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Alfalfa–organic amendments impact soil carbon sequestration and its lability in reclaimed loess

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Abstract

Soils derived from loess are fertile but susceptible to accelerated degradation in response to agricultural practices. The objective of our study was to evaluate the long-term effects of alfalfa (*Medicago sativa* L.) integrated with contrasting organic amendments (29 years) to rejuvenate degraded loess via total soil organic carbon (SOC) sequestration. The replicated study was conducted in concrete lysimeter plots (2 m long × 1 m wide × 60 cm deep) filled with degraded loess materials followed by planting of alfalfa with cattle manure (60 Mg/ha) or vermicompost (27 Mg/ha) amendments. After 29 years, SOC concentration increased by 5.3–6.2-fold under alfalfa–organic amendments compared to the control. A similar impact of alfalfa–organic amendments was observed on the humic acid, fulvic acid, and humin concentrations. There was an overestimation of SOC stocks (151 ± 48 kg/ha) when equivalent depth was used compared to equivalent mass of soil. While the SOC sequestration rates were 614 ± 129, 710 ± 69, and 744 ± 161 kg/ha/year at 0–10 cm depth under alfalfa, alfalfa–manure, and alfalfa–vermicompost treatments, respectively, the SOC sequestration rates decreased with depth. Significantly higher values of carbon pool index (CPI) and carbon management index (CMI) under alfalfa–organic amendments justified our results associated with the SOC sequestration; however, the SOC lability (CL) decreased under alfalfa–organic amendments, when compared to the control. A significant nonlinear inverse relationship ($R^2 = 0.80$) between the CPI and CL suggested that SOC sequestration is significantly dependent on its lability or vice versa. Our results suggested that the impact of alfalfa–organic amendments significantly rejuvenated the degraded loess soils via SOC sequestration.

KEYWORDS
carbon management index, fulvic acid, humic acid, manure, soil reclamation, vermicompost

1 | INTRODUCTION

Loess is one of the common soil-forming aeolian sedimentary deposits of the Quaternary period and covers around 10% of the land surface on Earth (Sergeev et al., 1986; Trofimov, 2001b; Li et al., 2020). It is a homogenous and porous structured material that primarily consists of fine quartz and felspar particles (Li et al., 2020). However, soils derived from loess and loess materials are more prone to accelerated erosion than other soils due to their low inherent stability and high susceptibility to degradation in response to agricultural practices

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(Trofimov, 2001b; Li et al., 2020). As a consequence, land abandonment and subsequent cessation of economic agricultural productivity occur due to erosion, drought, secondary salinity, and low soil productivity (Raiesi, 2012; Teryukov, 1996; Zhao et al., 2005).

Loess and loess-like deposits are widespread in Kazakhstan and considered fertile parent material deposits that form Chernozems, Kastanozem and Sierozems, or Cambisols (Sergeev et al., 1986; Trofimov, 2001a; Yelikbayev, 2014). About 60% of the soils derived from loess in Kazakhstan is affected by various degrees of degradation, especially accelerated loss of total soil organic carbon (SOC) by deep plowing, monocropping and summer fallowing, uncontrolled grazing practices, and in response to climate change effects (Li et al., 2015; Teryukov, 1996; Yelikbayev, 2014; Zhao et al., 2005).

SOC plays critical roles in soil formation processes and ecosystem services (Islam et al., 2021; Yelikbayev, 2010). It rejuvenates and maintains soil quality by improving biodiversity and efficiency, acts as a buffer and catalyst to support enzymatic functions, serves as food and energy sources for microbes, regulates chemical reactions and equilibrium, and creates favorable physical stability and hydrological properties (Amoakwah et al., 2022; Islam et al., 2021). With its complex nature, SOC contains diverse and multiple humified C pools that have variable turnover rates to carry out ecological functions (Amoakwah et al., 2020, 2022). The relative proportions of these SOC pools, such as humic acid (HA), fulvic acid (FA), and humin, are associated with the amount and quality of organic inputs into soil ecosystems and their temporal changes under contrasting soil management practices (Amoakwah et al., 2020; Stevenson, 1994). While HA, FA, and humin pools are themselves not biologically passive, they accumulate in various degrees of stability due to biochemical complexations with polyvalent cations and clays to carry out ecological functions (Amoakwah et al., 2022; De Mastro et al., 2019).

Restoring degraded agricultural soils formed on loess deposits is of vital importance for improving soil quality to support global food security (Gupta, 2019; Yelikbayev, 2014; Zhamalbekov, 1989). Soil degradation can be mitigated or reclaimed via SOC sequestration by adopting proactive restorative soil management practices (Amoakwah et al., 2022; Li et al., 2015). Research conducted in western Kazakhstan reported that long-term impacts of managed pastures integrated with legumes, shrubs, and perennial grasses improved soil fertility (Teryukov, 1996; Yelikbayev, 2014). On the loess plateau of China, it has been reported that native plant restoration was more useful in increasing SOC sequestration than tree plantations. The SOC storage was higher in the grasslands than in the forestlands, with a difference of 14.9 Mg/ha (Jin et al., 2014). A land use change from prairie to forest plantation improved the humification process by increasing SOC storage or vice versa (Amoakwah et al., 2022; Pizzeghello et al., 2017).

Legumes, manure, and vermicompost are some of emerging organic amendments to improve soil–plant ecosystems by recycling essential nutrients and accumulating intermediate carbon byproducts (Lim et al., 2016). Previous studies have reported that organic amendments such as animal manure and vermicompost increased plant growth, yield, and drought tolerance with an associated increase in SOC content to improve soil fertility (Azarmi et al., 2008; Ghosh et al., 2012; Li et al., 2021; Lin et al., 2019). Several long-term research studies have reported that chemical fertilization alone or in conjunction with crop residues, manures, and composts significantly increased SOC sequestration (Chaudhary et al., 2017; Hua et al., 2014; Li et al., 2021). Yan et al. (2013) reported that organic residues consist of rice straw, sago, and grass clippings containing about 22% to 32% HA content, used as green wastes to support growing crops. Thus, degraded soils derived from loess materials can be rejuvenated via increased SOC sequestration by integrating organic amendments with crops and leguminous pastures (Hua et al., 2014; Jin et al., 2014; Ngo et al., 2014).

SOC stock is commonly expressed as a SOC mass per unit surface area by multiplying its concentration at a fixed depth with concurrently measured bulk density (ρb) values (Ellert & Bettany, 1995; Razzaghi et al., 2022). While ρb is a dynamic property, soils under long-term diverse management practices often show changes in ρb (compaction); a higher or lower ρb means an added extra weight or smaller weight of soil for the same fixed depth (Razzaghi et al., 2022). A small change in ρb values can overestimate or underestimate SOC stocks when comparing the results from long-term contrasting management practices or over the years (Wilson & Warren, 2015). Differences in SOC stocks, as calculated based on equivalent depth, associated with differences in ρb can be corrected using the equivalent mass, which vary only due to variations in SOC concentration (Razzaghi et al., 2022; Wilson & Warren, 2015). To accurately calculate and predict SOC sequestration, it is much needed to adjust SOC concentration to an equivalent mass, based on depth correction. Until now, there is a lack of reported long-term studies to restore degraded loess soils by integrating legumes with organic amendments to calculate SOC sequestration, based on equivalent depth vs. equivalent mass, compared to the control.

While organic amendments affected directly, via the intrinsic properties of the organic compounds themselves, or indirectly, by influencing soil properties, we hypothesized that long-term contrasting alfalfa–organic amendments will quantitatively and qualitatively affect SOC dynamics. The specific objectives of our study (1991–2020) were to (1) evaluate the temporal effects (0, 3, 11, 18, and 29 years) of cattle manure and vermicompost amendments, with and without alfalfa, on the SOC, humin, HA, and FA concentrations, (2) measure the effects of alfalfa–organic amendments on ρb, depth distribution of SOC, humin, HA, and FA stocks and their sequestration rates, and (3) determine if differences in SOC sequestration rates affected its lability, when compared to the control, under similar loess materials and climatic conditions.

2 | MATERIALS AND METHODS

2.1 | Study site

A long-term field research study, to restore degraded loess-derived soils using contrasting alfalfa–organic amendments, was originally established in 1971 and later on restructured in 1991 at Kazakh National Agrarian University farm (43°17′N, 77°10′E at 998 mean sea

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level), Ile-Alatau, Almaty, Kazakhstan. Average annual monthly air temperatures at the site ranged between 6.4 and 9.8°C with annual rainfall ranging between 313 and 977 mm. The vegetation at the site was originally dominated by grass–forb mixtures with a predominance of *Stipa capillata*, *Festuca sulcata*, *Agropyron repens*, *Koeleria cristata*, *Bromus inermis*, *Dactylis glomerata*, *Poa bulbosa*, *Avena fatua*, *and motley grasses—Holosteum umbellatum*, *Tulipa patens*, *Inula grandis*, *Allium caesium*, *Artemisia dracunculus*, *Artemisia glauca*, *Viola suavis*, *Peania hybrida*, and *Origanum vulgare* (Yelikbayev, 2010; Yelikbayev, 2014).

The soil at the site is a degraded Kastanozem (US taxonomic classification equivalent of coarse loamy, mixed, active, mesic *Typic Dystropepts*) with poor soil quality to support economic agricultural productivity. To establish the experiment, about 90 m^2 of the Kastanozem area was excavated to a depth of 60 cm in the Ile-Alatau foothills of southeastern Kazakhstan in 1991 (Figure 1). The excavated loess materials had a particle density of 2.65–2.7 g/cm^3, ρb of 1.30–1.85 g/cm^3, $CaCO_3$ of 5.9%, pH of ~7.0, salts <0.1%, organic matters <0.3%, SiO_2 of 55.2%, Al_2O_3 of 11.64%, Fe_2O_3 + FeO of 4.0%, CaO of 10.3%, P_2O_5 of 0.14%, MgO of 2.2%, and K_2O + Na_2O of 4.36% (Yelikbayev, 2014).

2.2 | Experimental design

A completely randomized design with four treatments: (1) control (barley), (2) alfalfa (*Medicago sativa* L.), (3) alfalfa with cattle manure, and (4) alfalfa with vermicompost, was established in 1991. Each treatment was replicated thrice using 2-m-long × 1-m-wide × 60-cm-deep field micro-plot concrete lysimeters (Figure 1). Prior to imposing the treatments, all the concrete lysimeters were filled at 50 cm depth with the excavated loess materials. Vermicompost, produced from composting animal manures using *Eisenia foetida* (red Californian earthworm), and cattle manure were applied at the rate of 27 and 60 Mg/ha, respectively, on the undisturbed surface of the refilled loess materials in the concrete lysimeters. Alfalfa (in a 3-year period) and barley seeds were broadcasted and allowed to grow until harvest.

Vermicompost contained 49% organic carbon, 1.02% total N, 1.05% total P (P_2O_5), and 1.27% total K (K_2O). Cattle manure (semi-decomposed manure with bedding) had 30% organic carbon, 0.46% total N, 0.30% total P (P_2O_5), and 0.78% total K (K_2O) (Yelikbayev, 2014). While barley dry biomass contained 45% organic carbon, 0.62% N, 0.73% P_2O_5, and 1.58% K_2O, alfalfa dry biomass contained 40.2% organic carbon, 2.19% N, 1.73% P_2O_5, and 3.27% K_2O.

2.3 | Soil sampling, processing, and analysis

Composite soils were collected at 0–10 and 10–20 cm depths from the concrete lysimeters using a soil auger (internal diameter of 2.54-cm) in April 1991 prior to the application of treatments (time 0) and from soils under post-applied organic amendments in 1994 (3 years), 2002 (11 years), 2009 (18 years), and 2020 (29 years). A portion of the field-moist soil at each sampling was gently sieved through a 2-mm mesh to remove plant roots and organic residues, air-dried for a period of 15 days under shade at room temperature (25°C), processed, and analyzed for soil properties.

SOC was determined by following the Tyurin method (Kononova & Belchikova, 1961). Soil humic materials were extracted by a mixture of sodium pyrophosphate and sodium hydroxide, and the HA and FA fractions were separated and analyzed by following Ponomarev and Plotnikov method (Sokolov, 1975). (Swift, 1996). Humin was calculated after subtracting the FA and HA from the SOC content. Soil pH and electrical conductivity were measured by electrode methods. The ρb was determined by the standard core method from the relationship of oven-dried mass of a known volume of soil.



FIGURE 1 Long-term field experiment at Kazakh National Agrarian University farm at Ile-Alatau, Almaty, Kazakhstan, in 2002. (a) Degraded soils derived from loess materials and (b) experimental concrete lysimeter plots (photos taken by Bakhytzhan Yelikbayev). [Colour figure can be viewed at wileyonlinelibrary.com]

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2.4 | Vermicompost, manure, and plant analyses

Vermicompost, cattle manure, barley, and alfalfa samples were oven-dried at 65°C for a period of 24 h, ground, and 0.125-mm-sieved prior to analysis for total carbon, nitrogen, phosphorus, and potassium concentration using standard methods of analysis (Sokolov, 1975).

2.5 | Soil organic carbon stocks and sequestration

Initially, SOC, humin, HA, and FA stocks, based on equivalent soil depth (eq_{depth}), were calculated by multiplying their concentrations with soil depth (d) and concurrently measured ρb values (Ellert & Bettany, 1995; Razzaghi et al., 2022). A soil depth correction (deq) factor was calculated by dividing the ρb values of the control treatment (a higher ρb) with the ρb values of the alfalfa–organic treatments to convert SOC, humin, HA, and FA stocks into equivalent soil mass (eq_{mass}) basis (Razzaghi et al., 2022):

$SOCeq_{depth}$ stock (Mg/ha) = (ρb * d * SOC * 10000).

$SOCeq_{mass}$ stock (Mg/ha) = (ρb * SOC * 10000) * deq factor.

The SOC sequestration rates at different depths under alfalfa–organic amendments were assessed by plotting equivalent mass SOC, humin, HA, and FA stocks as a function of time (0, 3, 11, 18, and 29 years). A polynomial quadratic model ($f = y_0 + a^*x + b^*x^2$) was the best fit to calculate SOC sequestration rates in different pools at both soil depths.

2.6 | Soil organic carbon lability

The HA:FA, an indicator of the SOC quality and degree of humification, was calculated. The humification ratio (HR) was calculated (Saviozzi et al., 1994) as follows:

HR (%) = [(HA + FA) * 100]/SOC.

Using the data on SOC, humin, HA, and FA concentration, the C management index (CMI) was calculated (Islam et al., 2021) as follows:

CMI = (CPI × CLI).

CPI = [SOC in the treatment soil/SOC in the control soil].

CLI = [CL in the treatment soil/CL in the control soil].

Here, CL refers to the C lability, which was calculated as follows:

CL = [(HA + FA as a labile C pool)/(humin as a non-labile C pool)].

The labile C pool was considered as the portion of SOC that was individually measured as the FA and HA pools. The non-labile C pool was calculated by subtracting the values of HA and FA from the SOC values.

2.7 | Statistical analysis

Significant differences in the concentration, stock, and lability of SOC pools at different soil depths attributed to the impact of alfalfa–organic amendments were evaluated by a two-way analysis of variance procedure of the SAS® (SAS 2010). Both organic amendments and soil depth were considered as fixed predictor effects. For simple and interactive effects, treatment means were separated using the Duncan multiple range test (DMRT) with a $p \leq 0.05$. Nonlinear regression analyses were performed to calculate for SOC sequestration rates using SigmaPlot®.

3 | RESULTS AND DISCUSSION

3.1 | Soil organic carbon pools

While the initial SOC concentration of the refilled loess materials was only 1.4 g/kg, the impact of alfalfa–organic amendments variably, but significantly influenced the depth distribution of SOC pools (Table 1). The increase in SOC, humin, HA, and FA concentrations was more pronounced during the initial years compared to the later stages. Averaged across time and soil depth, SOC increased by 5.3-, 5.7-, and 6.2-fold under alfalfa alone, alfalfa–manure, and alfalfa–vermicompost amendments, respectively, when compared to the control (barley). A similar impact was observed on the humin concentration. Both HA and FA increased over time under all alfalfa–organic amendments; however, the impact was more pronounced on HA (>60%) than that on the FA (40%). The SOC, humin, HA, and FA concentrations were highest at surface depth (0–10 cm) compared to the subsurface depth (10–20 cm) under all treatments. A lack of significant alfalfa–organic amendments × time interaction indicated that the concentration of SOC pools increased over time in response to alfalfa–organic amendments.

Our results on SOC content and differentiation of its depths by the impact of organic amendments were agreed with the previous reports. A significant increase in concentration of SOC pools was due to complex, but synergistic, interactions of a legume-based cropping system with organic amendments over time. It was reported in a Central Asian regional study that SOC concentration increased by protective soil restorative practices and under ungrazed lands in Kyrgyzstan (Vladychensky et al., 1995). A pronounced increase in concentration of SOC, humin, HA, and FA across alfalfa–organic amendments confirms the greater contribution of deep-rooted perennial alfalfa on SOC dynamics. In other words, both cattle manure and vermicompost treatments synergistically complement the influence of alfalfa on SOC pools over time.

When compared to the control, a significant increase in concentration of SOC, humin, HA, and FA pools at both depths with a stratification corresponds to surface application of organic amendments and the contribution from alfalfa biomass (Roumet et al., 2016). Alfalfa, as a fine-rooted perennial legume, contributes a higher amount of N-enriched labile biomass to the soil (Roumet et al., 2016). Leaching and bioturbation of organic amendments and the deep rooting system of alfalfa might have increased the concentration of SOC, humin, HA, and FA pools at both depths when compared to the control (Roumet et al., 2016). The longer the vegetative period, the higher its contribution in the humification process associated with increased SOC

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TABLE 1 Long-term effects (1991–2020) of alfalfa–organic amendments on total soil organic carbon (SOC), humin, humic acid (HA), and fulvic acid (FA) concentrations at different soil depths.

Alfalfa–organic treatments	Time (year)	Depth (cm)	SOC (g/kg)	Humin	HA (mg/kg)	HA %	FA (mg/kg)	FA %
Control—barley	0	10	1.39	1.37	15.0	70.5	6.3	29.5
		20	0.82	0.80	13.3	67.9	6.3	32.1
Alfalfa	3	10	3.11	3.07	28.3	63.7	16.1	36.3
		20	2.06	2.04	16.2	65.1	8.7	34.9
	11	10	5.93	5.88	31.6	64.8	17.2	35.2
		20	4.33	4.29	21.5	60.4	14.1	39.6
	18	10	8.63	8.56	40.2	63.6	23.0	36.4
		20	5.08	5.04	25.0	63.0	14.7	37.0
	29	10	11.71	11.63	53.6	62.5	32.2	37.5
		20	5.82	5.77	28.3	60.2	18.7	39.8
Alfalfa–cattle manure	3	10	4.41	4.37	24.2	60.3	15.9	39.7
		20	2.60	2.57	21.9	60.3	14.4	39.7
	11	10	6.25	6.20	31.1	60.2	20.5	39.8
		20	4.72	4.68	23.5	60.3	15.4	39.7
	18	10	9.00	8.92	44.7	59.8	30.0	40.2
		20	5.46	5.41	27.1	60.2	17.9	39.8
	29	10	11.83	11.74	58.9	63.8	33.4	36.2
		20	6.34	6.29	31.5	60.1	20.9	39.9
Alfalfa–vermicompost	3	10	3.42	3.39	19.2	60.3	12.7	39.7
		20	2.91	2.88	15.5	54.6	12.9	45.4
	11	10	7.88	7.83	28.9	63.2	16.8	36.8
		20	4.26	4.22	23.5	61.3	14.8	38.7
	18	10	9.75	9.68	48.4	65.8	25.1	34.2
		20	6.80	6.75	28.6	59.5	19.5	40.5
	29	10	11.90	11.81	59.1	65.5	31.2	34.5
		20	7.72	7.67	32.8	60.1	21.8	39.9
Probability > F								
Treatment			<0.001	<0.001	0.003	<0.001	<0.001	<0.001
Time			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soil depth			0.008	0.009	<0.001	0.538	<0.001	0.534
Treatment × time			1.00	0.685	0.0454	0.889	1.00	1.00
Treatment × depth			0.72	0.711	0.098	0.961	0.219	0.948
Time × depth			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment × time × depth			0.991	0.993	<0.001	<0.009	<0.001	0.007

accumulation (Kuzyakov, 2002). It is reported that alfalfa roots and stubbles significantly increased the content of humic substances, especially at the surface soil depth (Ghimire et al., 2013; Zhamalbekov, 1989). Several previous studies have shown the concentration of SOC pools increased over time under undisturbed ecosystems, no-till with cover crops and organic amendments (Min et al., 2003). Liu et al. (2007) reported that long-term organic amendments and spontaneous vegetative succession increased SOC contents in the grasslands of China.

Despite a large amount of organic amendments with cattle manuring (60 Mg/ha), when compared to the vermicompost (27 Mg/ha), SOC, humin, HA, and FA concentrations in the loess materials were more pronounced in the latter treatment. This could be due to greater availability of nutrients and labile C from vermicompost for supporting microbial diversity and efficiency to activate loess's potentiality more effectively (Pathma & Sakthivel, 2012). Vermicompost, with its greater nutrient availability than cattle manures, might have increased both above- and below-ground biomass of alfalfa associated

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with the increased concentration of SOC, humin, HA, and FA (Alwaneen, 2016; Min et al., 2003; Ngo et al., 2014). A lower FA content but higher HA content indicated an accumulation of higher molecular weight aromatic and stabilized HA and humin compounds, which are formed due to complex physicochemical interactions with fine particles and polyvalent cations (Stevenson, 1994).

It was obvious that type and duration of organic amendments influenced SOC pools being greater in vermicompost than in cattle manure treatments. Previous studies investigating the effect of vermicompost have shown a faster maturation of the organic wastes complementary to increased SOC content (Atiyeh et al., 2000). Accordingly, partition of bulk SOC into humin, HA, and FA fractions can be directly affected more by the quality of organic amendments, as a portion of it is in partially decomposed organic form, so that returning new organic amendments and N-enriched alfalfa residue increases the labile SOC pool (Tiemann & Billings, 2011). Organic amendments also favor new SOC formation via efficient microbial C assimilation (anabolism) with a concomitant production of metabolites (Islam et al., 2021; Li et al., 2021).

3.2 | Soil organic carbon stocks

SOC, humin, HA, and FA stocks showed a slightly different pattern when calculated by taking equivalent depth (by using antecedent ρb) or equivalent mass (by using depth correction) into consideration (Table 2). The ρb values under unplowed alfalfa and alfalfa–organic amendments had initially increased but decreased over time to values that were lower when compared with the ρb values of the control. The impact of alfalfa–organic amendments on decreasing ρb was more pronounced at surface soil depth. Averaged across time and depth, the equivalent depth SOC pools did significantly increase by 5.6-, 2.1-, and 3-fold with respect to those SOC stocks under the control, and the highest increase was observed under alfalfa–vermicompost amendments followed by alfalfa–manure and alfalfa only. Over time, SOC and humin stocks increased by more than fivefold, HA by twofold, and FA by threefold than that of their initial values. With correction for equivalent mass, there were similar increases in SOC, humin, HA, and FA stocks than that of the respective SOC stocks under the control. The values of SOC, humin, HA, and FA stocks, calculated based on equivalent mass, were higher than the respective SOC pools, calculated based on equivalent depth.

The equivalent depth bulk SOC stock significantly and linearly accounted for variability (R^2) in equivalent mass bulk SOC stock with a slope ($\Delta y/\Delta x$) of 1.05 ± 0.005 (Figure 2a). However, there was a slight overestimation of bulk SOC stock by 151 ± 48 kg/ha when using the equivalent depth (due to variable ρb effect) to calculate and/or predict bulk SOC stock compared to the equivalent mass. A closely similar overestimation, like the bulk SOC stock, was observed for humin stock (Figure 2b). The equivalent depth HA stock accounted for 98.8% of the variations in equivalent mass HA stock [slope ($\Delta y/\Delta x$) of 1.06 ± 0.006 with an intercept (a), as a measure of overestimation, of 1.18 ± 0.28 kg/ha] (Figure 2c). Likewise, FA stock had a similar relationship between equivalent depth and equivalent mass ($R^2 = 0.998$, slope 1.06 ± 0.07, and an intercept of 0.69 ± 0.17 kg/ha) (Figure 2d).

Significantly higher SOC, humin, HA, and FA stocks under alfalfa–organic amendments, when compared to the control (barley), was due to the effects of ρb variations. A significant decrease in ρb suggested that the long-term organic amendments under undisturbed conditions (alike no-till) significantly improved soil physical properties (Hua et al., 2014). The lower values of ρb, especially ≥11 years, under the long-term alfalfa–organic amendments compared to the initial ρb of the loess materials (control at 0 year) were associated with the beneficial effects of low-density organic amendments and extensive rooting density of alfalfa on soil ecosystems. Organic matter, because of its low ρb (∼ 0.5–0.6 g/cm^3) and the ability to enhance soil macroaggregation as a transient binding agent, decreased ρb over time (Min et al., 2003; Ohu et al., 1994). However, a relatively higher ρb ≤ 3 years under alfalfa–organic amendments was due to transitional effects (alike no-till) from lack of soil mixing by plowing and compaction caused by natural processes. Diaz-Zorita (2000) reported a 10% increase in ρb within 2 years of no-till (undisturbed site) than that of the conventional plowing (disturbed site). Our results were also collaborated with results of other studies (Sundermeier et al., 2011).

A consistent increase in values of SOC, humin, HA, and FA stocks under alfalfa–organic amendments, calculated based on equivalent mass when compared to equivalent depth, was expected due to an adjustment in the equivalence of soil mass in response to the reclamation treatments (Don et al., 2011; Lee et al., 2009; Razzaghi et al., 2022; Wilson & Warren, 2015). In other words, an adjustment in soil mass equivalence accounted for higher and more realistic values of SOC, humin, HA, and FA stocks. Balbino et al. (2002) reported higher ρb (compaction) values resulted from degradation of soil aggregate stability when the native vegetation was replaced and/or converted for other land uses, which accounted for the overestimation of SOC stocks. A close relationship exists between the mass proportion of soil aggregates and the ρb to adjust equivalent mass, when compared to equivalent depth, for realistic measurement and prediction of SOC stocks (Volland-Tuduri et al., 2005). Razzaghi et al. (2022) reported that equivalent mass SOC stocks linearly and significantly accounted for the variability (R^2) in their respective C stocks calculated based on equivalent depth; however, the use of equivalent depth overestimated SOC storage when compared to equivalent mass. Lee et al. (2009) reported that the equivalent (fixed) depth unrealistically overestimated SOC stocks, while the equivalent mass reduced errors associated with SOC stocks estimation. Likewise, Don et al. (2011) indicated that without soil mass correction (due to ρb variations), SOC stock was underestimated by 28%, when comparing results among multiple sources or over time.

3.3 | Soil organic carbon sequestration

SOC sequestration in different pools was significantly impacted by alfalfa–organic amendments (Figure 3; Tables 4–7). Impact of alfalfa–

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TABLE 2 Long-term effects (1991–2020) of alfalfa–organic amendments on total soil organic carbon (SOC), humin, humic acid (HA), and fulvic acid (FA) stocks at different soil depths, calculated based on equivalent depth (eq_{depth}) and equivalent mass (eq_{mass}).

				$SOC_{eqdepth}$	SOC_{eqmass}	$Humin_{eqdepth}$	$Humin_{eqmass}$	$HA_{eqdepth}$	HA_{eqmass}	$FA_{eqdepth}$	FA_{eqmass}
Alfalfa–organic treatments	**Time (year)**	**Depth (cm)**	**ρb (g/cm^3)**	**Mg/ha**				**kg/ha**			
Control	0	10	1.25	1.90	1.90	1.87	1.87	20.6	20.6	8.6	8.6
		20	1.25	1.13	1.13	1.10	1.10	18.3	18.3	8.6	8.6
Alfalfa	3	10	1.27	4.22	4.26	4.16	4.19	38.4	38.7	21.9	22.1
		20	1.28	2.79	2.83	2.76	2.79	21.9	22.2	11.8	11.9
	11	10	1.24	7.94	8.12	7.87	8.05	42.3	43.2	23.0	23.5
		20	1.25	5.84	5.94	5.79	5.89	29.1	29.6	19.1	19.4
	18	10	1.22	11.60	11.80	11.51	11.71	54.1	55.1	30.9	31.5
		20	1.25	6.70	6.97	6.65	6.91	33.0	34.3	19.4	20.1
	29	10	1.20	15.25	16.02	15.14	15.91	69.8	73.3	41.9	44.0
		20	1.24	7.80	7.99	7.74	7.92	37.9	38.8	25.0	25.6
Alfalfa–cattle manure	3	10	1.28	5.94	6.04	5.89	5.99	32.6	33.1	21.4	21.8
		20	1.25	3.50	3.56	3.45	3.51	29.5	30.0	19.4	19.7
	11	10	1.24	8.35	8.57	8.28	8.50	41.5	42.6	27.5	28.2
		20	1.25	6.35	6.45	6.30	6.40	31.6	32.1	20.8	21.1
	18	10	1.24	12.08	12.35	12.00	12.26	60.1	61.4	40.3	41.2
		20	1.22	7.19	7.46	7.11	7.39	35.7	37.1	23.6	24.5
	29	10	1.20	15.39	16.23	15.27	16.11	76.6	80.8	43.5	45.9
		20	1.24	8.47	8.68	8.40	8.61	42.1	43.2	27.9	28.6
Alfalfa–vermicompost	3	10	1.27	4.60	4.68	4.56	4.63	25.9	26.3	17.1	17.4
		20	1.24	3.90	4.00	3.86	3.96	20.8	21.3	17.3	17.7
	11	10	1.23	10.50	10.78	10.45	10.73	38.5	39.5	22.4	23.0
		20	1.24	5.70	5.84	5.64	5.77	31.4	32.2	19.8	20.3
	18	10	1.23	13.00	13.34	12.90	13.24	64.5	66.2	33.5	34.4
		20	1.23	8.90	9.33	8.84	9.27	37.4	39.2	25.5	26.7
	29	10	1.20	15.47	16.28	15.35	16.15	76.9	80.9	40.5	42.6
		20	1.23	10.30	10.60	10.23	10.52	43.7	45.0	29.1	29.9
Probability > F											
Treatment		<0.001		<0.001		<0.001	<0.001	<0.001	0.003	<0.001	<0.001
Time		<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soil depth		0.011		0.001		0.008	0.001	0.009	<0.001	<0.001	<0.001
Treatment × time		1.00		1.00		1.00	1.00	1.00	0.016	0.049	1.00
Treatment × depth		0.805		0.743		0.713	0.738	0.708	0.106	0.094	0.233
Time × depth		<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment × time × depth		0.988		0.988		0.991	0.988	<0.992	<0.001	<0.001	<0.001

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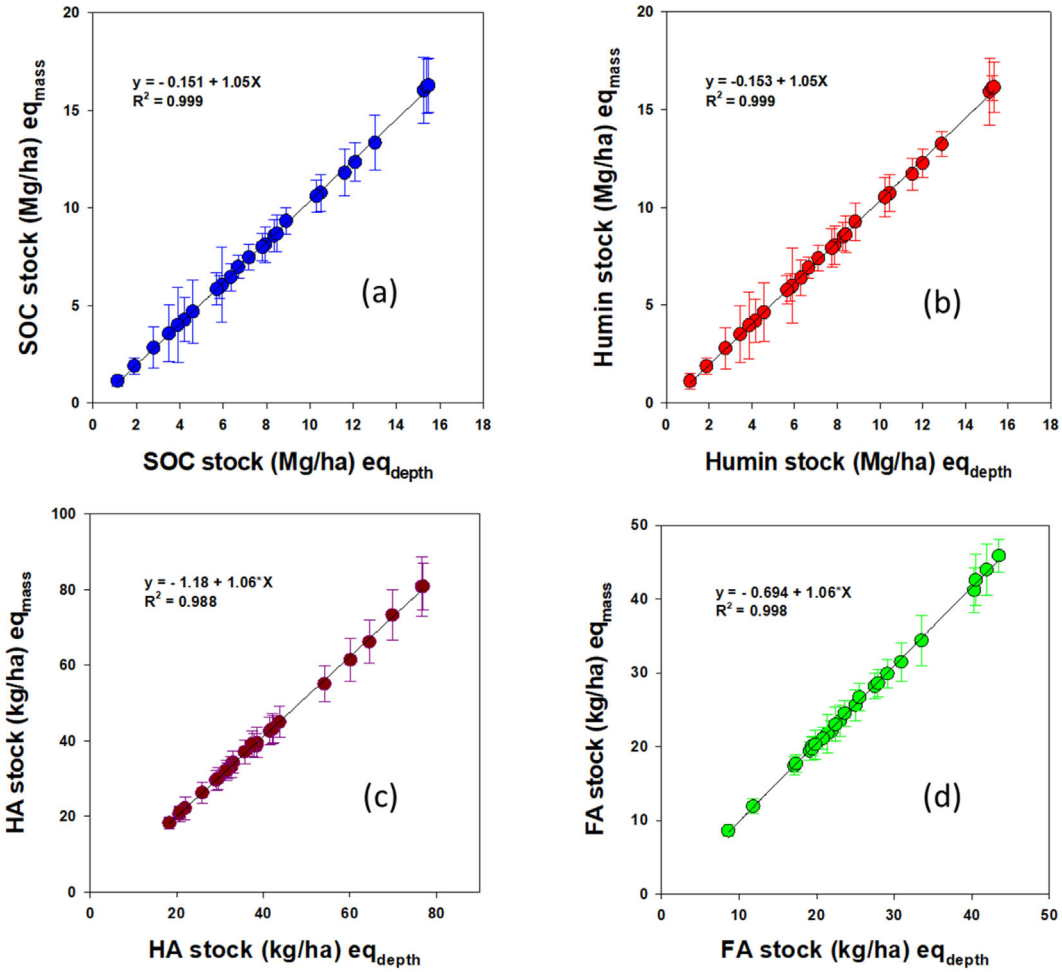


FIGURE 2 Relationship between equivalent depth and equivalent mass stocks of (a) total soil organic carbon (SOC), (b) humin, (c) humic acid (HA), and (d) fulvic acid (FA) in response to long-term effects (1991–2020) of alfalfa–organic amendments on soil (mean ± standard error). [Colour figure can be viewed at wileyonlinelibrary.com]

organic amendments on loess materials' capacity to sequester C at different depths was calculated by plotting SOC, humin, HA, and FA stocks as a function of time (0, 3, 11, 18, and 29 years). For SOC, humin, HA, and FA sequestration, a nonlinear quadratic regression model was the best fit for both 0–10 and 10–20 cm depths.

After analyzing the nonlinear regression equations over a 29-year period, bulk SOC sequestration rates were calculated to be 614 ± 129, 710 ± 169, and 744 ± 161 kg/ha/yr at 0–10 cm depth under alfalfa, alfalfa–manure, and alfalfa–vermicompost treatments, respectively, when compared to the control (Figure 3a, Table 4). Likewise, bulk SOC sequestration rates were calculated to be 410 ± 100, 453 ± 148, and 487 ± 143 kg/ha/yr at 10–20 cm depth under alfalfa, alfalfa–manure, and alfalfa–vermicompost treatments, respectively. A similar pattern, but slightly lower values of humin sequestration, was observed under all alfalfa–organic amendments (Figure 3b, Table 5).

The HA sequestration rates were calculated to be 5, 4.9, and 4.6 kg/ha/yr at 0–10 cm depth under alfalfa, alfalfa–manure, and alfalfa–vermicompost amendments, respectively (Figure 3c, Table 6). The HA sequestration rates were slightly less at 10–20 cm depth, that is, 3.2, 3.7, and 3.4 kg/ha/yr under alfalfa, alfalfa–manure, and alfalfa–vermicompost treatments, when compared to the control. In contrast, the FA sequestration rates were consistently lower than HA sequestration rates (Figure 3d, Table 7). The FA sequestration rates were calculated to be 2.7, 3, and 2.6 kg/ha/yr at 0–10 cm depth under alfalfa, alfalfa–manure, and alfalfa–vermicompost systems, respectively. Slightly lower values of FA sequestration rates were calculated at 10–20 cm depth under alfalfa, alfalfa–manure, and alfalfa–vermicompost treatments, when compared to the control.

Our results on SOC sequestration closely resembled those of previous studies (Johnston et al., 2017; Powlson et al., 2011; Wang et al., 2018; Li et al., 2021). Powlson et al. (2011) reported that soil has a finite capacity for C storage, which justified our results that SOC sequestration in different pools at both depths did not increase linearly and indefinitely. Johnston et al. (2017) reported that SOC sequestration rates peaked initially and then declined progressively over time. Lower SOC rates at 10–20 cm depth indicate that further SOC sequestration is likely in subsurface soil over time in response to alfalfa–organic amendments.

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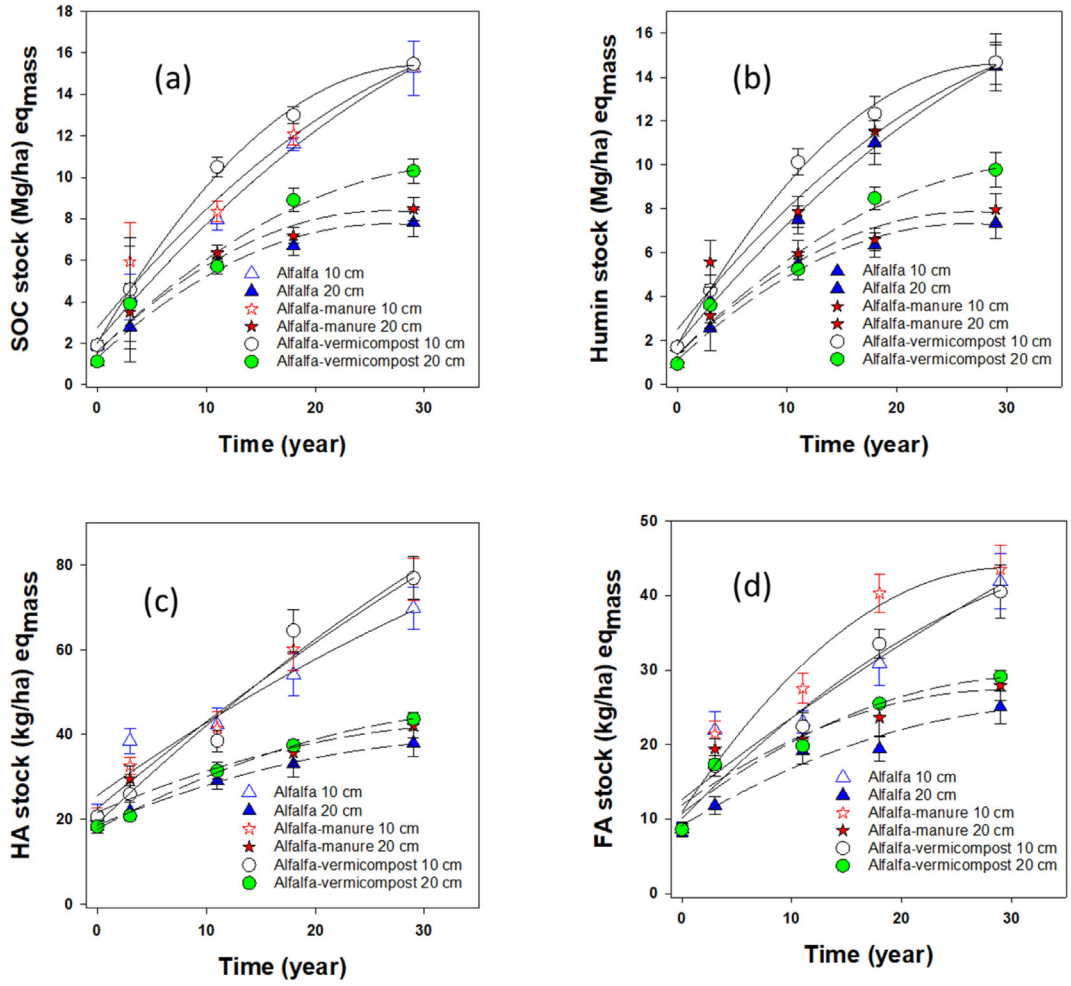


FIGURE 3 Relationship between equivalent mass stocks of (a) total soil organic carbon (SOC), (b) humin, (c) humic acid (HA), and (d) fulvic acid (FA) and time (years) to calculate carbon sequestration rates in response to long-term effects (1991–2020) of alfalfa–organic amendments on soil (mean ± standard error). [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Long-term effects (1991–2020) of alfalfa–organic amendments on total soil organic carbon (SOC) sequestration at different depths, calculated based on equivalent soil mass.

Alfalfa–organic treatments	Depth (cm)	Regression equation	R^2	SOC sequestration rate (kg/ha/yr)
Alfalfa	10	$y = 2.08 + 0.625*X - 0.0059*X^2$	0.996	641 ± 129
	20	$y = 1.30 + 0.483*X - 0.0091*X^2$	0.981	410 ± 100
Alfalfa–cattle manure	10	$y = 2.75 + 0.644*X - 0.0072*X^2$	0.948	710 ± 169
	20	$y = 1.56 + 0.514*X - 0.0097*X^2$	0.956	453 ± 148
Alfalfa–vermicompost	10	$y = 1.20 + 0.910*X - 0.0155*X^2$	0.996	744 ± 161
	20	$y = 1.58 + 0.522*X - 0.0076*X^2$	0.945	487 ± 143

While SOC sequestration is the quantitative and qualitative balance of organic C inputs and their temporal decomposition by soil microbes (Hua et al., 2014), the use of organic amendments is a promising approach to enhance SOC sequestration; however, its effectiveness for SOC sequestration has varied among studies (Ghosh et al., 2012; Hua et al., 2014; Li et al., 2021; Wang et al., 2018). Based on the results of a long-term (1982–2011) study, Hua et al. (2014) reported that mean SOC sequestration rates were calculated to be 200, 390, 500, 510, and 970 kg/ha/yr in the NPK, NPK + ½ wheat straw, NPK + wheat straw, NPK + pig manure, and NPK + cattle manure treatments, respectively, when compared to the 20 kg/ha/yr in the control. Likewise, Li et al. (2021) indicated that the largest average SOC sequestration rates were 1.28 Mg/ha/yr followed by 840 and 230 kg SOC/ha/yr under manure compost, straw return, and

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TABLE 4 Long-term effects of soil remedial management practices on humin carbon sequestration at different depths of loess (1991–2020).

Alfalfa–organic treatments	Depth (cm)	Regression equation	R^2	Humin sequestration rate (kg/ha/yr)
Alfalfa	10	$y = 1.82 + 0.605*X - 0.0058*X^2$	0.997	600 ± 115
	20	$y = 1.11 + 0.469*X - 0.0088*X^2$	0.982	382 ± 90
Alfalfa–cattle manure	10	$y = 2.50 + 0.625*X - 0.0058*X^2$	0.947	682 ± 144
	20	$y = 1.35 + 0.485*X - 0.0090*X^2$	0.950	417 ± 104
Alfalfa–vermicompost	10	$y = 1.80 + 0.887*X - 0.0154*X^2$	0.993	707 ± 151
	20	$y = 1.37 + 0.499*X - 0.0071*X^2$	0.938	454 ± 96

TABLE 5 Long-term effects (1991–2020) of alfalfa–organic amendments on humic acid (HA) carbon sequestration at different soil depths, calculated based on equivalent soil mass.

Alfalfa–organic treatments	Depth (cm)	Regression equation	R^2	HA sequestration rate (kg/ha/yr)
Alfalfa	10	$y = 25.62 + 1.829*X - 0.0112*X^2$	0.873	5.0 ± 1.8
	20	$y = 18.54 + 1.092*X - 0.0148*X^2$	0.997	3.2 ± 1.3
Alfalfa–cattle manure	10	$y = 22.54 + 2.139*X - 0.0091*X^2$	0.966	4.9 ± 1.6
	20	$y = 21.63 + 1.121*X - 0.0149*X^2$	0.774	3.7 ± 1.6
Alfalfa–vermicompost	10	$y = 18.73 + 2.455*X - 0.0141*X^2$	0.939	4.6 ± 1.4
	20	$y = 17.67 + 1.412*X - 0.0177*X^2$	0.994	3.4 ± 1.3

TABLE 6 Long-term effects (1991–2020) of alfalfa–organic amendments on fulvic acid (FA) carbon sequestration at different soil depths, calculated based on equivalent soil mass.

Alfalfa–organic treatments	Depth (cm)	Regression equation	R^2	FA sequestration rate (kg/ha/yr)
Alfalfa	10	$y = 12.6 + 1.153*X - 0.0054*X^2$	0.821	2.7 ± 0.9
	20	$y = 9.11 + 0.890*X - 0.0123*X^2$	0.926	1.9 ± 0.7
Alfalfa–cattle manure	10	$y = 10.92 + 2.215*X - 0.0374*X^2$	0.926	3.0 ± 1.2
	20	$y = 11.89 + 1.041*X - 0.0175*X^2$	0.672	2.3 ± 0.9
Alfalfa–vermicompost	10	$y = 10.02 + 1.514*X - 0.0157*X^2$	0.949	2.6 ± 1.2
	20	$y = 10.75 + 1.133*X - 0.0174*X^2$	0.862	2.2 ± 0.8

control plots, respectively. These results indicated that SOC sequestration rate changes were related to the amount and quality of organic C inputs. The C-enriched recalcitrant organic compounds (i.e., lignin and polyphenol) in manure compost led to higher SOC sequestration rates (Di Lonardo et al., 2017; Ghosh et al., 2012).

3.4 | Soil organic carbon lability

The HA:FA ratio, as one of the measures of SOC quality, was impacted in response to alfalfa–organic amendments (Table 3). Averaged across depth, the HA:FA ratio narrowed over time by 1.3-, 1.7-, and 1.4-fold under alfalfa, alfalfa–manure, and alfalfa–vermicompost treatments, respectively, when compared to the control (initial values). The average decrease in HA:FA ratio was 1.5-fold over time. Likewise, the HR of SOC decreased over time, and the decrease was more pronounced under alfalfa–vermicompost (by 2.5-fold) followed by alfalfa–manure (2.2-fold) and alfalfa (2.1-fold) with an average decrease by 2.3-fold, compared to the control.

The CPI, an indicative measure of SOC accumulation or depletion over time, did increase at both depths under alfalfa–organic amendments (Table 3). The CPI values were higher by 5.3-fold under alfalfa, 5.7-fold under alfalfa–manure, and 6.3-fold under alfalfa–vermicompost, with an average increase by 5.7-fold, when compared to the control. Both carbon lability and carbon lability index (CL and CLI) decreased at both depths by the impact of alfalfa–organic amendments, and the decrease was more pronounced under alfalfa–vermicompost than that of the alfalfa and alfalfa–manure treatments. The CMI, as composite measures of both SOC accumulation or depletion and lability, was significantly higher under alfalfa–manure (2.6-fold) followed by 2.4-fold under alfalfa–vermicompost and 2.3-fold under alfalfa, with an average increase by 2.4-fold, when compared to the control. However, a significant nonlinear inverse relationship between CPI (SOC accumulation) and CL was observed,

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TABLE 7 Long-term effects (1991–2020) of alfalfa–organic amendments on total soil organic carbon (SOC) accumulation and lability at different soil depths, calculated based on equivalent soil mass.

Alfalfa–organic treatments	Time (year)	Depth (cm)	HA/FA	HR	CPI	CL	CLI	CMI
Control	0	10	2.39	1.54	1.00	0.016	1.00	1.00
		20	2.12	2.38	1.00	0.024	1.00	1.01
Alfalfa	3	10	1.76	1.43	2.24	0.014	0.93	2.08
		20	1.86	1.21	2.51	0.012	0.50	1.26
	11	10	1.84	0.82	4.27	0.008	0.53	2.27
		20	1.52	0.82	5.28	0.008	0.34	1.80
	18	10	1.75	0.73	6.21	0.007	0.47	2.94
		20	1.70	0.78	6.19	0.008	0.32	2.00
	29	10	1.67	0.73	8.43	0.007	0.47	3.98
		20	1.51	0.81	7.10	0.008	0.33	2.37
Alfalfa–cattle manure	3	10	1.52	0.91	3.17	0.009	0.59	1.86
		20	1.52	1.40	3.17	0.014	0.58	1.84
	11	10	1.51	0.83	4.50	0.008	0.53	2.40
		20	1.52	0.82	5.75	0.008	0.34	1.96
	18	10	1.49	0.83	6.47	0.008	0.54	3.48
		20	1.51	0.83	6.65	0.008	0.34	2.27
	29	10	1.76	0.78	8.51	0.008	0.50	4.29
		20	1.51	0.83	7.74	0.008	0.34	2.64
Alfalfa–vermicompost	3	10	1.52	0.93	2.46	0.009	0.60	1.49
		20	1.20	0.98	3.55	0.010	0.63	2.24
	11	10	1.72	0.58	5.67	0.006	0.24	1.36
		20	1.58	0.90	5.19	0.009	0.58	3.02
	18	10	1.92	0.75	7.02	0.008	0.31	2.18
		20	1.47	0.71	8.30	0.007	0.46	3.78
	29	10	1.90	0.76	8.56	0.008	0.31	2.68
		20	1.51	0.71	9.42	0.007	0.29	2.75
Probability > F								
Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Soil depth	0.499	0.006	0.56	0.001	0.296	<0.001		
Treatment × time	1.00	1.00	1.00	1.00	1.00	1.00		
Treatment × depth	0.961	<0.001	0.959	<0.001	0.798	0.101		
Time × depth	<0.001	<0.001	<0.001	<0.001	0.005	<0.001		
Treatment × time × depth	<0.001	1.00	0.765	1.00	0.084	<0.001		

which accounted for more than 80% (R^2) of the variability in SOC lability (Figure 4).

The differences in HA:FA ratios were expected due to the variations in C lability and microbial palatability as influenced by the impact of alfalfa–organic amendments (Amoakwah et al., 2020, 2022). The progressively narrower HA:FA ratios (1.5–1.8) under the alfalfa–organic amendments compared to the initial HA:FA ratios (2.1–2.4) under the control were associated with a greater proportional accumulation of FA over the prevalence of HA in loess materials (Amoakwah et al., 2020; Navarrete et al., 2010; Stevenson, 1994). It is reported that greater content of humified materials in organic amendments, including vermicompost, influences the rate of their mineralization and partition in different SOC pools (Campitelli & Ceppi, 2008). Likewise, a progressive decrease in HR values under alfalfa–organic amendments, especially alfalfa–vermicompost when compared to the control, suggested a slightly more humified and stable SOC quality (Amoakwah et al., 2020; Saviozzi et al., 1994).

Significantly higher SOC contents under all alfalfa–organic amendments were expected to translate into higher CPI and CMI values. Results showed that higher CPI values, rather than C lability values, significantly contributed to an increase in the CMI values at both depths under all alfalfa–organic amendments when compared to

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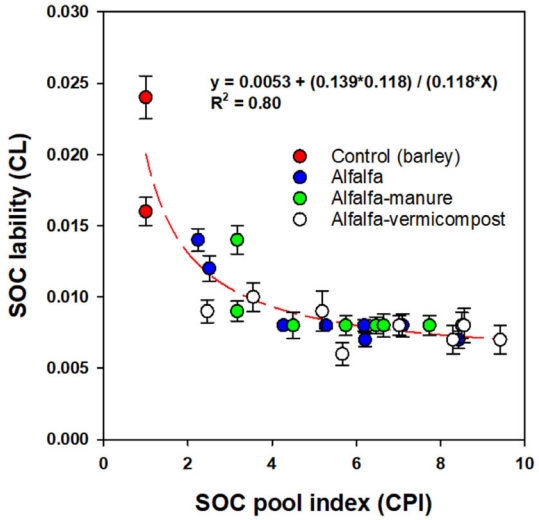


FIGURE 4 Relationship between total soil organic carbon (SOC) pool index (accumulation) and carbon lability (CL) by the impact (1991–2020) of alfalfa–organic amendments on soil (mean ± standard error). [Colour figure can be viewed at wileyonlinelibrary.com]

the control. The higher CPI values under alfalfa–organic amendments resulted in bulk SOC accumulation due to the impact of C-enriched organic amendments, but consequently affected the SOC lability. A nonlinear relationship between CPI and CL indicated that SOC sequestration is inversely and significantly related to its lability or vice versa. Therefore, organic amendments increased the CMI, thus indicating that the loess-based soils were being reclaimed over time due to increased SOC sequestration (Chaudhary et al., 2017; Li et al., 2021; Lin et al., 2019).

4 | CONCLUSIONS

Long-term effects of perennial vegetation with contrasting organic amendments, such as alfalfa, alfalfa–manure, and alfalfa–vermicompost significantly increased the concentration of SOC, humin, HA, and FA to rejuvenate degraded loess-derived soils. Nutritionally enriched vermicompost, when applied at 27 Mg/ha together with alfalfa, had more pronounced effects on SOC pools, especially SOC (higher by 9%) and humin (higher by 7%) when compared to the nutritionally poor semi-decomposed cattle manures applied at 60 Mg/ha with alfalfa. A similar impact of alfalfa–organic amendments was observed on SOC stocks when calculated based on equivalent depth and equivalent mass; however, the equivalent depth significantly overestimated SOC, humin, HA, and FA sequestration rates than that of the equivalent mass. However, there was a slightly higher proportion of SOC partitioned into humin fraction under alfalfa–organic amendments, as reflected by higher CPI and CMI values with a significant nonlinear inverse relationship between SOC accumulation (CPI) and its lability (CL). While SOC is one of the core indicators of soil quality, there is a lack of availability of information associated with the long-term effects of contrasting organic amendments on SOC sequestration and its lability across geographic regions, and therefore, further research is needed to address this knowledge gap.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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