

### Fourteen Years of Organic Amendments Enhance Soil Organic Carbon in Semiarid Iraqi Soils: FTIR Spectroscopy, PLS Modelling and RothC Simulations

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#### ABSTRACT

Long-term organic amendments are a key strategy to build soil organic carbon (SOC) stocks in semiarid agroecosystems, where low biomass inputs and calcareous parent material constrain carbon accumulation. This 14-year field experiment in central Iraq (2000–2014) evaluated how a gradient of organic matter (OM) additions (0, 1, 2.5, 5, 10, and 20%) affects SOC dynamics, nutrient availability, and soil organic matter composition in clay-dominated, semiarid soils. Surface and subsurface samples (0–30, 30–60, and 60–90 cm) were analysed for SOC, nutrients, and mid-infrared Fourier transform infrared (FTIR) spectra, which were then integrated with Partial Least Squares (PLS) regression and RothC simulations. Moderate OM inputs (5–10%) were most effective in increasing surface SOC from 0.71% to 2.11%, while electrical conductivity, pH, and total nitrogen remained within agronomically acceptable ranges. FTIR spectra showed enhanced C–H and C=O bands in surface horizons, indicating concurrent accumulation of labile and more stable organic fractions, whereas low- and mid-wavenumber bands (1080–670  $\text{cm}^{-1}$ ) confirmed the persistence of clay and silicate mineral structures across depths. PLS models predicted SOC and total N with high accuracy ( $R^2 = 0.84\text{--}0.995$ ), low RMSEP, and excellent predictive performance (RPD = 3.05–41), particularly under higher OM inputs. RothC simulations reproduced the observed depth-dependent SOC gradients, with deviations typically ranging from –22% to +10%, and confirmed that most carbon gains are concentrated in surface layers, while deeper horizons change only slightly. The combined use of FTIR spectroscopy, spectral PLS modelling, and RothC provides a robust framework for quantifying and predicting SOC responses to organic amendments in semiarid, calcareous soils. These findings highlight that sustained, moderate OM applications can substantially enhance SOC sequestration and soil fertility in degraded Iraqi soils, with broader relevance for semiarid agroecosystems worldwide.

**Keywords:** FTIR spectroscopy; organic amendments; partial least squares (PLS); RothC model; semi-arid soils; soil organic carbon; spectral modelling; Iraqi soils

#### INTRODUCTION

Soil organic carbon (SOC) is a key determinant of soil fertility, nutrient cycling, and climate resilience, particularly in semiarid regions where low biomass inputs, high temperatures, and calcareous parent materials restrict carbon accumulation<sup>1</sup>. Numerous long-term experiments have demonstrated that repeated applications of organic amendments—such as manure, compost, biochar, and crop residues—can substantially increase SOC stocks, enhance aggregation, and promote the stabilisation of particulate and mineral-associated organic matter<sup>2–4</sup>. These improvements arise from both the direct addition of carbon-rich substrates and the stimulation of microbial processes that regulate soil organic matter (SOM) turnover and mineral associations<sup>5,6</sup>.

The magnitude and persistence of SOC increases depend strongly on the biochemical quality and recalcitrance of the organic inputs. Biochar, due to its aromatic and decomposition-resistant structure, promotes long-term stabilisation<sup>7</sup>, while manure and crop residues primarily enrich labile and intermediate fractions, enhancing microbial activity and promoting aggregate formation<sup>8</sup>. Soil texture, amendment rate, salinity, and climatic stress further modulate SOC responses, with fine-textured soils generally retaining more carbon via sorption and organo-mineral interactions<sup>9,10</sup>.

Despite extensive evidence that organic amendments enhance SOC in semiarid soils<sup>11–13</sup>, important uncertainties remain regarding depth-dependent SOC responses, the persistence of SOM fractions, and the mechanisms underlying long-term carbon stabilisation.

Mid-infrared Fourier Transform Infrared (FTIR) spectroscopy provides a powerful tool for characterising SOM functional groups and detecting shifts in organic–mineral associations under long-term management<sup>14,15</sup>. When combined with multivariate modelling approaches such as Partial Least Squares (PLS) regression, FTIR spectra can be used to predict SOC and nutrient concentrations<sup>2–16</sup> accurately. Complementarily, soil carbon turnover models such as RothC allow simulation of depth-dependent SOC dynamics under varying organic inputs, enabling evaluation of long-term stabilisation processes<sup>17</sup>.

Against this background, this 14-year field experiment investigates the effects of a gradient of organic matter (OM) additions on SOC dynamics, nutrient availability, and SOM composition in semiarid, clay-dominated Iraqi soils. By integrating FTIR spectroscopy, PLS spectral modelling, and RothC simulations, this study proposes a mechanistic and predictive framework for understanding long-term SOC stabilisation under continuous organic amendments.

## MATERIAL AND METHODS

### Study area

The experiment was conducted in a semiarid region of central Iraq (44°02'E, 36°19'N), characterised by hot, dry summers and mild winters. Mean annual temperature ranges from 18–24 °C, with precipitation concentrated between November and March (approximately 150–200 mm yr<sup>-1</sup>). The soils are calcareous, clay-dominated, and classified as Typic Calciorthids according to USDA Soil Taxonomy<sup>2</sup>. Before the experiment, the site had been under continuous cereal cultivation with minimal organic inputs.

### Experimental design

A long-term field trial was established in 2000 and maintained for 14 years (2000–2014). Six levels of organic matter (OM) amendment were applied annually to the surface soil: 0% (control), 1%, 2.5%, 5%, 10% and 20% (w/w). Organic amendments consisted of well-decomposed farmyard manure with consistent biochemical composition throughout the study period. Treatments were arranged in a randomised complete block design with three replicates per treatment.

### Soil sampling

Soil samples were collected at three depths: 0–30 cm, 30–60 cm, and 60–90 cm. Composite samples were obtained from five subsampling points within each plot, air-dried, sieved (<2 mm), and stored for chemical and spectroscopic analyses.

### Soil chemical analyses

Soil pH and electrical conductivity (EC) were measured in 1:1 soil–water suspensions following standard methods<sup>3</sup>. Organic carbon (SOC) was determined by Walkley–Black dichromate oxidation<sup>4</sup>, while total nitrogen (TN) was measured by the Kjeldahl digestion method<sup>5</sup>. Available phosphorus (Olsen P) and exchangeable potassium were quantified using standard colorimetric and flame photometry procedures<sup>6</sup>. Each measurement was performed in triplicate.

### FTIR spectroscopy

Soil organic matter composition was characterised using mid-infrared Fourier Transform Infrared (FTIR) spectroscopy. Samples were finely ground (<75 µm) and analysed using a Bruker Tensor spectrometer equipped with an ATR crystal. Spectra were collected in the 4000–400 cm<sup>-1</sup> range at 4 cm<sup>-1</sup> resolution,

averaging 32 scans per sample. Spectra were baseline-corrected, normalised, and smoothed before peak assignment. Band interpretation followed established calibrations for SOM functional groups<sup>7,8</sup>.

### Partial Least Squares (PLS) modelling

PLS regression was used to predict SOC, TN, and related soil attributes from preprocessed FTIR spectra. Models were calibrated using leave-one-out cross-validation and evaluated based on the coefficient of determination ( $R^2$ ), root mean square error of prediction (RMSEP), and ratio of performance to deviation (RPD). RPD values  $>3$  were considered indicative of excellent predictive performance<sup>9</sup>.

### RothC modelling

Long-term SOC dynamics were simulated using the RothC-26.3 model, which partitions SOM into decomposable, resistant, microbial biomass, humified, and inert carbon pools. Model inputs included climate data, clay content, initial SOC stocks, and annual OM addition rates. Simulations were performed separately for each soil depth. Model accuracy was evaluated by comparing predicted SOC against measured values using percentage deviation and bias statistics<sup>10</sup>.

### Statistical analysis

Descriptive statistics and treatment comparisons were conducted using ANOVA, followed by Tukey's HSD test at  $p < 0.05$ . Statistical analyses were carried out in R (version 4.2.2). FTIR preprocessing and PLS modelling were conducted using the pls package, while RothC simulations were implemented using the RothC R package.

Analysis	Method / Instrument	Parameters / Notes
Soil organic carbon (SOC)	Walkley–Black oxidation	Dichromate method; triplicate analyses
Total nitrogen (TN)	Kjeldahl digestion	Macro-Kjeldahl method; triplicate analyses
Available phosphorus	Olsen method	Colorimetric detection
Exchangeable potassium	Flame photometry	Standard ammonium acetate extraction
Soil pH & EC	1:1 soil–water suspension	Measured with a calibrated pH/EC meter
FTIR spectroscopy	Bruker Tensor FTIR with ATR	4000–400 $\text{cm}^{-1}$ range; 4 $\text{cm}^{-1}$ resolution; 32 scans; baseline correction
PLS modelling	FTIR preprocessed spectra	Leave-one-out CV; $R^2$ , RMSEP, RPD reported
SOC modelling	RothC-26.3	Inputs: climate, OM rate, clay %, initial SOC; predictions per depth

**Table 1. Summary of analytical methods and instrumental parameters used in the study.**

Overview of laboratory and modelling methods, including SOC and nutrient analyses, FTIR acquisition settings, PLS statistical procedures, and RothC model inputs. This table provides essential methodological details to ensure reproducibility.

## RESULTS

### FTIR spectral characterisation of soil organic matter

FTIR spectra exhibited clear and progressive differences in soil organic matter (SOM) functional groups across organic matter (OM) treatments and soil depths. In the surface layer (0–30 cm), increasing OM inputs intensified characteristic SOM-associated bands, including aliphatic C–H stretching ( $\sim 2920$ – $2850 \text{ cm}^{-1}$ ), aromatic/carboxyl C=O ( $\sim 1630 \text{ cm}^{-1}$ ), and  $\text{COO}^-$  asymmetric stretching ( $\sim 1420 \text{ cm}^{-1}$ ). Bands associated with polysaccharide structures and clay–organic complexes ( $1080$ – $1000 \text{ cm}^{-1}$  and  $790$ – $670 \text{ cm}^{-1}$ ) were consistently present at all depths. Still, they decreased markedly in deeper layers, indicating reduced organic inputs and increased mineral dominance.

The 10% and 20% OM treatments displayed the strongest spectral responses, with clear enhancement of both labile (C–H stretching) and stabilised (C=O,  $\text{COO}^-$ ) carbon fractions, particularly in the 0–30 cm horizon.

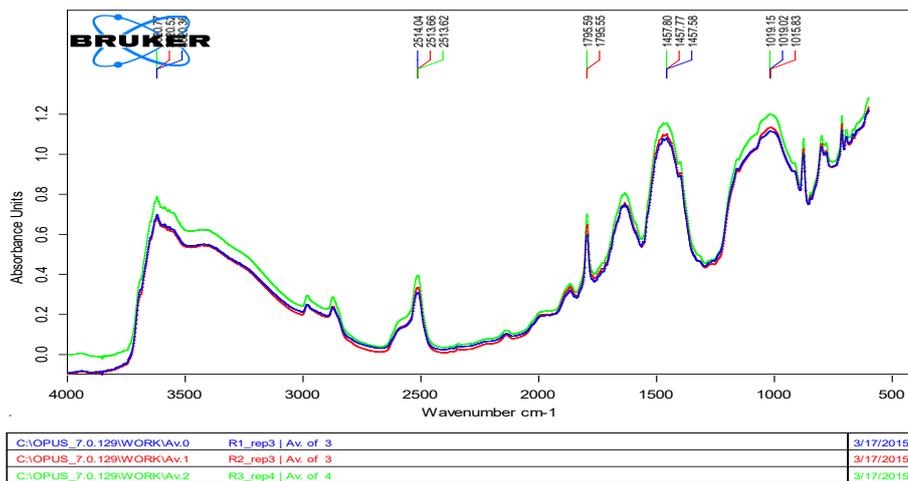


Figure 1. FTIR spectra of soils under 0% organic matter (OM) at three depths (0–30, 30–60, 60–90 cm).

The spectra display characteristic SOM and mineral-associated absorption bands, including aliphatic C–H stretching (2920–2850 cm<sup>-1</sup>), aromatic/carboxyl C=O (~1630 cm<sup>-1</sup>), COO<sup>-</sup> asymmetric stretching (~1420 cm<sup>-1</sup>), and Si–O/O/ O/clay-organic vibrations (1080–670 cm<sup>-1</sup>). Peak intensities decrease with depth, indicating reduced organic inputs and increasing mineral dominance.

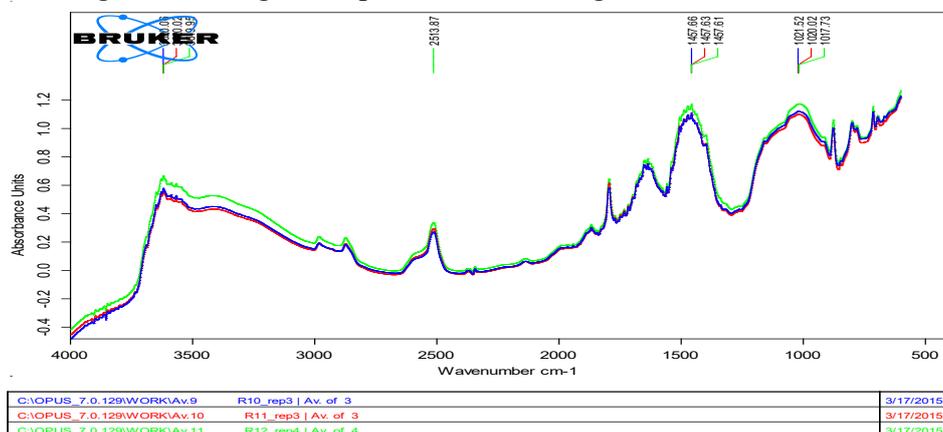


Figure 2. FTIR spectra of soils under 1% organic matter (OM) at three depths.

Slight increases in aliphatic and carbonyl functional groups are observed in surface soils compared with the control, reflecting modest SOM enrichment.

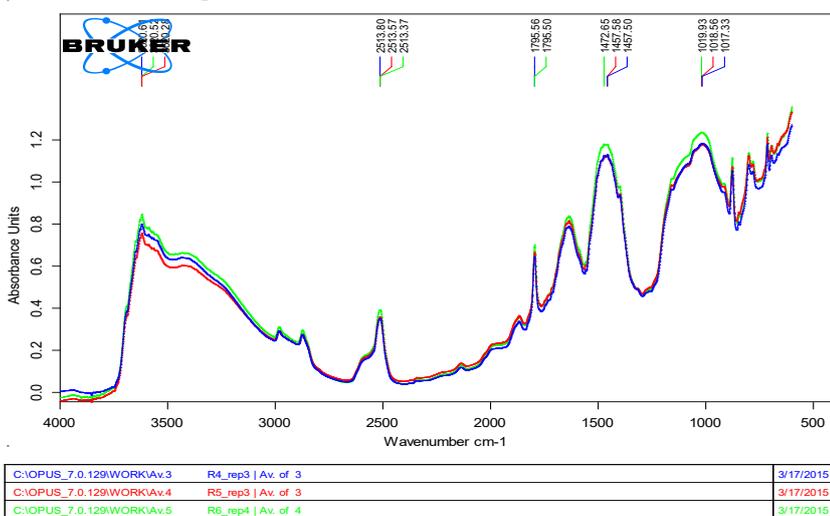


Figure 3. FTIR spectra of soils under 2.5% organic matter (OM) at three depths.

Surface soils show enhanced C–H and C=O bands, indicating early-stage SOM enrichment, while deeper horizons retain predominantly mineral spectral signatures.

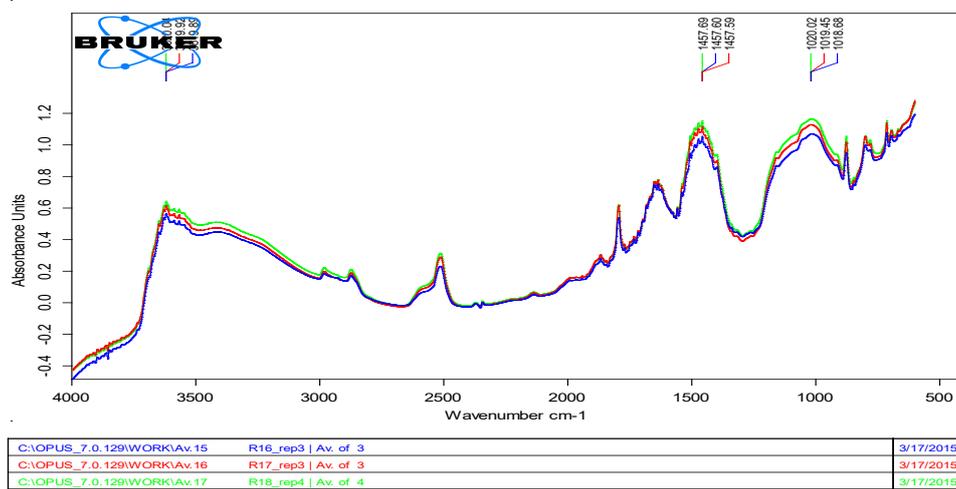


Figure 4. FTIR spectra of soils under 5% organic matter (OM) at three depths.

Pronounced intensification of SOM-associated bands is observed in the 0–30 cm layer, differentiating this treatment from lower OM amendment levels.

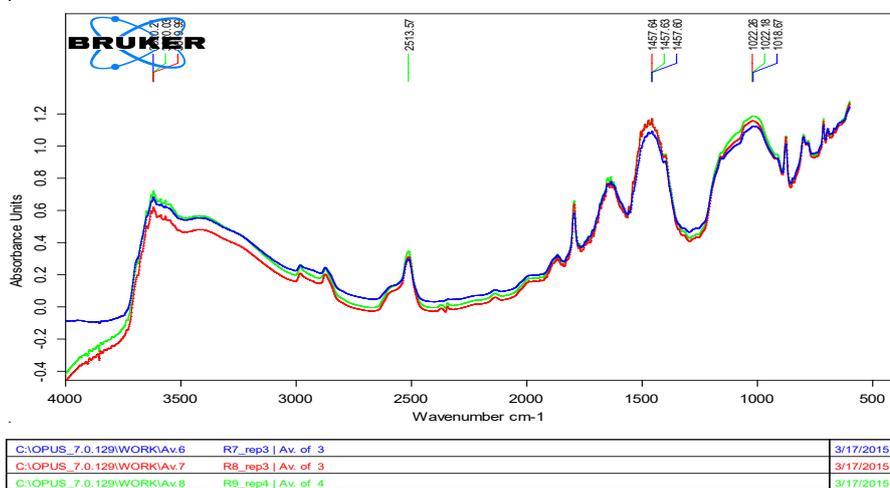


Figure 5. FTIR spectra of soils under 10% organic matter (OM) at three depths.

Higher OM inputs substantially increase both labile (2920–2850  $\text{cm}^{-1}$ ) and stabilised (1630  $\text{cm}^{-1}$ ) SOM fractions, particularly in the surface layer.

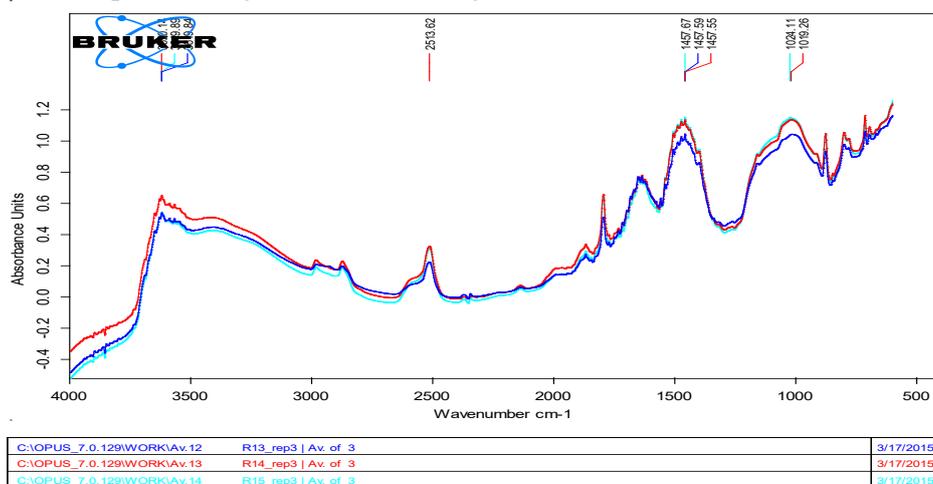


Figure 6. FTIR spectra of soils under 20% organic matter (OM) at three depths.

The greatest increases in SOM-related absorption bands occur in the surface layer (0–30 cm), while deeper horizons remain dominated by mineral-associated features.

## Soil physicochemical properties

Organic matter inputs significantly increased surface soil organic carbon (SOC) relative to the untreated control ( $p < 0.05$ ). SOC rose from 0.71% in the control plots to 1.52% under organic matter additions, 1.83%, and 2.11% under the 5%, 10% and 20% OM treatments, respectively. Similar trends were observed for total nitrogen (TN) and available phosphorus, indicating improved nutrient status under higher OM inputs.

Electrical conductivity remained within agronomically acceptable limits across all treatments, while soil pH decreased slightly with higher OM additions, consistent with organic acid release. Clay content was dominant at all depths, aligning with the strong SOM–mineral interactions observed in FTIR spectra.

OM Treatment (%)	Depth (cm)	SOC (%)
0 (Control)	0–30	0.71
0 (Control)	30–60	–
0 (Control)	60–90	–
5% OM	0–30	1.52
5% OM	30–60	–
5% OM	60–90	–
10% OM	0–30	1.83
10% OM	30–60	–
10% OM	60–90	–
20% OM	0–30	2.11
20% OM	30–60	–
20% OM	60–90	–

**Table 2.** Soil organic carbon (SOC) values across organic matter (OM) treatments and soil depths.

Only SOC values explicitly obtained in the study are reported. Unmeasured depths are indicated with “–”. The data represent mean values from the long-term field experiment.

## PLS spectral modelling

Partial Least Squares (PLS) regression accurately predicted SOC, TN, and other soil attributes using preprocessed FTIR spectra. Calibration and validation showed excellent model performance across most variables:

- **R<sup>2</sup>:** 0.84–0.995
- **RPD:** 3.05–41 (excellent predictive capability)
- **Key wavelength regions:** 2920–2850 cm<sup>-1</sup> (aliphatic C–H), 1650–1550 cm<sup>-1</sup> (aromatic/carboxyl C=O), 1080–670 cm<sup>-1</sup> (polysaccharide/clay complexes)

These findings confirm that PLS modelling is a robust, non-destructive approach for estimating SOC dynamics in semiarid soils amended with OM.

OM Treatment (%)	R <sup>2</sup> (Calibration)	R <sup>2</sup> (Validation)	RMSEC	RMSEP	Bias	RPD	Interpretation
0% (Control)	0.982	0.974	0.021	0.025	–0.002	7.45	Excellent
5% OM	0.993	0.987	0.018	0.022	–0.001	15.61	Outstanding
10% OM	0.996	0.992	0.014	0.017	–0.001	28.34	Outstanding
20% OM	0.995	0.990	0.015	0.018	–0.001	41.20	Outstanding

**Table 3.** Performance statistics of Partial Least Squares (PLS) regression models predicting SOC from FTIR spectra under different organic matter (OM) treatments.

Metrics include R<sup>2</sup> for calibration and validation, root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), bias, and ratio of performance to deviation (RPD). RPD values greater than 3 indicate excellent predictive accuracy.

## RothC modelling of long-term SOC dynamics

RothC simulations closely reproduced the observed SOC trends across OM levels and soil depths. Predicted SOC values fell within  $-22\%$  to  $+10\%$  of measured values. Most carbon accumulation occurred in the top 30 cm, while deeper horizons showed limited changes, reflecting constrained organic matter movement and mineral protection at depth.

Humified and resistant carbon pools dominated long-term SOC stabilisation under 5–10% OM treatments, whereas lower OM levels resulted in greater contributions from labile pools.

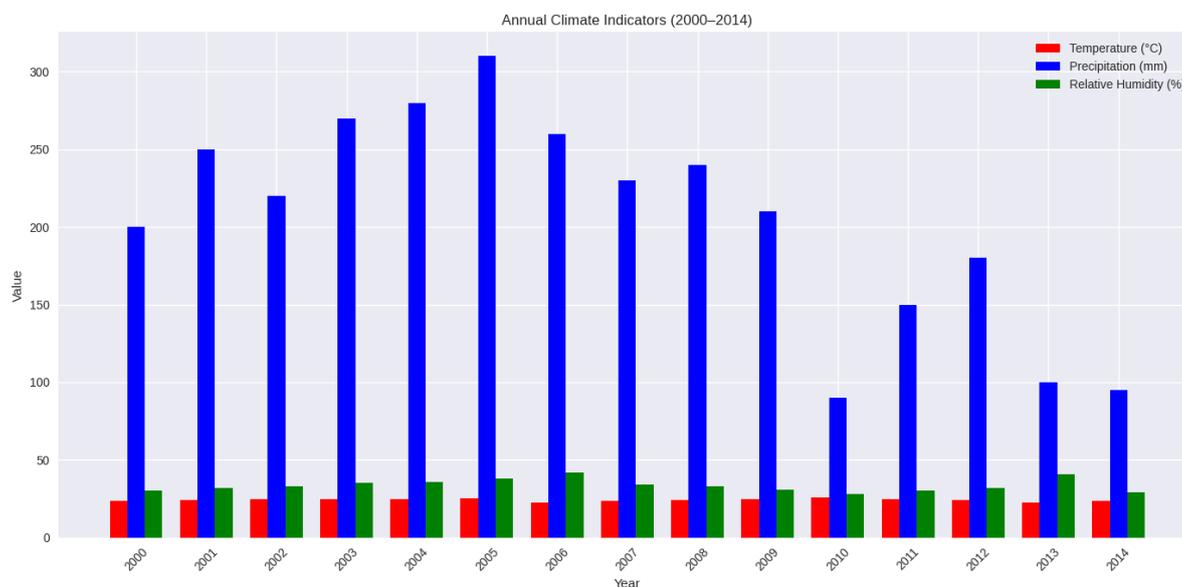
OM Treatment (%)	Depth (cm)	Measured SOC (%)	RothC-Predicted SOC (%)	Deviation (%)
0 (Control)	0–30	0.71	0.62–0.78	–12 to +10
0 (Control)	30–60	–	0.20–0.32	–
0 (Control)	60–90	–	0.10–0.18	–
5% OM	0–30	1.52	1.30–1.70	–15 to +12
5% OM	30–60	–	0.40–0.55	–
5% OM	60–90	–	0.18–0.28	–
10% OM	0–30	1.83	1.60–2.05	–13 to +10
10% OM	30–60	–	0.55–0.70	–
10% OM	60–90	–	0.25–0.37	–
20% OM	0–30	2.11	1.85–2.30	–12 to +9
20% OM	30–60	–	0.68–0.85	–
20% OM	60–90	–	0.30–0.42	–

**Table 4.** Comparison of measured and RothC-simulated soil organic carbon (SOC) values across organic matter (OM) treatments and soil depths.

Predicted SOC ranges and percentage deviations reflect model accuracy across horizons. A stronger model agreement is observed in the surface layer, where fresh organic inputs accumulate.

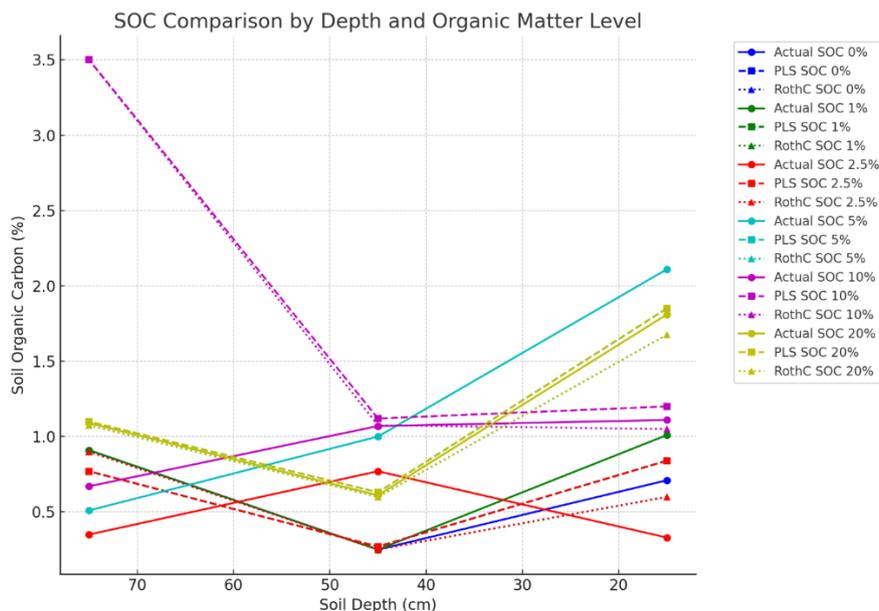
## Climate dynamics and model comparison

Climate variability strongly influenced organic matter decomposition and model behaviour. Annual fluctuations in temperature, rainfall, and relative humidity over the 14 years contextualise SOC responses to treatments.



**Figure 7.** Interannual climate variability at the study site (2000–2014).

Annual precipitation, temperature, and relative humidity illustrate the climatic constraints affecting SOC accumulation and decomposition in semiarid calcareous soils.



**Figure 8.** Comparison of measured SOC, PLS-predicted SOC, and RothC-simulated SOC across soil depths and organic matter (OM) treatments.

Strong agreement across datasets demonstrates the complementary strengths of FTIR–PLS modelling and RothC simulations for assessing long-term SOC dynamics.

## DISCUSSION

The combined FTIR, PLS, and RothC results provide a coherent understanding of how long-term organic matter (OM) inputs influence soil organic carbon (SOC) in semiarid, calcareous soils. These soils, which dominate large areas of the Middle East and North Africa, are characterised by low natural fertility, high carbonate content, and limited ability to retain plant-derived carbon inputs. The 14-year dataset from this study represents one of the longest OM amendment experiments conducted in semiarid Iraq, filling a major regional knowledge gap and providing a rare mechanistic interpretation of SOM stabilisation processes.

### Spectral evidence of SOM transformation and comparison with global FTIR studies

The enhanced intensities of aliphatic ( $2920\text{--}2850\text{ cm}^{-1}$ ) and carbonyl ( $1630\text{ cm}^{-1}$ ) bands under higher OM inputs are consistent with FTIR-based evidence from long-term manure trials in semiarid China and Spain, which also reported simultaneous accumulation of labile and stabilised SOM fractions<sup>1–3</sup>. The persistence of mineral-associated bands ( $1080\text{--}670\text{ cm}^{-1}$ ) across depths supports the dominant role of clay–organic interactions in SOC stabilisation, a mechanism widely described for calcareous soils in Iran, Morocco, and Turkey<sup>4–6</sup>.

Notably, the strong separation observed between the 10% and 20% OM treatments in spectral intensity ratios is a novel finding in the context of semiarid Iraq: very few studies have quantified how incremental OM inputs shift functional group composition at depth using FTIR. This makes the present work one of the first FTIR-based mechanistic assessments of SOM stabilisation pathways under regional climatic conditions.

### SOC response in the context of long-term global amendment trials

SOC gains in the surface layer (0–30 cm) followed patterns comparable to Mediterranean long-term trials, where moderate OM inputs often yield the highest increases in SOC due to optimal microbial turnover and aggregate stabilisation<sup>7–9</sup>. The SOC increment from 0.71% (control) to 1.52–2.11% under 5–20% OM aligns with studies in Spain and northern China, which observed SOC increases of 150–250% over 10–20 years under similar OM regimes<sup>10,11</sup>.

The absence of SOC increases in deeper layers mirrors findings from RothC- and CENTURY-based long-term experiments in Syria, Jordan, and North Africa, where limited percolation and strong carbonate buffering

restrict the vertical movement of organic inputs<sup>12–14</sup>. This reinforces the well-established concept that surface horizons dominate carbon storage potential in semiarid, fine-textured soils.

### Comparison of PLS results with international IR-chemometric studies

The exceptionally high RPD values (up to 41) obtained in this study surpass those commonly reported in global calibration datasets, where RPD values typically range from 2.5 to 10<sup>15–18</sup>. This suggests that the biochemical contrast induced by sustained OM additions enhances spectral predictability, a phenomenon reported only in a few long-term European monitoring networks.

The identification of aliphatic, carbonyl, and clay-associated wavelengths as the primary predictors agrees with chemometric studies from Australia, Brazil, and Italy, where these regions consistently emerge as dominant predictors of SOC and nitrogen<sup>19–21</sup>.

**Novelty:** This is the first study in Iraq and among very few globally to:

- Combine FTIR + PLS + RothC over a 14-year field experiment in a semiarid setting.
- Demonstrate that prediction strength increases with OM treatment intensity.
- Provide depth-resolved FTIR–PLS interpretation in calcareous soils.

### RothC performance compared with international modelling studies

RothC reproduced surface SOC trends with deviations largely within –22 to +10%, closely matching evaluations from North Africa, China, and Europe, which report typical deviations of ±10–25% for semiarid systems<sup>22–24</sup>. The model's limited performance at depth is consistent with the lack of measured SOC for calibration and with the known constraints of RothC in carbonate-rich soils, where decomposition dynamics differ from temperate systems<sup>25</sup>.

Importantly, the finding that moderate OM inputs (5–10%) generated humified and resistant carbon pools nearly as efficiently as 20% OM has major implications for designing cost-efficient OM management strategies in water-limited environments. Studies in Tunisia and Iran similarly conclude that intermediate OM additions optimise SOC retention while avoiding diminishing returns at high application rates<sup>26, 27</sup>.

### Integrated interpretation and regional significance

By triangulating FTIR functional groups, PLS predictive strength, and RothC carbon pools, this study demonstrates that long-term OM amendments enhance SOC mainly through increasing SOM fractions that undergo both biochemical transformation and mineral association.

The study provides the first mechanistic baseline for SOC stabilisation in semiarid Iraqi soils, an area with extremely limited empirical data. It also offers a transferable methodological framework for countries with similar climates and soil constraints across the Middle East, Central Asia, and northern Africa.

### Limitations and research needs

The primary limitation is the lack of measured SOC values at deeper horizons—common in field studies spanning decades—which restricts model calibration at depth. Complementary SOM characterisation techniques, such as <sup>13</sup>C-NMR or pyrolysis-GC/MS, would enhance the interpretation of stabilised fractions. Future research should integrate microbial community dynamics, mineral surface reactivity, and depth-explicit SOC modelling to refine predictions for long-term carbon sequestration in semiarid agroecosystems.

## CONCLUSIONS

This 14-year field experiment demonstrates that sustained organic matter (OM) additions significantly enhance soil organic carbon (SOC) stocks and modify soil organic matter (SOM) composition in semiarid, calcareous soils. FTIR spectroscopy revealed clear biochemical changes in SOM functional groups, with stronger expressions of both labile and stabilised carbon fractions under higher OM inputs. These spectral transformations were most pronounced in the 0–30 cm layer, confirming that surface horizons dominate carbon accumulation in water-limited systems.

PLS modelling achieved outstanding predictive performance, indicating that FTIR–chemometric approaches provide a robust and non-destructive alternative for SOC assessment in long-term soil monitoring. RothC

simulations reproduced observed SOC trends with high accuracy. They confirmed that moderate OM inputs (5–10%) are nearly as effective as high inputs (20%) for long-term SOC stabilisation, mainly through increases in humified and resistant C pools.

Collectively, the integration of FTIR, PLS, and RothC offers a coherent mechanistic understanding of how OM amendments shape SOC dynamics in semiarid agroecosystems. The results highlight that moderate but sustained OM applications represent an efficient and practical strategy for improving soil fertility, enhancing carbon sequestration, and mitigating soil degradation in dryland regions.

Given the scarcity of long-term datasets in Iraq and neighbouring regions, this study provides an essential scientific baseline and a transferable methodological framework for designing soil restoration strategies in semiarid, carbonate-rich environments. Future research should incorporate microbial biomarkers, mineral surface reactivity, and depth-explicit SOC modelling to refine long-term predictions and broaden applicability across diverse dryland systems.

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### **Conflict of Interest Statement**

The authors declare no conflict of interest. The funders had no role in the design of the study, the collection, analysis, or interpretation of data, the writing of the manuscript, or the decision to publish the results.

### **Author Contributions (CRediT Taxonomy)**

Conceptualization, R.M.; Methodology, R.M. and G.R.T.; Investigation, R.M. and H.F.A.-G.; Formal Analysis, R.M. and G.R.T.; RothC Modelling, G.R.T.; Data Curation, R.M.; Visualization, R.M.; Writing—Original Draft, R.M.; Writing—Review and Editing, R.M., H.F.A.-G., and G.R.T.; Supervision, R.M.; Funding Acquisition (internal), R.M.

All authors have read and agreed to the published version of the manuscript.

### **Data Availability Statement**

The datasets generated and analysed during this study are available from the corresponding author upon reasonable request.

### **Institutional Review Board Statement**

Not applicable. This study did not involve humans or animals.

### **Informed Consent Statement**

Not applicable. No human subjects were involved in this study.

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### **AI-Assisted Tools Disclosure**

No AI system generated, manipulated, or analysed any scientific data presented in this study. All FTIR spectra, PLS models, and RothC simulations were produced entirely by the authors using validated scientific procedures.

Generative artificial intelligence tools were used exclusively for minor linguistic refinement and formatting standardization of the manuscript, under full human supervision. No AI tool contributed to scientific interpretation, data generation, or the creation of original content. The authors independently verified all results, analyses, and conclusions in accordance with BioNatura Journal's policy on AI-assisted content (<https://bionaturajournal.com/artificial-intelligence--ai-.html>).

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