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The formation of humic acid and micro-aggregates facilitated long-time soil organic carbon sequestration after *Medicago sativa* L. introduction on abandoned farmlands

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ABSTRACT

The substantial carbon sequestration observed in abandoned farmland assumes a pivotal role in mitigating the impacts of global warming. However, it remains unclear how to effectively manage abandoned farmlands to achieve this goal, especially over the long term, and understand the underlying mechanisms. The introduction of legumes can augment vegetation coverage, mitigate soil erosion, and ameliorate soil quality. A long-term study has been conducted in the semiarid region of the Loess Plateau since 2003, with a focus on revegetation strategies involving the introduction of alfalfa (*Medicago sativa* L.) and sweet clover (*Melilotus officinalis* L.), while natural abandonment (fallow) served as a control. In this study, we utilized a physical–chemical combination approach to investigate the carbon sequestration process within the 0–20 cm soil layer. Our results demonstrated that, in comparison to the fallow, alfalfa introduction significantly increased soil organic carbon (SOC), light fraction organic carbon (LFOC), and heavy fraction organic carbon (HFOC) concentrations, whereas no significant differences were observed when comparing with sweet clover. The amount and proportion of humic acid (HA) within HFOC were notably higher in alfalfa fields compared to fallow fields. While soil wet-sieved macro-aggregates (>2 mm and 0.25–2 mm) showed no significant difference between alfalfa and fallow fields, soil wet-sieved micro-aggregates were significantly more abundant in alfalfa fields. These wet-sieved micro-aggregates displayed high levels of humification across all fields. As soil wet-sieved aggregate size increased, the proportions of humin (HM) and HA decreased, while the proportion of fulvic acid (FA) increased. We concluded that the formation of HM, HA, and soil wet-sieved micro-aggregates played key roles in promoting long-term soil carbon storage following alfalfa introduction. These findings enhance our understanding of soil ecosystem responses to future climate change and underscore the significance of species selection in restoration processes to effectively mitigate global warming.

1. Introduction

In recent decades, the concentration of carbon dioxide (CO_2) in the atmosphere has steadily increased due to the combustion of fossil fuels, leading to global warming (Peters et al., 2013; Shakun et al., 2012). This rise in CO_2 levels has already resulted in an average global temperature increase of approximately 1.1 °C above pre-industrial levels (Armstrong McKay et al., 2022). The consequences of global warming are far-reaching, negatively impacting species diversity and crop production (Deutsch et al., 2018; Harrison, 2020; Wiens, 2016). Moreover, the

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melting of glaciers is contributing to rising sea levels, posing a significant threat to millions of people worldwide (Kerr, 2007). Within terrestrial ecosystems, soil plays a pivotal role as the largest carbon reservoir, with a size 4.5 times greater than plant storage and 3.3 times larger than the atmospheric pool (Lal, 2004). Consequently, it holds substantial potential for mitigating global warming (Melillo et al., 2002; Zhou et al., 2012).

Approximately 40 % of the world's land area is arid or semiarid, characterized by low plant cover (Lal, 2019; Shakun et al., 2012). The Loess Plateau, the most severely eroded region in China (Fu, 1989), is ecologically vulnerable and susceptible to global warming impacts (Miao et al., 2016). Soil erosion and organic matter decomposition from tillage contribute to soil carbon loss, leading to increased CO_2 in the atmosphere. To combat soil erosion, the Chinese government initiated the "Grain for Green" project, converting farmland into grasslands or forests (Deng et al., 2014; Wang et al., 2021). This revegetation effort boosts plant cover and above/belowground biomass (Li et al., 2018; Song et al., 2022; Zheng et al., 2019), resulting in improved soil organic carbon (SOC) levels (Song et al., 2020; Zhang et al., 2021). Consequently, the project safeguards the local environment, controls soil erosion, and contributes to climate change regulation.

SOC comprises a variety of organic compounds with different stability, decomposition rates, and turnover rates (Guan et al., 2018; Li et al., 2017). The response of various SOC fractions to changes in land use varies significantly (Dalal et al., 2021; Luo et al., 2019; Su et al., 2021). From a physical density perspective, SOC can be categorized into two groups: unstable SOC (light fraction organic carbon) and chemically complex SOC (heavy fraction organic carbon, primarily composed of humic substances) (Li et al., 2022; Song et al., 2014; Yuan et al., 2016). Humic substances, which are the most stable organic compounds in soil, exhibit slow turnover rates and long residence times (Guimaraes et al., 2013; Kotze et al., 2016). The humic substances can be further chemically divided into humin (HM), humic acid (HA), and fulvic acid (FA) depending on their solubility in acid and alkaline solutions (Guan et al., 2018; Guimaraes et al., 2013). The more evolved soils have usually more HA than FA, indicating the accumulation of higher weight aromatic molecules and humin compounds (Amoakwah et al., 2022). Despite their long residence times, the chemical composition of humic substances may undergo changes in response to alterations in land use. A deeper understanding of these changes in the chemical composition of SOC is essential for elucidating the actual and potential mechanisms of the response to future temperature rising.

Soil aggregates serve as the fundamental building blocks of soil structure and play a crucial role in the physical protection and stabilization of SOC (Guan et al., 2018; Six et al., 2004; Six et al., 2000). The abandonment and subsequent revegetation of farmland can alter soil nutrient cycling and contribute to the maintenance or improvement of soil physical structures (Nadal-Romero et al., 2016). The formation of soil aggregates is closely linked to the quantity and quality of SOC (Nie et al., 2018), and the carbon associated with soil aggregates is an important indicator of SOC sequestration (Bai et al., 2020b). Generally, the formation of soil aggregates and the sequestration of SOC are mutually reinforcing processes. Previous studies have investigated the dynamics of soil aggregates in various land use types, including forests, woodlands, exclosures, grazing lands, cultivated lands, and paddy fields (Okolo et al., 2020; Tang et al., 2022; Zhong et al., 2019). However, there is still a lack of understanding regarding the chemical fraction (HM, HA, and FA) transformations occurring within soil aggregates and the relationship between soil density fractions and soil aggregates during SOC sequestration. Especially, most previous studies focus on these changes in the short term, but we have a limited understanding of the formation of soil carbon fractions in the long term.

On the Loess Plateau, leguminous alfalfa has become a prominent forage crop due to its remarkable adaptability to the local environment, making it an ideal choice for revegetation efforts. Our previous study has demonstrated that the introduction of alfalfa can increase the content of chemically complex heavy fraction organic carbon (HFOC) from 2003 to 2013, which is critical for soil carbon sequestration (Yuan et al., 2016). Nevertheless, the specific factors contributing to this increase in HFOC have remained elusive. In this study, we employed a combination of physical and chemical fractionation methods to address the following objectives: 1) Identify the components responsible for the increase in HFOC; 2) investigate the process of converting plant inputs into HFOC and the subsequent internal transformation of HFOC following alfalfa introduction; and 3) Evaluate the impact of alfalfa introduction on soil carbon sequestration through the formation of soil aggregates on abandoned farmland in semiarid regions.

2. Materials and methods

2.1. Study site

Since 2003, a long-term *in situ* revegetation experiment has been conducted at Gansu Dryland Agroecology Observation and Research Station. Our study site was in zhonglianchuan, Lanzhou, Gansu Province, China. The soil was characterized as Heima according to FAO classification. The mean annual precipitation from 2003 to 2018 is 314 mm and the mean air temperature is 7.5 °C. Soil erosion is rampant in this area, and plants are sparse. The detailed soil condition can be seen in (Song et al., 2022; Song et al., 2021). There are several common agricultural practices, including crops with peas (*Pisum sativum* L.), corn (*Zea mays* L.), potato (*Solanum tuberosum* L.), and spring wheat (*Triticum aestivum* L.).

2.2. Experiment design

Regeneration of abandoned farmlands was undertaken in 2003 on three different landscape positions. Before being abandoned, the farmlands were cultivated with spring wheat. They are situated in southeast-facing (12–16°), northeast-facing (10–14°), and horizontal-facing (4–8°) slopes with the same altitude. The abandoned farmlands in each landscape were divided into three plots (35 × 40 m) and next to each other. Three revegetation methods were allocated randomly on the plots: (1) natural revegetation (also called "fallow"), (2) alfalfa (*Medicago sativa* L., a perennial legume species, with a seeding rate of 22.5 kg ha^{-1}), (3) sweet clover (*Melilotus Officinalis* L., a biennial legume species, with a seeding rate of 11.3 kg ha^{-1}) introduction. Totally, 9 of main the plots were obtained (3 landscape positions × 3 revegetation methods). To obtain the highest aboveground biomass, these seeding rates are according to the recommendations of local farmers. It is important to note that no artificial activities, such as tillage, replanting, fertilizing, grazing, harvesting, and irrigation, were performed during the revegetation process.

2.3. Soil sampling

In October 2016, the soil samples of 0–20 cm were taken with a soil auger (inner diameter: 4 cm) to determine SOC concentration, LFOC concentration, HFOC concentration and other related soil properties. Each plot was divided into three subplots in the upper, middle, and lower positions along the slope. In each subplot, three soil samples were collected and mixed. Totally, 27 soil samples were collected (3 revegetation methods, 3 landscapes, and 3 subplots). After removing all visible litter and roots, soil samples were sieved through a 2-mm aperture screen, and then air-dried at room temperature.

In April 2017, the PVC tubes (diameter 7 cm, height 20 cm) were used to sample undisturbed soil for aggregate determination. In each subplot, one soil aggregate sample was taken (a total of 27 as described above). PVC tubes were taken directly to the laboratory, and soil was gently moved out with less disturbance. We removed the extruded soil from the PVC column's outer ring and peeled off the soil in the inner ring based on the soil texture. The diameter of soil samples was then less than

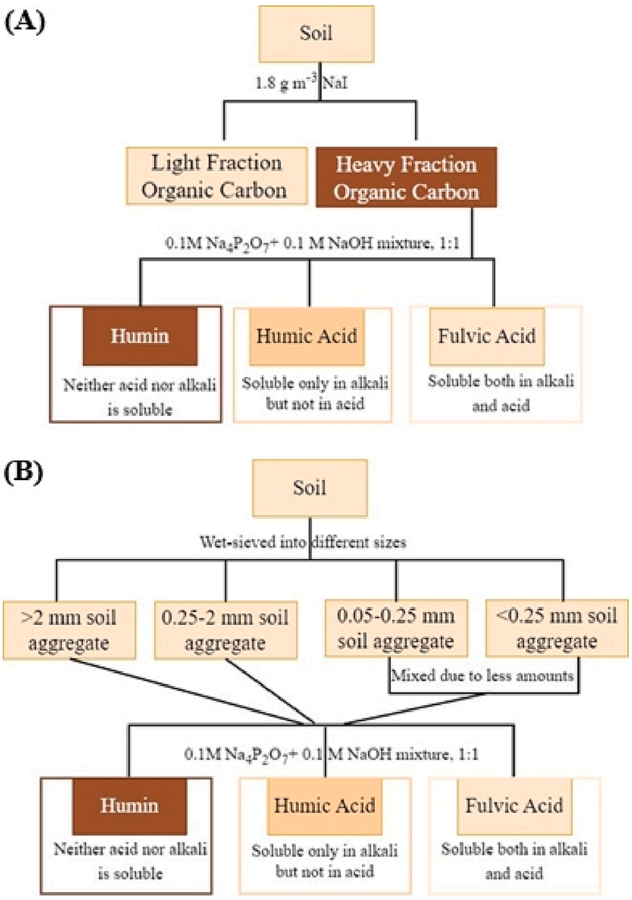

Fig. 1. The sequential fraction procedure of Soil Density-Chemical (A) and Soil Aggregates-Chemical (B) methods.

10 mm. All the soil samples were air-dried.

2.4. The determination of SOC and L/HFOC

The density fraction method was employed to isolate the LFOC and HFOC as described in Gregorich and Ellert (1993). In summary, 20 g of air-dried (<2 mm) was mixed with 50 mL of NaI solution (density: 1.8 g mL^{-1}) in a centrifuge tube. Subsequently, the tubes underwent agitation using a reciprocal shaker for 10 min, followed by centrifugation at 3000 rpm for 30 min. Afterward, the floating material (light-fraction organic matter, density $< 1.8\ mL^{-1}$) was isolated by collecting it on a 0.45 μm hydrophilic polyvinylidene fluoride filter under vacuum conditions. Thorough rinsing with 0.5 mol/L $CaCl_2$ and distilled water was performed to eliminate additional NaI. The heavy fraction remaining at the bottom of the centrifuge tube, underwent multiple rinses with deionized water (about 300 mL). The light and heavy fraction were dried at 60 °C to constant weights and then ground in a mortar and pestle for analysis (Yuan et al., 2016). The Walkley–Black method was used to measure SOC, LFOC, and HFOC concentrations (Nelson and Sommers, 1982).

2.5. The determination of soil aggregates

Soil aggregates were separated according to Wang et al., 2017 with minor modifications. Briefly, air-dried soil samples (<10 mm) were wet-sieved into different sizes (>2 mm, 0.25–2 mm, 0.05–0.25 mm, and < 0.05 mm). The sieves were installed on a reciprocating shaker in order with a cover. Soil samples (around 70 g) were added to the top of the sieves. The piped water was filled carefully, about 5 cm higher than the cover of the sieves. The soil samples were soaked in the water for 30 min and then shaken at 35-cycle/min. The length of the oscillation stroke is 3.5 cm. After shaking for 30 min, the soil retained in the sieves were the corresponding sizes. And then, we collected the soil in plastic bottles and dried it at 65 °C to constant weight.

The mean weight diameter (MWD) and geometric mean diameter (GMD) were calculated as follows,

$$MWD = \sum_{i=1}^{n} X_i W_i \tag{1}$$

$$GMD = EXP\left(\frac{\sum_{i=1}^{n} Y_i * lnX_i}{\sum_{i=1}^{n} Y_i}\right) \tag{2}$$

Where X_i is the proportion of soil retained on each sieve, W_i is the mean diameter of soil aggregates in different sizes (mm), and Y_i is the weight of the sample of soil aggregates in different sizes (g).

2.6. The determination of HA, FA, and HM in HFOC and soil aggregates

A modified version of Kumada was used to determine the HM, HA, and FA concentrations in HFOC or soil aggregates as described in Fig. 1 (Kumada, 1987; Loke et al., 2021; Zhang et al., 2022).

2.6.1. The preparation of mixed solution

HFOC or soil aggregates (<0.05 mm was mixed to 0.05–0.25 mm due to less weight for separation) samples weighing 5.0 g were placed into a conical flask, 100 mL of sodium pyrophosphate and sodium hydroxide mixture (0.1 M $Na_4P_2O_7$ + 0.1 M NaOH mixture, 1:1) was added, and the solution held for 1 h in boiling water after shaking for 5 min. In the next step, the solution was filtered and collected after it had recovered to room temperature. FA and HA were contained in this solution.

2.6.2. The determination of HM

In a test tube, 10 mL filtrate from 2.6.1 was added along with quartz sand, and the pH was adjusted to 7 by adding 0.5 mol/L H_2SO_4 until the solution became cloudy. The sample was dried in a boiling water bath. Lastly, the organic carbon of HA and FA solutions was determined by the Walkley-Black method. The soil HM is computed by subtracting the HA and FA content from soil aggregate-associated C or HFOC content.

2.6.3. The separation and determination of HA and FA

30 mL Filtrate from 2.6.1 was added to the conical flask, heated almost to boiling, and pH was adjusted to 1–1.5 with 0.5 mol/L H_2SO_4. The conical flask should be kept at 80 °C for 30 min and overnight in a thermostatic incubator to fully separate the HA. Before filtering, moisten the filter paper with 0.025 M H_2SO_4, and then purge the precipitate and flask with 0.5 M H_2SO_4 until the solution turns transparent. The precipitate is HA. We then dissolved the precipitate in 0.05 M hot NaOH until the filtrate was colorless in the 100 mL volumetric flask. After the volume was fixed, 50 mL of the filtrate was added to the test tubes. The pH was adjusted to 7 until the solution clouded with 0.5 mol/L H_2SO_4. HA content is determined using the same procedure as in 2.6.2. The soil FA is the HA and FA content minus the HA content. The humification degree of soil aggregate sizes was calculated as the ratio of HA and FA concentrations (Raiesi, 2021)

2.7. Statistical analysis

Prior to analysis, all data were assessed for normal distribution and homoscedasticity using the Q-Q plot and Levene's test, respectively. Differences in SOC, LFOC, HFOC, soil aggregates, MWD, GMD, humification degree, HA, HM, and FA among the various revegetation methods were evaluated using one-way analysis of variance (ANOVA) with landscapes as blocks in Genstat 18.0 software (VSN International, Hemel Hempstead, United Kingdom). Significant differences were determined using the Tukey test with a significance level of $P < 0.05$.

To examine the direct and indirect effects of soil water content (SWC), LFOC, microorganisms, and soil conditions on HFOC composition (HFOCs) within all treatments, partial least squares path modeling (PLSPM) was performed using the “PLSPM” package in R software (version 3.6.2). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were selected as representatives of microorganisms, while SOC, total nitrogen (TN), and total phosphorus (TP) were considered as indicators of soil conditions. HFOCs encompassed HM, HA, and FA. The PLSPM was validated based on goodness-of-fit (GOF) statistics. A higher GOF value indicates a better fit, and if GOF is > 0.7, the fitness is considered excellent (Tian et al., 2019).

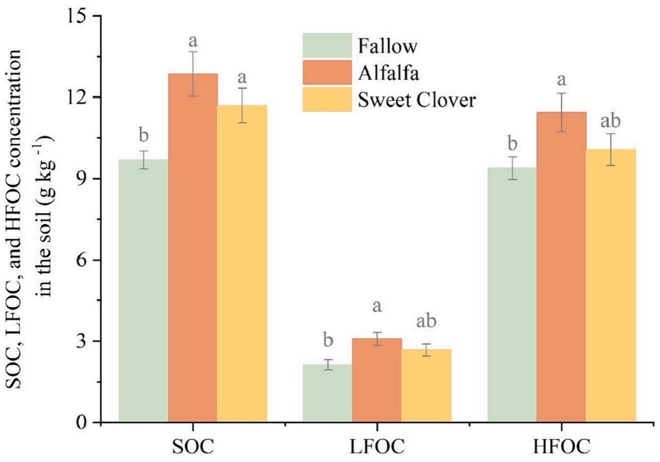

Fig. 2. The soil organic carbon (SOC), light fraction organic carbon (LFOC), and heavy fraction organic carbon (HFOC) concentrations in fallow, alfalfa, and sweet clover fields in 2016. The lower-case letters indicate the significant difference at $P < 0.05$; bars represent the standard error, N = 9).

3. Results

3.1. The effect of legume introduction on SOC, LFOC, and HFOC

In 2016, the SOC, LFOC, and HFOC concentrations in fallow fields were 9.69 ± 0.34 g kg^{-1}, 2.14 ± 0.20 g kg^{-1}, and 9.39 ± 0.42 g kg^{-1}, respectively. Alfalfa introduction in the same year had increased the SOC, LFOC, and HFOC concentration to 12.86 ± 0.83 g kg^{-1}, 3.01 ± 0.25 g kg^{-1}, and 11.44 ± 0.70 g kg^{-1} compared with fallow ($P < 0.05$) (Fig. 2). Sweet clover also increased the SOC concentration to 11.69 ± 0.65 g kg^{-1} ($P < 0.05$). However, it was not observed that LFOC and HFOC differed significantly in fallow and sweet clover fields (Fig. 2).

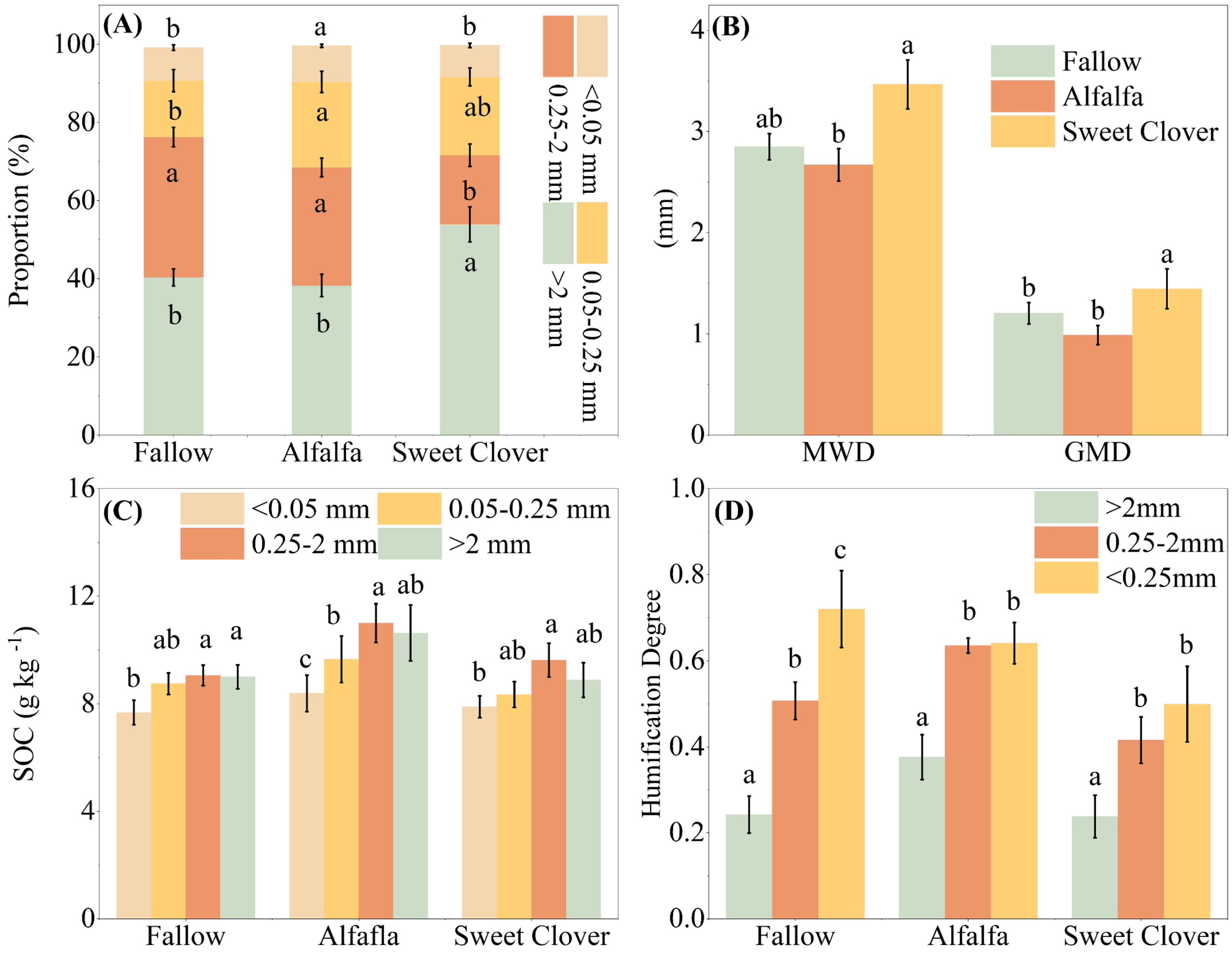

Fig. 3. The proportion of soil aggregates in different sizes (A), mean weight diameter (MWD) and geometric mean diameter (GMD) (B), soil organic carbon (SOC) in different aggregates sizes (C), and humification in different aggregates sizes (D) of fallow, alfalfa, and sweet clover fields in 2017. The lower-case letters indicate the significant difference at $P < 0.05$; bars represent the standard error, N = 9).

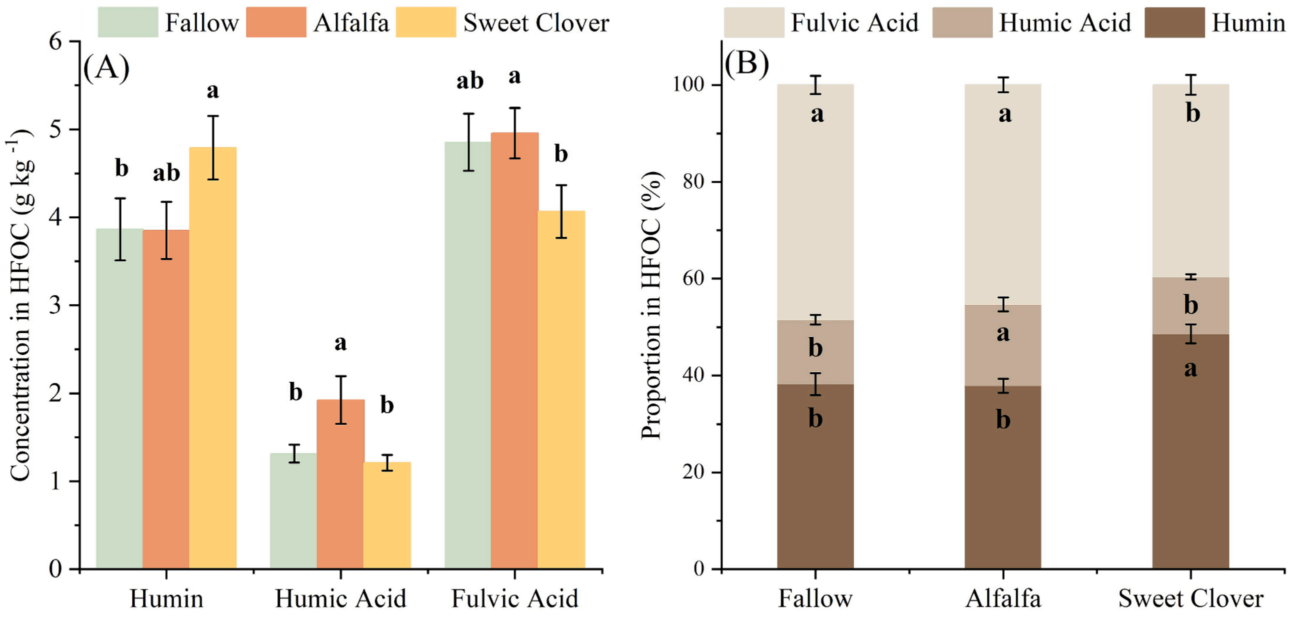

Fig. 4. The humin (HM), humic acid (HA), and fulvic acid (FA) concentrations (A) and proportion (B) in heavy fraction organic carbon (HFOC) of fallow, alfalfa, and sweet clover fields in 2016. The lower-case letters indicate the significant difference at $P < 0.05$; bars represent the standard error, N = 9).

3.2. The effect of legume introduction on the distribution of soil aggregates

In 2017, compared with fallow, the introduction of alfalfa significantly increased soil micro-aggregate (<0.05 mm and 0.05–0.25 mm) proportions from 22.87 % to 31.10 % ($P < 0.05$) (Fig. 3A). Soil macro-aggregate (>2 mm and 0.25–2 mm) proportion decreased without a significant difference (Fig. 3A). The proportion of soil macro-aggregate (>2 mm) in sweet clover fields was 53.91 ± 4.49 %, significantly greater that in fallow fields, but no difference was observed on soil micro-aggregate (<0.05 mm and 0.05–0.25 mm) (Fig. 3A).

In alfalfa fields, soil aggregates showed the lowest MWD and GMD levels, but there was no significant difference between alfalfa fields and fallow fields (Fig. 3B). In all treatments, SOC concentration would increase as aggregate size increased. The SOC concentration in different aggregate sizes was greatest in alfalfa fields than the related sizes in fallows and sweet clover fields (Fig. 3C).

The soil aggregate size was associated with a markable decrease in humification degree (Fig. 3D). The humification degree of < 0.25 mm, 0.25–2 mm, and > 2 mm in fallow fields was 0.72 ± 0.09, 0.51 ± 0.04, and 0.24 ± 0.04, in alfalfa fields, was 0.64 ± 0.05, 0.63 ± 0.02, and 0.37 ± 0.05, in sweet clover fields was 0.50 ± 0.09, 0.42 ± 0.05, and 0.24 ± 0.05, respectively (Fig. 3D).

3.3. The effect of legume introduction on HM, HA, and FA in HFOC

The amount and proportion of HA in HFOC of fallow fields were 1.32 ± 0.10 g kg^{-1} and 13.28 ± 1.01 %, respectively. Alfalfa introduction increased the amount and proportion of HA in HFOC significantly (1.92 ± 0.27 g kg^{-1} and 16.81 ± 1.45 %, $P < 0.05$) (Fig. 4A, B). The amount and proportion of FA and HM displayed no marked difference between fallow and alfalfa fields (Fig. 4 A, B). Sweet clover introduction increased the amount and proportion of HM in HFOC (4.79 ± 0.36 g kg^{-1} vs. 3.86 ± 0.35 g kg^{-1}, 48.60 ± 1.97 % vs. 38.22 ± 2.22 %, $P < 0.05$) whereas declined the proportion of FA in HFOC (39.63 ± 2.03 % vs. 48.49 ± 1.86 %, $P < 0.05$) compared with fallow (Fig. 4 A, B).

3.4. Factors influencing HFOC composition (HFOCs)

The composition of heavy fraction organic carbon (HFOCs) is influenced by multiple factors including soil water content (SWC), light fraction organic carbon (LFOC), microorganisms, and soil conditions, collectively explaining 77.5 % of the total variance in HFOCs. SWC exhibited a direct positive effect on both LFOC (path coefficient = 0.57, $P < 0.01$) and microorganisms (path coefficient = 0.38, $P < 0.05$). HFOCs were directly regulated by microorganisms (path coefficient = 0.80, $P < 0.01$) and soil conditions (path coefficient = 0.30, $P < 0.05$). Additionally, SWC had indirect effects on HFOCs through its influence on LFOC, microorganisms, and soil conditions, while LFOC indirectly influenced HFOCs through its impact on microorganisms and soil conditions (Fig. 5).

3.5. The distribution of HM, HA, and FA in soil aggregates

Generally, the concentration and proportion of HA and HM decreased with the particle size, while the concentration and proportion of FA increased in all three treatments (Fig. 6). The HM and FA contributed almost 80 % to soil aggregates in different sizes (Fig. 6).

4. Discussion

SOC has garnered significant attention due to its crucial role in the global carbon cycle and climate change. Consistent with findings from other studies (Fang et al., 2021; Li et al., 2019; Yuan et al., 2016), our study demonstrates that the introduction of alfalfa significantly increases SOC and HFOC concentrations (Fig. 2). This effect can be attributed to various factors. On the one hand, during revegetation, organic compounds from litter, root biomass, and secretions are incorporated into the soil, contributing to increased SOC (Cotrufo et al., 2015; Song et al., 2021). The formation of soil aggregates through plant litter and root secretions can inhibit organic matter decomposition by microorganisms. Also, alfalfa introduction can alleviate nitrogen limitation in the early stages of the regeneration process when farmland activities are abandoned (Lan et al., 2020), supporting a long-term plant biomass input. As vegetation cover and biomass increase, and tillage decreases after abandonment, soil nutrient loss is minimized, and SOC is enhanced (Wang et al., 2018). On the other hand, the fresh organic matter inputs may also promote the decomposition of stable organic carbon and reduce SOC (Fontaine et al., 2007; Kuzyakov, 2010). In our field study site, farmland SOC levels are extremely low (Zhang et al., 2020), presenting an opportunity for rapid SOC improvement. It is noteworthy that

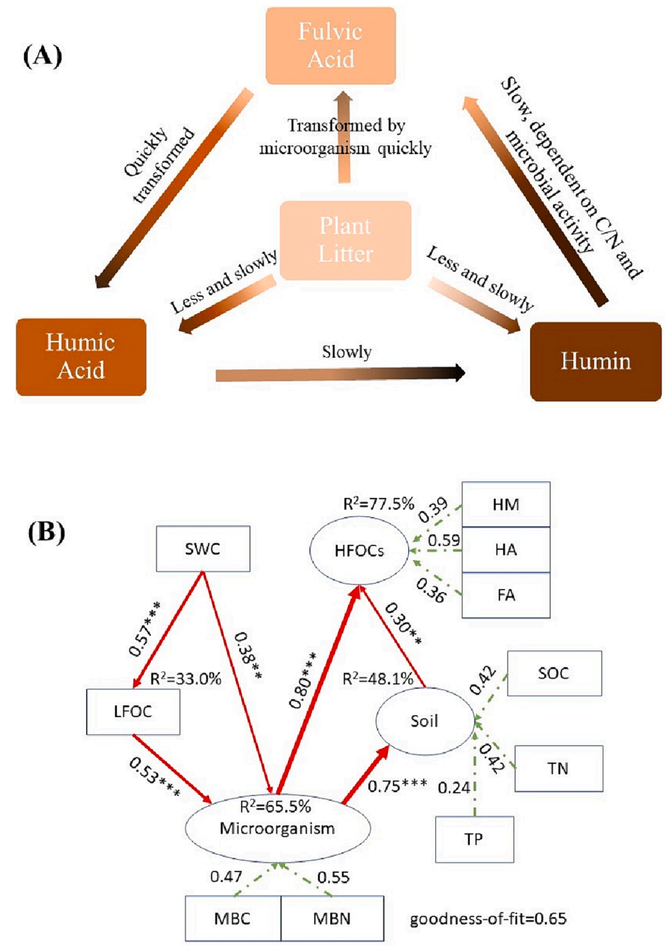

Fig. 5. **(A)** The transformation frame of HM, HA, and FA in HFOC (The darker the rectangle, the higher the degree of humification). **(B)** Partial least squares path modeling (PLSPM) indicated the relationship between soil conditions, microorganism condition, SWC (soil water content), LFOC (light fraction organic carbon) and HFOCs (heavy fraction organic carbon compounds). The circles and squares denote latent and observable variables, respectively. Green lines signify the correlation between latent variables and their associated observable variables, while red lines represent the causal paths. Numbers on the lines are the effects values. (MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, SOC: soil organic carbon, TN: total nitrogen, TP: total phosphorus, HFOC: heavy fraction organic carbon, HM: humin, HA: humic acid, FA: fulvic acid, (“**” and “***” besides lines represent significant difference at $P < 0.05$ and $P < 0.01$, respectively).

plant productivity in semi-arid regions is primarily limited by soil moisture. Alfalfa introduction reduced soil water storage of 1.4–5 m profile (Song et al., 2022), indicating deep water consumption for SOC sequestration. The trade-off between deep soil water storage and top SOC needs to be further explored.

Previous studies have shown that the proportion of soil macro-aggregates increases with revegetation on abandoned farmlands (Raiesi, 2012; Wei et al., 2013), and this increase correlates positively with SOC. With the revegetation of abandoned farmland and the reduction in tillage, along with increased plant litter and root secretions, soil macro-aggregate formation and SOC are favored. Our results from short-term experiments (5-year revegetation) demonstrate this result (Guo et al., 2010). However, after 15 years of revegetation, the long-term experiment results reversed. In 2017, compared to fallow and sweet clover fields, the percentage of soil macro-aggregates decreased while micro-aggregates increased in the alfalfa field, despite the SOC concentration remaining high (Fig. 2A, Fig. 3A). Alfalfa is a highly water-consuming species that produces a large number of roots to seek and absorb water in the semiarid region of the Loess Plateau (Song et al., 2021). Root secretions during this process can facilitate the formation of soil macro-aggregates (Dijkstra et al., 2021). However, root growth can also disrupt soil macro-aggregates and accelerate their turnover rate, leading to contradictory short- and long-term results (Dijkstra et al., 2021; Wang et al., 2020). This highlights the necessity of long-term experiments to study the mechanisms of soil carbon sequestration as they evolve over time. By encapsulating SOC in micro-aggregates and mixing it with inorganic matter after alfalfa introduction, it can be chemically protected from microbial decomposition, facilitating long-term storage.

The introduction of alfalfa increased the amount and proportion of humic acid (HA) in HFOC, indicating that HA plays a key role in improving HFOC (Fig. 4A, B). A study conducted near our experiment field in Ningxia, China, suggested that root cellulose drived soil carbon sequestration by enhancing fulvic acid (FA) during the restoration process (Bai et al., 2020a). This discrepancy may be attributed to differences in method selection for whole soil separation and HFOC separation. HFOC in our study site contains a significant proportion of insoluble HM (Fig. 4A), not consistent with other studies due to variations in environmental conditions (Bai et al., 2020a; Raiesi, 2021), suggesting the presence of organic-mineral complexes in alkaline soil environments. The association of SOC with mineral fractions provides chemical protection against biodegradation (Bruun et al., 2010; Six et al., 2002). We established a framework for the transformation process of FA, HA, and HM in HFOC (Fig. 5A). FA is abundant in plant inputs, including litter and roots (Bai et al., 2020a; Wang et al., 2022), and therefore, plant litter and root inputs can rapidly increase the concentration of FA, serving as a source of microbial energy. Through microbial decomposition, organic matter enters the soil and undergoes degradation. This decomposition eventually leads to the formation of HA and HM. The transformation of HA to HM is a slow process, resulting in the accumulation of HA and an increase in its amount and proportion. The analysis of partial least squares path modeling (PLSPM) indicated that soil conditions, microbial activities, and LFOC collectively influence HFOC composition (Fig. 5B). LFOC is directly related to plant inputs (Tan et al., 2007), and is mainly determined by soil moisture in our study site. Increased soil water content enhances productivity (0.57, $P < 0.01$) and microbial activity (0.38, $P < 0.05$), leading to higher LFOC. Microbial biomass carbon plays a crucial role in regulating carbon transformation among different pools, from labile to stabilizable (0.80, $P < 0.01$) (Bautista-Cruz et al., 2018; Six et al., 2006). The indirect effects of LFOC on microorganisms and microorganisms on HFOCs are 0.53 and 0.80, respectively ($P < 0.01$, Fig. 5B), indicating that LFOC indirectly affects HFOC through microorganisms. The soil conditions also had an impact on HFOC (0.30, $P < 0.05$). Therefore, the quality and transformation of soil organic matter in a specific area can be influenced by soil properties and environmental conditions (substrate quality). There may be an equilibrium point for stable SOC in certain regions, and this issue requires further exploration.

In our study, the concentrations and proportions of HA and HM increased as aggregate size decreased (Fig. 6). However, FA, which is more directly related to plant inputs, exhibited an opposite trend (Fig. 6). This results was supported by aggregate hierarchy model, plant inputs primarily contribute to carbon associated with macroaggregates and micro-aggregates can be bound together into macro-aggregates through organic binding agents derived from plants (Six et al., 2000; Six et al., 2004; Tisdall and Oades, 1982). Consequently, macro-aggregates contain a higher proportion of plant-derived carbon and are less stable compared to other soil aggregates with small sizes (Zhong et al., 2017). We clearly demonstrated deep humification of soil micro-aggregates with a higher HA/FA ratio (Fig. 3D). These finding further confirms the HFOC transformation depicted in Fig. 5A.

We integrated the relationship between HFOC and soil aggregates in Fig. 7. The introduction of alfalfa leads to an increase in plant detritus. The plant detritus contains a significant amount of FA (Bai et al., 2020a;

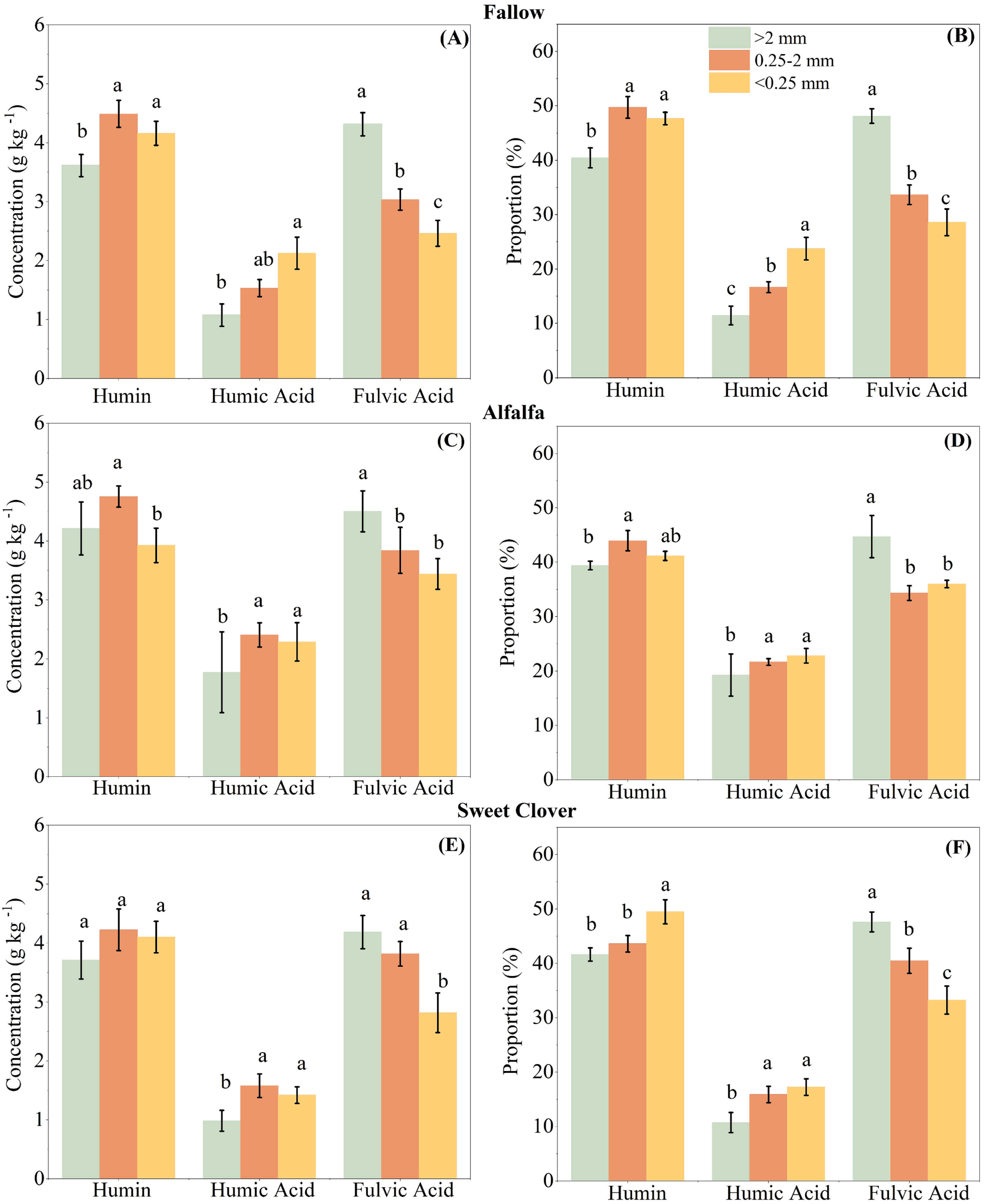

Fig. 6. The humin (HM), humic acid (HA), and fulvic acid (FA) concentrations and proportion in different size of soil aggregates of fallow (A and B), alfalfa (C and D), and sweet clover (E and F) fields in 2017. The lower-case letters indicate the significant difference at $P < 0.05$; bars represent the standard error, N = 9).

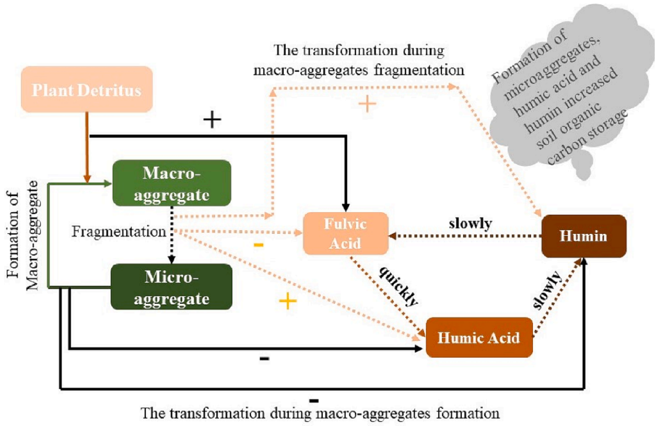

Fig. 7. The frame of humin, humic acid, and fulvic acid transformation during the soil aggregates formation and fragmentation (The path indicated by '+' signifies a promotional effect, whereas the '-' denotes a weakening effect. The dashed lines represent the fragmentation of soil aggregates; the solid lines represent the formation of soil aggregates).

Wang et al., 2022). Consequently, the input of plant detritus directly increased FA concentration. The abundant biomass benefits microbial activity, which can transform FA into HA and HM, stimulating HFOC transformations. Additionally, the plant detritus accelerates the turnover of soil aggregates, promoting the soil macro-aggregate formation. During the formation of macro-aggregates, the concentrations of HM and HA decrease, indicating that HM and HA may be the main compounds influencing the "priming effect". Moreover, the decrease in HM and HA concentrations could be attributed to the slow formation of HM within macro-aggregates, where gas exchange and oxygen penetration are more limited, and microbial communities are less diverse (Bach et al., 2018). On the other hand, during the process of micro-aggregate formation, the concentrations of HM and HA increase, while FA concentration and the degree of humification decrease, promoting the formation of a more stable SOC. These dynamics collectively contribute to long-term SOC storage.

5. Conclusion

The introduction of alfalfa on abandoned farmlands led to a significant increase in SOC, LFOC, and HFOC after being planted for 16 years. This improvement in HFOC was primarily attributed to the increase in the amount and proportion of HA. Additionally, alfalfa introduction increased the proportions of soil micro-aggregates, accompanied by an increase in the amount and proportion of HM and HA, while the particle size of soil aggregates decreased. Conversely, FA exhibited an opposite trend. The accumulation of HM and HA within soil micro-aggregates, which are highly humified, played a crucial role in enhancing HFOC. The input of plant detritus facilitated the turnover of soil aggregates, promoting the stabilization and renewal of different compounds within HFOC. The formation of soil micro-aggregates, along with the increase in soil HA and HM, contributed to the long-term storage of SOC following alfalfa introduction, which is beneficial to the sustainable development of semi-arid region and contributes to achieving net-zero emissions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CRediT authorship contribution statement

Xin Song: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Zi-Qiang Yuan:** Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Chao Fang:** Writing – original draft, Writing – review & editing. **Zhen-Hong Hu:** Writing – original draft, Writing – review & editing. **Feng-Min Li:** Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision. **Jordi Sardans:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Josep Penuelas:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization.

Declaration of competing interest

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

Data availability

Data will be made available on request. The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

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