4. Discussion

4.1 Effects of manure application on SOC fractions in greenhouse

Manure application in greenhouses significantly increased SOC content, playing a key role in enhancing soil fertility. However, with continuous application, SOC in loam stabilized after 20 years, whereas in sandy loam equilibrium was reached within only 2 years (Fig. 1). This stabilization likely reflects a new balance in SOC, where additional carbon inputs are offset by the higher mineralization rates characteristic of greenhouse soils (Stewart et al., 2007). The difference in equilibrium time can be attributed to soil texture: fine-textured soils (e.g., loam) provide more binding sites that enhance SOC stability and slow decomposition, while sandy loam, with limited binding capacity, undergoes faster SOC decomposition and therefore reaches equilibrium more rapidly (Wiesmeier et al., 2019; Mao et al., 2024).

Manure application significantly increased all SOC fractions, with MAOC emerging as the dominant stable pool (Figs. 2, 3), consistent with previous findings highlighting MAOC's central role in long-term carbon sequestration (Mustafa et al., 2022; Zhou et al., 2024). With prolonged manure application, EOC, cPOC, and fPOC and their relative proportions in SOC tended to stabilize (Fig. 2, 3). Under greenhouse conditions, manure-derived EOC and POC exhibit rapid microbial turnover, and with prolonged manure application their inputs and losses approach a dynamic balance, thereby stabilizing both their contents and their relative contributions to SOC (Zheng et al., 2022; Zhou et al., 2024). DOC and MBC accounted for less than 2.5% of SOC, showing no significant differences across application durations or soil textures, serving primarily as indicators of microbial metabolism rather than major carbon reservoirs (Francioli et al., 2016; Li et al., 2021; Yan et al., 2023). Loam exhibited higher levels of EOC, POC, and MAOC, with the increasing contribution of MAOC under prolonged manure application reflecting a greater availability of mineral binding and protective sites and, consequently, a superior capacity for organic matter accumulation and stabilization (Zhou et al., 2024; Ling et al., 2025). In contrast, sandy loam, with limited mineral protection, retained SOC mainly in particulate forms, resulting in a much lower sequestration potential, thereby explaining the contrasting SOC fraction distributions between the two soils.

4.2 Effects of manure application on SOC chemical composition

Manure application reshaped SOC chemical structures via texture-specific pathways. In the loam, the application of manure decreased the proportion of alkyl C and O-alkyl C in SOC, while increasing the proportions of aromatic C and carbonyl C (Fig. 5a, Table 2). O-alkyl C and alkyl C are more readily biodegraded than aromatic and carbonyl C and can be transformed into more stable carbonyl- and aromatic-enriched structures via microbial oxidation and free-radical reactions (Panettieri et al., 2014; Kubar et al., 2018; Lan et al., 2022). In loam, rapid and extensive depletion of labile O-alkyl and alkyl C during the initial stage of manure application temporarily shifted SOC composition toward less hydrophobic structures; however, as this selective decomposition persisted over successive microbial turnover cycles, the remaining SOC pool became progressively enriched in more hydrophobic and decomposition-resistant carbon (Xu et al., 2020), driving SOC composition toward greater stability and leading to a subsequent recovery of HI and A/OA (Fig. 5c). In sandy loam, the proportion of alkyl C gradually increased with prolonged manure application, whereas the proportions of O-alkyl C, aromatic C, and carbonyl C show an overall decreasing trend (Fig. 5b, Table 2). This is likely because of the lower content of clay and silt in the sandy loam, which reduces the physical protection of soil particles on SOC

(An et al., 2021; Yao et al., 2022). In sandy loam, manure application significantly increased HI and A/OA while decreasing AI (Fig. 5d), indicating that SOC stabilization was mainly associated with an increased contribution of alkyl C and enhanced hydrophobicity, rather than with the accumulation of chemically resistant aromatic C (Almendros and González-Pérez, 2025). These divergences reflected two stabilization mechanisms: mineral protection in loam (clay-silt adsorption promoting recalcitrant structure accumulation) and hydrophobic protection in sandy loam (alkyl C enrichment reducing microbial accessibility) (Almendros and González-Pérez, 2025; Yao et al., 2022). Greenhouse's warm and humid conditions accelerated labile carbon (O-alkyl C, alkyl C) decomposition, further amplifying texture-driven differences in SOC chemical composition (Tan et al., 2025).

4.3 Relationships between SOC fractions and stability-related indices

Mineral protection capacity determined the strength and direction of correlations between SOC fractions (Datta et al., 2017; Yan et al., 2023). In loam, MAOC showed significant positive correlations with EOC, cPOC, and fPOC (Fig. 6a). This pattern reflects the higher mineral protection capacity of loam, whereby labile carbon is retained during decomposition and can associate with clay and silt surfaces, ultimately facilitating MAOC formation (Six et al., 2002; Ji et al., 2024; Si et al., 2024). In contrast, the sandy loam, with its low clay and silt contents, exhibited a weaker SOC accumulation capacity, where SOC was positively correlated only with EOC and MAOC, but negatively correlated with fPOC (Fig. 4b). This finding differs from the results of Rocci et al. (2021), who reported that increases in both MAOC and POC directly contributed to SOC pool enhancement. However, in the sandy loam soil under greenhouse conditions, the negative correlation between POC and MAOC indicates that POC accumulation did not contribute to effective carbon stabilization but instead likely promoted MAOC loss through priming effects. This interpretation is consistent with previous studies showing that inputs of labile or low-quality organic carbon, particularly in soils with limited mineral protection or dominated by low-reactivity clays, can offset newly formed MAOC (Angst et al., 2023; Liang et al., 2023; Elias et al., 2024).

This study also found that the correlations between SOC fractions, functional groups, and stability indices differed significantly between loam and sandy loam (Fig. 6). Previous studies have shown that fine-textured soils preferentially retain microbially transformed and chemically restructured SOC (e.g., aromatic and carboxyl groups) within MAOC, whereas coarse-textured soils mainly preserve partially decomposed plant-derived residues with higher proportions of O-alkyl C (Jindaluang et al., 2013; Niu et al., 2024; Witzgall et al., 2021). In this study, EOC, POC, and MAOC in loam showed significant positive correlations with SOC stability indices such as AI and HI (Fig. 6a), indicating that even metabolically active fractions may contribute to long-term SOC stabilization through mineral associations when mineral adsorption sites are abundant. In contrast, the organic carbon fractions in sandy loam were mainly positively correlated with alkyl C, HI, and A/OA, while fPOC showed no significant relationships with SOC chemical structures or stability indices (Fig. 6b), indicating that these particulate organic carbons were not effectively adsorbed or protected. This finding aligns with Witzgall et al. (2021), who observed in controlled litter decomposition experiments that fine-textured soils preferentially retained microbially restructured SOC, whereas coarse-textured soils released more CO_2 and retained only limited plant-derived fragments.

4.4 Sequestration pathways of SOC in greenhouse

Across the two greenhouse , MAOC was the primary driver of SOC accumulation, but the contribution pathways of SLOC and POC differed markedly (Fig. 7). In loam, although EOC can be partially transformed into residues and incorporated into MAOC through microbial metabolism, its contribution is not significant. This does not imply the absence of such a process; rather, the abundant clay and mineral binding sites in loam make the microbial reprocessing and mineral association of POC the predominant sources of MAOC, thereby masking the effect of EOC incorporation (Liu et al., 2025; Zhou et al., 2024). In contrast, limited clay and sorption sites in sandy loam markedly reduce the conversion of POC into MAOC, leaving POC largely as particulate residues (Christy et al., 2023; Fohrafellner et al., 2024). This explains why there was no significant path detected between POC and MAOC in sandy loam, while POC still directly promoted SOC content through its own accumulation. Under such conditions, the molecular characteristics of EOC, such as small molecules rich in carboxyl and phenolic hydroxyl groups, enable rapid diffusion and preferential occupation of scarce mineral binding sites, making it a key precursor of MAOC (Christy et al., 2023). This is consistent with several studies reporting that in soils with low fine particle content, inputs of labile or soluble organic carbon contribute relatively more to the stable carbon pool (Peng et al., 2025; Simon et al., 2025). In summary, the results of this study indicate that the long-term stabilization of SOC in greenhouse soils is not the accumulation of a single carbon pool, but rather a synergistic process in which labile carbon fractions serve as substrates that are gradually incorporated into stable pools through microbial metabolism and transformation, thereby achieving long-term preservation.

The results of this study provide guidance for the management of manure application in greenhouse soils with different textures. It should be noted that this study did not include structural characterization of submicron-scale mineral–OC complexes (e.g., X-ray absorption fine structure spectroscopy), and therefore could not distinguish the differences among clay mineral types (e.g., montmorillonite vs. kaolinite) in the formation and stabilization of MAOC. In addition, this study primarily analyzed the relationship between SOC fractions and stabilization, lacking direct evidence on the microbial communities driving carbon transformation. Future studies could integrate metagenomic sequencing techniques to elucidate the community structure and metabolic traits of functional microorganisms that play key roles in carbon transformation across soils with different textures, thereby advancing the understanding of SOC stabilization processes in greenhouses through a multi-scale approach.