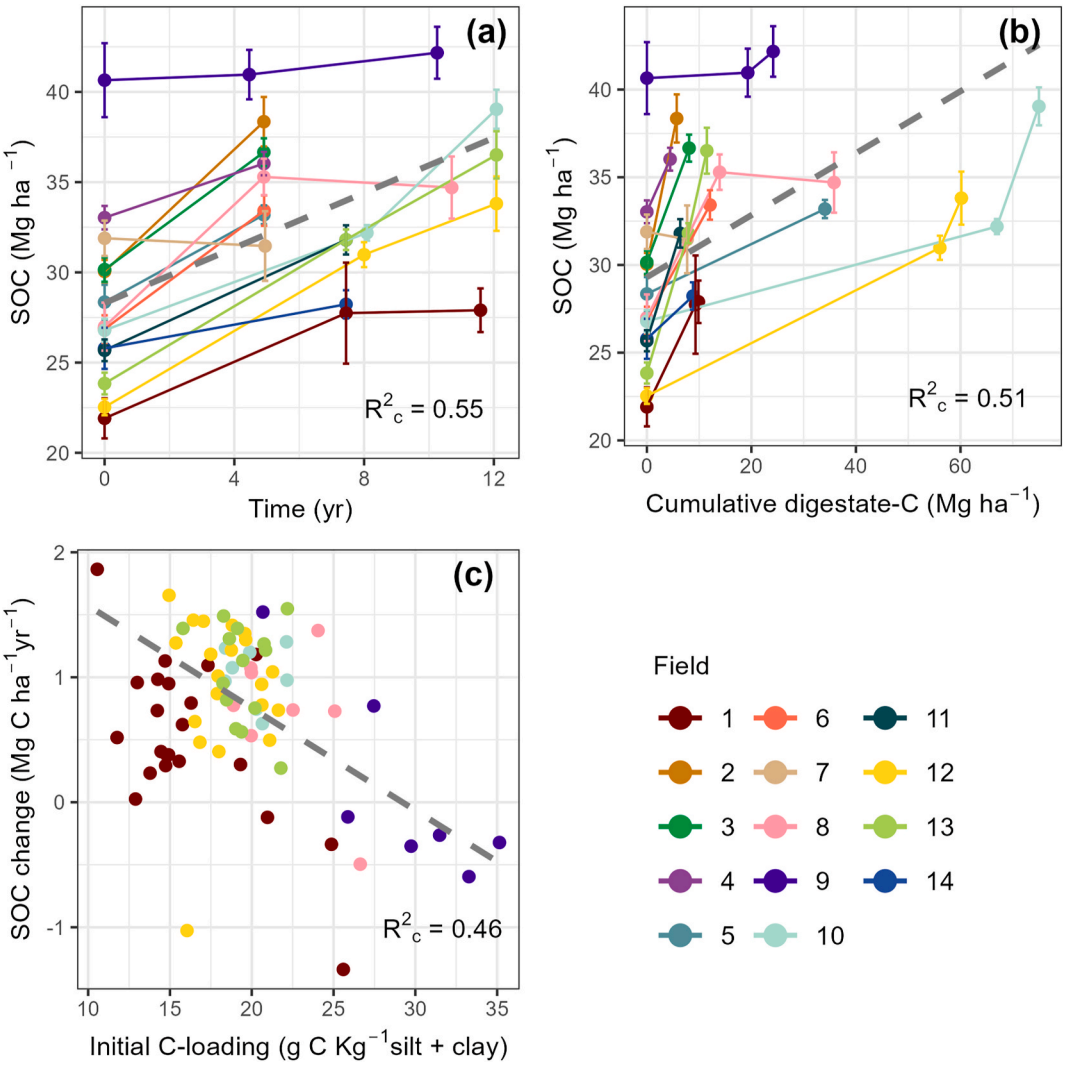

Fig. 2. Soil organic carbon (SOC) stocks of 14 fields (Fig. 1c) across time **(a)** and in relation to cumulative anaerobic digestate-carbon input (digestate-C) **(b)**. In panels **(a)** and **(b)**, each point represents the mean SOC stock, with error bars indicating the standard error of the mean. Panel **(c)** illustrates the relationship between SOC changes from T_0 to T_{12} and the C-loading at T_0. Gray dashed lines show the fitted models for fixed effects: **(a)** SOC (Mg ha^{-1}) = 28 (Mg ha^{-1}) + 0.8 time (y); **(b)** SOC (Mg ha^{-1}) = 29 (Mg ha^{-1}) + 0.18 digestate-C (Mg ha^{-1}); **(c)** SOC change (Mg ha^{-1} y^{-1}) = 2.4 (Mg ha^{-1} y^{-1}) – 0.08 initial C-loading (g C Kg^{-1} silt + clay). R^2_c: conditional coefficient of determination. Diagnostic plots of model residuals are shown in Fig. S4.

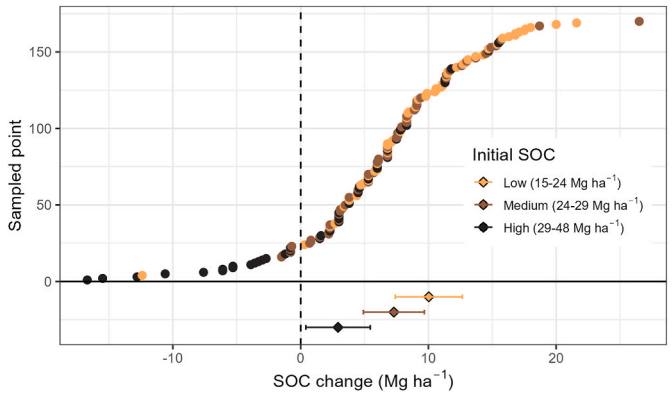

Fig. 3. SOC changes between the first (T_0) and last sampling time (T_6 or T_{12}) (Fig. 1c). The vertical black dash line represents no change. Diamonds and error bars below the 0-y-axis black line correspond to the fixed effects of initial SOC and the 95 % confidence intervals of the linear mixed model. Initial SOC categories differed significantly ($p < 0.01$).

5–10 Mg ha^{-1}), and 3 Mg ha^{-1} (CI_{95} = 0.4–5 Mg ha^{-1}), for low, medium, and high initial SOC categories, respectively (Fig. 3). All initial SOC categories differed significantly ($p < 0.05$), and no CI_{95} included zero, indicating SOC increases across all categories. However, the lower limit for the high initial SOC group was close to zero (0.4 Mg ha^{-1}).

3.2. Carbon and N isotopes in soil and digestate

We selected three fields with contrasting amounts of digestate-C applied – low, medium, and high rates – to analyze soils for $\delta^{13}C$ and $\delta^{15}N$ (Fig. 4). The solid $\delta^{13}C_{AD}$ was −14.7, and $\delta^{15}N_{AD}$ was 10.1. We observed that as the digestate rate increased, the $\delta^{13}C_{soil}$ and $\delta^{15}N_{soil}$ from these fields also increased and approached the digestate δ ($\delta^{13}C$ = −14.7, and $\delta^{15}N$ = 10.1) (Fig. 4). The same pattern was observed for the 15–30 cm soil depth (Supplemental, Fig. S3).

3.3. Digestate effects on soil test phosphorus and potassium

Five to eight years of digestate applications increased extractable STP and STK (Fig. 5). Soil test phosphorus increased by 7 mg P kg^{-1} y^{-1} (CI_{95} = 6–8 mg kg^{-1} y^{-1}) and STK by 10 mg K kg^{-1} y^{-1} (CI_{95} = 8–12 mg

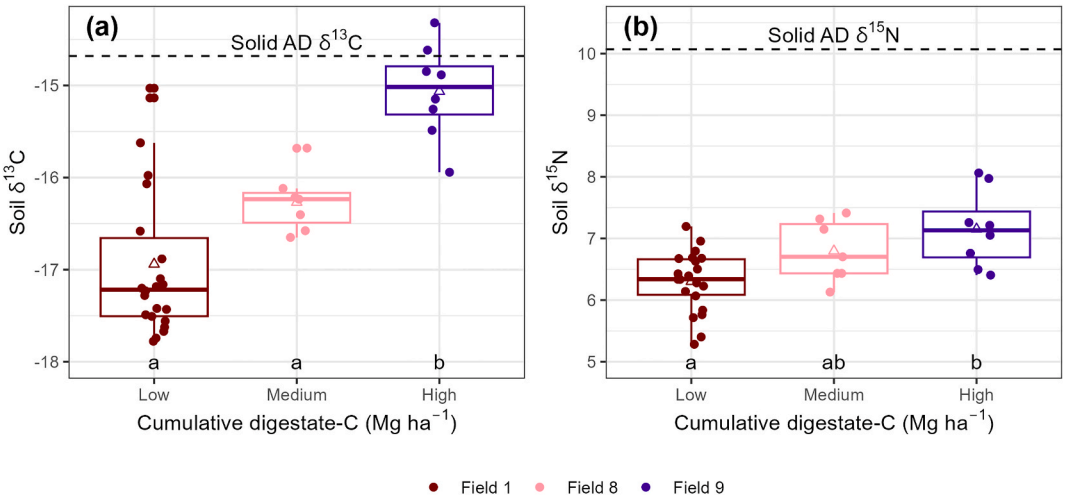

Fig. 4. Soil carbon-13 isotope ($\delta^{13}C$) and nitrogen-15 isotope ($\delta^{15}N$) at 0–15 cm depth and under three different levels of cumulative anaerobic digestate-C (digestate-C) applications. The dashed lines are the $\delta^{13}C$ (a) and $\delta^{15}N$ (b) of solid digestate. The rates are: Low, 9.9 (Field 1); Medium, 24.2 (Field 8); High, 35.8 Mg ha^{-1} (Field 9). Letters indicate significant differences among digestate rates (p-value <0.05). Triangles indicate the mean values.

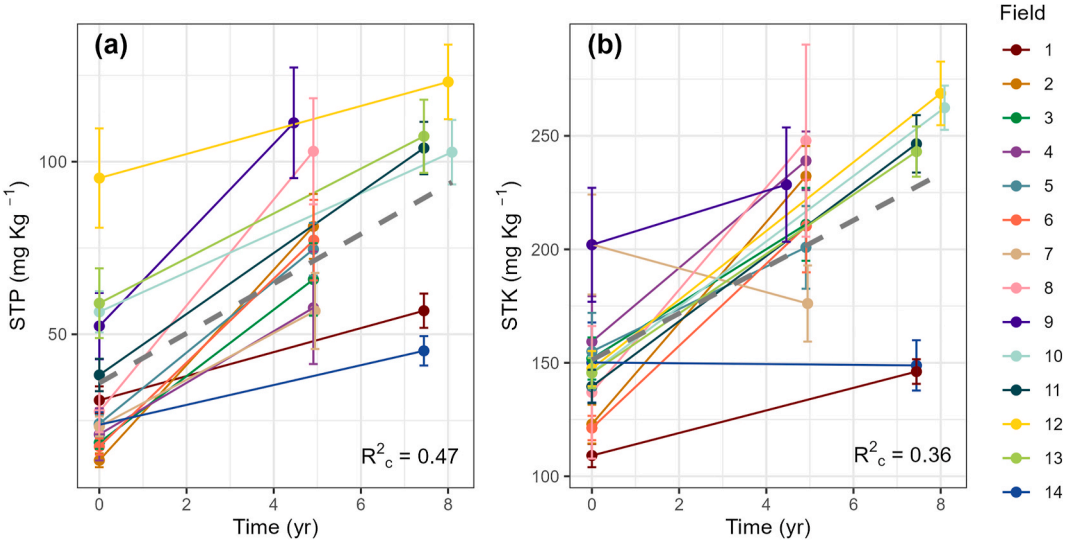

Fig. 5. Soil test **(a)** phosphorus (STP) and **(b)** potassium (STK) over time. The points represent the P and K averages for each field. Error bars are the standard error of the mean in the panel, and the gray dashed lines represent the fitted models for fixed effects: STP (mg kg^{-1}) = 36 (mg kg^{-1}) + 7 time (yr) (a) and STK (mg kg^{-1}) = 151 (mg kg^{-1}) + 10 time (yr) (b). Diagnostic plots of model residuals are shown in Fig. S4.

K kg^{-1} y^{-1}). Initially, the fields had an average STP of 43 mg P kg^{-1}, which doubled to 89 mg P kg^{-1} after 8 years of digestate applications. STK started at an average of 151 and reached 233 mg K kg^{-1}.

4. Discussion

4.1. New SOC formation derived from digestate inputs

We estimated that SOC increased by 0.18 Mg ha^{-1} per each Mg ha^{-1} of digestate-C applied to the soil, corresponding to a digestate-SOC_{FE} of 18 % (Fig. 4). This estimate is valid only under the assumption that digestate-C additions have minimal effects on crop biomass production and the endogenous SOC mineralization rate. Digestate can reduce endogenous SOC mineralization [14,16], an effect known as negative priming [17]. However, this phenomenon is typically short-lived [47] and is, therefore, likely to have a limited impact in the long term. Through isotope analysis, we further observed that the more digestate added, the greater the increase in soil $\delta^{13}C$ and $\delta^{15}N$, approaching the δ values of solid digestate (Fig. 4). This result thus supports the hypothesis that all the SOC increases are due to new SOC formation derived from digestate. Furthermore, observing a similar pattern of $\delta^{13}C_{soil}$ and $\delta^{15}N_{soil}$ at 15–30 cm depth indicates digestate is also increasing SOM in this deeper layer, where we were unable to quantify direct SOC changes due to lack of sampling at T_0 (Supplemental, Fig. S3).

Considering the abovementioned assumptions, the estimated digestate-SOC_{FE} is 18 % (CI_{95} = 14–21 %). However, this value may be underestimated as potential SOC increases in the 15–30 cm soil depth were not accounted for (Supplemental, Fig. S3). The 18 % digestate-SOC_{FE} is above the 6–14 % manure-SOC_{FE} reported in meta-analyses under field conditions [48,49]. However, those meta-analyses encompass a wide range of environmental conditions, soil types, and management practices, which limit the possibility of direct comparison. In contrast, our estimate is lower than the digestate-SOC_{FE} of 30–60 % observed in laboratory studies [13,14] and the digestate-SOC_{FE} of 68 % estimated from a systematic review including incubations, field trials, and modeling [50].

Since most of the fields received solid and liquid digestates (Table S1), it is impossible to distinguish the SOC_{FE} between these digestate fractions in our study. However, the physical and chemical compositions of solid and liquid digestate differ significantly (e.g., C:N ratios of organic matter are 8 for liquid and 27 for solid, Table 1), which is expected to influence SOC_{FE} [51,52]. In long-term field studies in Canada, the SOC_{FE} of solid cattle and swine manure averaged 26 %, whereas liquid manure was much smaller, at only 5 % [53]. The digestate-SOC_{FE} of 18 % in our study falls within this range and jointly accounts for liquid and solid digestate. Thus, our results align with these findings, although whether liquid or solid digestate-SOC_{FE} is different than what would be achieved through the direct input of maize residue, cattle manure, and/or organic waste to crop fields is unclear.

4.2. Initial SOC effect on digestate-C accumulation

The initial SOC effect on SOC changes was evaluated in terms of absolute mass difference rather than ratios to avoid statistical artifacts (Fig. 3; [54]). When SOC changes are normalized by initial SOC stock, relative changes appear larger in SOC-poor soils due to the smaller denominator [54]. Nevertheless, understanding relative SOC changes remains relevant, for instance, for carbon inventories using IPCC equations [55]. Accordingly, mean initial SOC stocks were 22, 26, and 33 Mg C ha^{-1} for the low, medium, and high categories. Based on these initial SOC levels and the estimated changes (Fig. 3), the relative SOC changes were 47 %, 28 %, and 9 % for the low, medium, and high SOC categories, respectively.

The greater SOC response in soils with the lowest initial SOC and the lower response in soils with the highest initial SOC (Fig. 3) can be partly explained by the effect of C-loading on digestate-SOC_{FE} (Fig. 2c). The likelihood of forming organo-mineral compounds that stabilize SOC increases as C-loading decreases [56,57]. Consequently, digestate-SOC_{FE} increases as C-loading decreases, triggering the higher response of SOC to digestate-C inputs (Fig. 2c). In addition, beyond the interaction between soil organic matter and silt + clay particles, other stabilization mechanisms also contribute to SOC persistence over the long term. For instance, negatively charged SOM can bind with polyvalent cations, especially Ca^{2+}, to protect SOC from decomposition [58,59]. Given that digestate residues contain significant amounts of Ca^{2+} and Mg^{2+} (Table 1), their addition to soil could enhance SOC stabilization by promoting this type of organo-mineral association in low-SOC soils.

4.3. Accumulation of plant-available nutrients with digestate application

As is common with manure application, digestate application rates were designed to meet crop N requirements and avoid groundwater quality problems created by N leaching [60]. However, this approach can still result in excess nutrients beyond plant demand. Soil P is of particular concern because of links to water quality issues, and even has specific terminology, “legacy P” [61,62].

The N:P ratio required for maize production is around six, meaning that the crop needs to uptake 6 kg of N per kg of P [63]. The N:P ratio of liquid and solid digestate is 3 (Table 1). Therefore, applying digestate to meet N needs could supply twice the necessary plant-available P, creating a nutrient surplus and resulting in P accumulation. For immobile nutrients, like P and K, repeated application inevitably leads to their accumulation in the soil beyond crop needs (Fig. 5; [64]).

According to Iowa State University, STP values between 18 and 26, and STK values between 170 and 220, are classified as optimal range for maize and soybean needs [65]. After 12 years of digestate applications STP values are 89 mg P kg^{-1} and STK are 223 mg K kg^{-1} on average. Therefore, the availability of these nutrients is far beyond crop requirements. For STP it is not just that there are surplus nutrients and further application is not needed, but that it is also a water quality concern.

Phosphorus loss in runoff from agricultural fields can lead to surface freshwater eutrophication [66]. Since water erosion is a surface process, STP concentrations in the topsoil have been shown to be closely linked to dissolved P concentrations in runoff [67]. While several states in the United States have estimated critical levels of STP ranging from 75 to 200 mg P kg^{-1} [68], P losses are also influenced by additional factors such as soil tillage, crop rotation, soil cover, local climate, and topography. Therefore, STP monitoring should be combined with assessments of potential runoff and erosion to evaluate the freshwater contamination risks [67,69]. However, STP levels in six of the 14 fields analyzed exceeded 100 mg kg^{-1}, indicating that STP levels should be carefully considered in digestate application management. Although we assessed the 0–15 cm soil layer, P stratification may exacerbate surface P losses by concentrating STP in the uppermost portion of the profile [70,71].

4.4. Final considerations

Agricultural systems must be redesigned to meet global goals, such as mitigating climate change and sustaining agricultural productivity, while also addressing local socio-environmental goals, like improving water quality and soil health and enhancing local economies. Integrating anaerobic digestion technologies on farms can contribute to helping meet these goals. In addition to generating renewable energy, utilizing the digestate by-product in crop fields has several benefits:

i) **Nutrient reuse and recycling.** Applying digestate to fields enhances essential soil nutrients for crop production (Table 1). By recycling nutrients from waste, digestates promote more circular agricultural systems and decrease the need for synthetic fertilizers. However, not all nutrients in digestate will be taken by plants, and some may be lost. For instance, N losses can occur through ammonia volatilization or nitrate leaching, while P may become immobilized depending on soil pH and mineral composition [72,73]. These processes can reduce nutrient use efficiency and lead to environmental impacts. Over-application of digestate or additional P and K fertilizers can be financially inefficient and result in a surplus of plant-available nutrients. The excessive application of certain nutrients, particularly P (Fig. 5), may pose water quality concerns. We recommend regular soil testing to monitor nutrient levels and the use of water quality mitigation techniques like cover crops or reduced tillage. Additional technologies can potentially be integrated to extract P from digestate for application in fields where it is needed.

ii) **Soil C sequestration.** The digestate application to the soil is effective in increasing SOC storage in commercial fields (Fig. 2a). A portion of this C originates from waste that would otherwise decompose in landfills, releasing GHGs. Thus, retaining part of this C in soils could be claimed as a GHG reduction. Nonetheless, soil C sequestration must be considered only as a part of a broader GHG balance analysis for the system [74]. For example, C and N cycles are tightly coupled in soils, and we do not yet fully understand the impact of digestate applications on nitrous oxide (N_2O) emissions, a GHG nearly 300 times more potent than CO_2. Nitrogen inputs to soil usually increase N_2O, potentially offsetting the benefits of SOC accumulation [75]. On the positive side, reducing synthetic fertilizer use or replacing fossil fuels with renewable fuels are ways to avoid GHG emissions. Therefore, a comprehensive system or life-cycle analysis on total emissions is essential to assess the net GHG impact of integrating anaerobic digestion on farms.

iii) **Soil health improvements.** Soil organic carbon is a key indicator of soil health [76,77]. On a global scale, it has been observed that crop productivity and soil functions decline when SOC falls below 2 % [7,8,78,79]. This is likely because of improvements in soil structure and functioning that co-occur with increases in SOC [80]. In this study, the initial estimated SOC stock of 28 Mg C ha^{-1} equates to a SOC concentration of 1.7 %, assuming an

average bulk density of 1.1 Mg m^{-3} across fields (Table 2). This value is below the proposed critical threshold of 2 % C. The estimated SOC accumulation rate of 0.8 Mg C ha^{-1} yr^{-1} (0.05 % C yr^{-1}) with digestate applications suggests that, after 10 years, SOC could rise from 1.7 % to 2.2 %, exceeding the threshold. Therefore, these SOC increases are likely to have improved soil health.

CRediT authorship contribution statement

S.H. Villarino: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **M.D. McDaniel:** Writing – review & editing, Supervision, Conceptualization. **M.J. Blauwet:** Writing – review & editing. **B. Sievers:** Investigation, Conceptualization. **L. Sievers:** Investigation, Conceptualization. **L.A. Schulte:** Writing – review & editing, Supervision, Funding acquisition. **F.E. Miguez:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2025.101942.

Data availability

Data will be made available on request.

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