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RESEARCH ARTICLE

Chemical fertilizer and liming-induced changes in aluminum, iron oxides and soil organic carbon fractions: Implications for carbon sequestration in an upland red soil

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Highlights

- Lime application significantly increased dissolved organic carbon in acidic red soil.
- Liming decreased the predominance of free Al and Fe oxides in stabilizing soil organic carbon under acidic conditions.
- The soil carbon sequestration rate exhibited a negative correlation with easily oxidizable organic carbon with and without lime in red acidic soil.

Abstract

Lime application represents an established approach for ameliorating soil acidity, and understanding its effects on the interactions between aluminum (Al) and iron (Fe) oxides and soil organic carbon (SOC) fractions is essential for promoting sustainable agricultural practices that enhance carbon sequestration. This investigation examined the interactions among Al and Fe oxides and SOC fractions under long-term fertilization and liming. A long-term field experiment was implemented with five treatments: CK (no fertilizer), N (nitrogen fertilizer), NCa (N plus lime), NPK (nitrogen, phosphorus, and potassium fertilizer), and NPKCa (NPK plus lime). Soil samples were obtained from three depths: 0–10, 10–20, and 20–30 cm. The findings revealed that lime application increased SOC by 20.84% under the N treatment but decreased SOC by 9.97% under NPK. At the 0–10 cm depth, dissolved organic carbon (DOC) was substantially higher under NCa (410.51 mg kg^{-1}) and NPKCa (372.83 mg kg^{-1}) compared with CK. Particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) demonstrated consistent enhancement under NPK and NPKCa across all soil depths compared with CK. DOC exhibited significant positive correlations with both aluminum (Ald), reactive aluminum (Alo) and aluminum (Alp), indicating a key role of organically bound and reactive Al in carbon dynamics. Compared to the CK treatment, SOC stock increased significantly by 43.49% under NPK and by 36.82% under NPKCa. Structural equation modeling demonstrated that lime application mitigated the negative effects of free Al (Ald) on carbon sequestration, while Fe oxides (Fed) contributed positively to SOC stabilization. DOC showed no significant impact on carbon sequestration rate (CSR), while easily oxidizable carbon (EOC) negatively affected CSR directly. These results highlight the crucial role of lime in improving acidic soil conditions and enhancing the stability and sequestration of soil organic carbon.

Keywords: nitrogen fertilizer, lime, carbon fraction, carbon sequestration, aluminum and iron oxide

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1. Introduction

Red soils, encompassing approximately 2.18×10^6 km^2 across southern China, are highly weathered and derived from granite (Shi *et al.* 2010; Zhang *et al.* 2020). The region exhibits a warm, humid climate with abundant rainfall, frequent rainstorms, and extended precipitation periods. These tropical weathering conditions have facilitated the development of deep soil profiles, occasionally exceeding 50 m, composed primarily of residual granite material (Xue *et al.* 2019). Extensive weathering has resulted in the accumulation of aluminum and iron oxides, which constitute key components of these soils (Hong *et al.* 2024). The elevated concentrations of aluminum (Al) and iron (Fe) oxides serve a crucial function in soil organic matter dynamics through the formation of stable organo-mineral complexes. These complexes inhibit microbial decomposition of soil organic carbon (SOC), thereby enhancing its persistence. Kleber *et al.* (2021) demonstrated that Al and Fe oxides stabilize SOC through adsorption and chemical bonding, substantially reducing its vulnerability to microbial degradation. This stabilization mechanism is particularly significant for long-term carbon sequestration in red acidic soils, where mineralogical composition exerts a primary influence on organic matter stability (Bao *et al.* 2022).

SOC comprises various fractions that differ in stability and residence time within the soil matrix (Rahmati *et al.* 2020). These fractions can be differentiated using physical, chemical, and biological fractionation methods, each providing distinct insights into SOC dynamics. Physical fractionation separates SOC based on density, particle size, and spatial distribution, effectively distinguishing between labile (active) and stable (inactive) carbon pools (Ma *et al.* 2021). Chemical fractionation examines the solubility of organic matter, utilizing various reagents to separate SOC into components such as dissolved organic carbon (DOC), a biologically available fraction comprising organic acids, phenols, and carbohydrates. Hydrolysable organic carbon can be further subdivided into active and inactive pools using chemical reagents (Kurek *et al.* 2022). Advanced techniques including $KMnO_4$ oxidation or enzymatic oxidation enable differentiation between labile and recalcitrant organic carbon fractions (Li *et al.* 2023). Biological fractionation quantifies microbial biomass carbon (MBC) and assesses organic carbon bioavailability through mineralization processes, frequently utilizing carbon dioxide (CO_2) evolution as an indicator (Kandeler 2024). These biological measurements reflect the interaction between microbial communities and SOC components. Understanding the influence of different management practices on SOC fractions is essential for advancing soil carbon sequestration strategies (Six *et al.* 2002; Zhang *et al.* 2025). For instance, Mi *et al.* (2016) observed that straw and manure incorporation significantly increased particulate organic carbon (POC) and easily oxidizable carbon (EOC) compared to total SOC. While POC and DOC demonstrate greater susceptibility to rapid turnover and loss, mineral-associated organic carbon (MAOC) represents a more stable, long-term carbon sink, serving a vital role in sustained carbon sequestration (Si *et al.* 2024).

The application of chemical fertilizers represents a common practice for enhancing crop productivity (Yahaya *et al.* 2023). However, excessive or imbalanced nitrogen fertilizer use has resulted in widespread soil acidification, particularly in red soils with inherently low buffering capacity. A significant pH decline of approximately 0.5 units in major Chinese agricultural soils between the 1980s and 2000s illustrates the severity of acidification due to long-term N fertilization (Guo *et al.* 2010). As soil pH decreases, aluminum and iron solubility increases, destabilizing organo-mineral complexes and exposing SOC to microbial decomposition (Violante *et al.* 2005; Tamrat *et al.* 2019). This process contributes to SOC loss through several pathways, including aggregate disruption, desorption of MAOC, and enhanced microbial access to labile pools such as POC and DOC (Bailey *et al.* 2019). SOC destabilization denotes the transition of carbon from protected forms to more labile states, increasing its accessibility for microbial decomposition and eventual loss as CO_2. Several mechanisms contribute to this process: (1) physical disturbances, including tillage, freeze-thaw, and wetting-drying cycles, which disrupt soil aggregates and release occluded SOC; (2) chemical processes, comprising desorption and changes in pH or redox conditions, that enhance the bioavailability of MAOC and DOC; (3) enhanced pore connectivity, facilitating microbial access to POC and DOC; and (4) microbial factors, such as alterations in microbial activity or carbon use efficiency, which accelerate SOC decomposition into CO_2 rather than promoting its stabilization in microbial biomass (Bailey *et al.* 2019).

To mitigate acidification and restore soil fertility, liming practices — such as the application of calcium carbonate — are widely implemented in acid-prone agricultural regions (Thakuria *et al.* 2016; Butterly *et al.* 2022). By increasing soil pH and reducing Al toxicity, lime enhances plant growth and nutrient availability. Significantly, liming may influence SOC stability through modifying the interactions between organic matter and Fe/Al oxides. For example, Shen *et al.* (2024) demonstrated that lime application suppressed the dissolution of Fe and Al oxides, potentially enhancing SOC stabilization through organo-mineral complex formation. However, liming may also increase the solubility of certain labile carbon pools such as DOC, thereby affecting their contribution to long-term carbon sequestration (Rowley *et al.* 2018). These contrasting effects emphasize the importance of better understanding how liming modulates SOC fractions and their interactions with mineral phases, particularly in highly weathered red acidic soils.

Although SOC storage plays a crucial role in mitigating climate change, understanding of how imbalanced applications of chemical fertilizers and lime influence SOC fractions in red acidic soils remains limited. Previous research has established the stabilizing role of aluminum and iron oxides in SOC dynamics (Cloy *et al.* 2014; Yu *et al.* 2019; Song *et al.* 2022). However, the specific interactions between these oxides and distinct SOC fractions under varying soil management practices require further investigation. Additionally, the combined effects of unbalanced fertilization and liming on these interactions have not been systematically

examined. Addressing these knowledge gaps is essential for advancing our understanding of SOC stabilization mechanisms in red acid soils and for optimizing sustainable land management strategies. This study aims to evaluate the influence of chemical fertilizer and lime applications on Al and Fe oxides, SOC fractions, and carbon sequestration in highly weathered acidic red soils. The specific objectives are to: (1) determine the distribution of SOC fractions under different fertilization and liming treatments, (2) investigate the interactions between Al and Fe oxides and SOC fractions, and (3) assess how these interactions affect SOC sequestration potential. We hypothesize that lime application increases soil pH, reduces exchangeable aluminum, and promotes the formation of stable aluminum and iron oxides, thereby enhancing SOC stabilization in acidic upland soils. Furthermore, labile fractions such as POC and DOC contribute less to long-term carbon storage due to rapid microbial decomposition, highlighting the critical role of soil pH and aluminum/iron oxides in regulating SOC dynamics in acidic upland environments. This study provides valuable insights into how long-term chemical fertilization and liming influence the interactions among soil pH, aluminum and iron oxide fractions, and SOC fractions, thereby affecting carbon sequestration in acidic soils. These findings contribute to a deeper understanding of SOC stabilization mechanisms under red acidic soil.

2. Materials and methods

2.1. Experimental site and Soil samples

The soil variables presented in Table 1 were obtained from a long-term field fertilization experiment conducted at the Chinese National Observation and Research Station in Qiyang, Hunan Province, China. Five treatments were selected for this study: CK (no fertilizer), N (nitrogen only), NCa (nitrogen plus lime), NPK (nitrogen, phosphorus, and potassium), and NPKCa (NPK plus lime). These treatments were arranged in a completely randomized block design and maintained continuously for 13 years. Fertilizer application rates were established at 300 kg N yr^{-1}, 120 kg P_2O_5 yr^{-1}, and 120 kg K_2O ha^{-1} yr^{-1}. Fertilizers were applied directly into the planting rows during crop growing seasons, with an annual distribution of 30% for wheat and 70% for maize. Urea, calcium superphosphate, and potassium chloride served as the sources of nitrogen, phosphorus, and potassium, respectively. The lime application rate for each treatment was determined based on the difference between the soil pH after long-term fertilization and the initial baseline pH. Quicklime (CaO) was used as the liming material and incorporated uniformly into the 0–20 cm soil layer at rates of 2,550 kg ha^{-1} in 2010, 1,500 kg ha^{-1} in 2014, and 1,500 kg ha^{-1} in 2022. Soil samples were collected in 2023 following the maize harvest, using an auger at three depths: 0–10, 10–20, and 20–30 cm.

Table 1 Location, climate conditions and initial surface soil properties of the long-term experimental site

Parameter[1)]	Measurements
Initiation year	1990
Year of lime application	2010, 2014 and 2022[2)]
Latitude (N)	26°46´´
Longitude (E)	112°53´´
Climate	Subtropical monsoon
Mean annual temperature (°C)	17.8
Mean annual precipitation (mm)	1,430
Cropping system	Wheat–maize
Soil texture	Clay
Soil pH	5.69
SOC (g kg^{-1})	13.52
TN (g kg^{-1})	1.09
AN (mg kg^{-1})	82.03
TP (g kg^{-1})	0.47
AP (mg kg^{-1})	14.21
TK (g kg^{-1})	14.2
AK (mg kg^{-1})	103.21

[1)] SOC, soil organic carbon; TN, total nitrogen; AN, available nitrogen; TP, total phosphorus; AP, available phosphorus; TK, total potassium; AK, available potassium.
[2)] 2,550 kg ha^{-1} for 2010; 1,500 kg ha^{-1} for 2014; 1,500 kg ha^{-1} for 2022.

2.2. Soil chemical analysis

Soil pH was measured using a glass electrode in a soil-to-water suspension ratio of 1:2.5. Soil organic matter content was determined using the potassium dichromate ($K_2Cr_2O_7$) oxidation method, following the procedure described by Nelson and Sommers (1983). Total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were analyzed according to the methods outlined by Lu (1999). Available phosphorus was determined using the Olsen method (Olsen 1954), while available potassium was extracted with ammonium acetate (CH_3COONH_4) and measured by flame photometry, as described by Page (1982). Soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were determined by extracting fresh soil samples with 1 mol L^{-1} KCl (soil-to-solution ratio of 1:5). The suspension underwent shaking for 60 min and filtration through Whatman No. 40 filter paper. The filtrates were analyzed for NO_3^- and NH_4^+ using a flow injection analyzer (Seal AutoAnalyzer, Germany). Exchangeable calcium (Ca^{2+}) and magnesium (Mg^{2+}) were extracted with 1 mol L^{-1} ammonium acetate (pH 7) and measured using atomic absorption spectroscopy. Exchangeable aluminum (Al^{3+}) was extracted with 0.1 mol L^{-1} barium chloride ($BaCl_2$) and quantified *via* atomic absorption spectroscopy.

2.3. Determination of aluminum and iron oxides

Air-dried soil samples were passed through a 0.25-mm sieve prior to the extraction of aluminum and iron fractions. Free iron and aluminum oxides were extracted using the dithionite citrate–bicarbonate method, following the procedures described by Mehra (1960) and McKeague and Day (1966). For the extraction of organically complexed Al/Fe, 1 g of soil was combined with 100 mL of 0.1 mol L^{-1} sodium pyrophosphate solution (pH 10), shaken for 16 h, then centrifuged at 2,500×g for 10 min. The supernatant underwent filtration through a 0.45-μm membrane filter (Bottinelli *et al.* 2017). Reactive Al and Fe, including non-crystalline and organically bound forms, were extracted by combining 1 g of soil with 100 mL of 0.2 mol L^{-1} oxalic acid–ammonium oxalate solution (pH 3). The mixture underwent

shaking in the dark for 4 h, centrifugation at 2,500×g for 15 min, and filtration through a 0.45-μm membrane (Vaezi *et al.* 2017). The concentrations of Al and Fe in all extracts were determined using inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 7000, Perkin-Elmer). Calibration standards were prepared through serial dilution of a stock standard solution containing 1,000 mol L^{-1} of each element. The instrument operated under the following conditions: radio frequency power of 1.3 kW, plasma gas flow rate of 15.0 L min^{-1}, auxiliary gas flow rate of 1.5 L min^{-1}, and nebulizer gas flow rate of 0.7 L min^{-1} (Wang *et al.* 2019).

2.4. SOC fractions

Soil MBC was determined using the fumigation-extraction method as described by Wu *et al.* (1990). In this procedure, 12.5 g of fresh soil underwent immediate extraction with 0.05 mol L^{-1} K_2SO_4 solution, while a paired sample was fumigated with chloroform in the dark for 24 h prior to extraction. The K_2SO_4 extracts were analyzed using a TOC/TN analyzer (multi-C/N 3100, Analytik Jena AG, Germany), and MBC was calculated based on the difference in carbon concentration between fumigated and non-fumigated extracts.

POC and MAOC fractions were determined following the wet-sieving procedure described by Marriott and Wander (2006). For each sample, 20 g of air-dried soil was suspended in 100 mL of 5% (w/v) sodium hexametaphosphate solution and shaken at 180 r min^{-1} for 18 h. The suspension underwent wet-sieving using a 0.053 mm mesh sieve. The fraction retained on the sieve (>0.053 mm) was dried at 60°C and weighed to determine POC content, while the fraction passing through the sieve (<0.053 mm) was dried and weighed to quantify MAOC. Subsequently, the organic carbon content in each fraction was analyzed using an elemental analyzer (EA3100, Euro Vector, Milan, Italy). Easily oxidizable organic carbon was determined using the potassium permanganate ($KMnO_4$) oxidation method described by Weil *et al.* (2003), based on the dithionite-citrate-bicarbonate procedure of McKeague and Day (1966). Specifically, 2.5 g of air-dried soil was reacted with 2 mL of $KMnO_4$ solution for 1 h, and the EOC content was quantified spectrophotometrically by measuring the reduction of $KMnO_4$.

2.5. Calculations

Free non-crystalline Al and Fe were calculated by:

$$\text{Free non-crystalline Al (Alnc)} = \text{Alo} - \text{Alp} \quad (1)$$

$$\text{Free non-crystalline Fe (Fenc)} = \text{Feo} - \text{Fep} \quad (2)$$

Free crystalline Al and Fe were calculated as:

$$\text{Free crystalline Al (Alc)} = \text{Ald} - \text{Alo} \quad (3)$$

$$\text{Free crystalline Fe (Fec)} = \text{Fed} - \text{Feo} \quad (4)$$

where Alo and Feo represent reactive Al and Fe oxides, respectively; Alp and Fep represent organically bound Al and Fe, respectively; and Ald and Fed represent total free Al and Fe oxides, respectively. All Al and Fe oxides are expressed in mg kg^{-1} (Wang *et al.* 2019; Ren *et al.* 2025).

SOC stocks (t ha^{-1}) were estimated using the following equation:

$$\text{SOC stock} = C \times BD \times H/10 \quad (5)$$

where C is soil C content (g kg^{-1}), BD is soil bulk density (g cm^{-3}), and H is soil depth (cm). carbon sequestration rate (CSR) (t ha^{-1} yr^{-1}) was calculated using the following equation (Zhang *et al.* 2012):

$$CSR = (SOC_t - SOC_0)/T \quad (6)$$

where SOC_t and SOC_0 represent the stock of OC (t ha^{-1}) at time t and in the initial year (2010), respectively. T represents the duration of the experiment (13 years).

2.6. Statistical analysis

Data analysis was conducted using SPSS version 20 (SPSS Inc., USA). The normality of the data was verified prior to analysis. Analysis of variance (ANOVA) was performed to assess differences in soil indicators among fertilization treatments. Correlation analyses examining the relationships between aluminum and iron oxides and carbon fractions were conducted using OriginPro 2021. To evaluate the relative importance of soil properties influencing SOC sequestration rate and SOC stock, boosted regression tree models were developed using the rfPermute package in R version 4.3.3 (Elith *et al.* 2008). A partial least squares structural equation model (PLS-SEM) was constructed using SmartPLS 4 to elucidate the direct and indirect effects of key variables on SOC. The variables were selected based on the study's core hypothesis and supported by preliminary correlation and multicollinearity tests, ensuring the statistical robustness and clarity of the model.

3. Results

3.1. Effect of lime and chemical fertilizer on soil pH and SOC

Nitrogen fertilization significantly decreased soil pH compared to the control treatment (Table 2). At soil depths of 0–10, 10–20, and 20–30 cm, the N treatment showed the lowest pH values of 3.69, 3.80, and 3.97, respectively. In contrast, lime application substantially increased soil pH across all depths ($P \leq 0.05$) (Table 2). The NCa treatment elevated soil pH by 1.92, 1.93, and 1.14 units at 0–10, 10–20, and 20–30 cm depths, respectively. Similarly, the NPKCa treatment increased pH by 0.74, 0.31, and 0.18 across these depths. Lime addition also significantly affected exchangeable cations, including magnesium (Ex. Mg^{2+}), calcium (Ex. Mg^{2+}), hydrogen (Ex. H^+), and aluminum (Ex. Al^{3+}) (Table 3). The nitrogen-only treatment demonstrated elevated levels of Ex. Al^{3+} and Ex. H^+ but the lowest concentrations of Ex. Mg^{2+} and Ex. Mg^{2+}.

3.2. Effects of lime and chemical fertilizer application on Al and Fe oxides

The aluminum and iron oxide fractions (Ald, Alo, Alp, Alnc, Alc, Fed, Feo, Fep, Fenc, and Fec) demonstrated consistent distribution patterns across soil depths and treatments. The concentrations of iron fractions consistently exceeded their corresponding aluminum fractions across all extraction methods. Under the N treatment, Ald concentrations measured 5.13, 5.46, and 5.30 g kg^{-1} at the respective depths, while Fed levels were higher at 30.48, 31.76, and 30.15 g kg^{-1}. Similarly, under the NCa treatment, Ald

Table 2 Soil chemical properties in different treatments[1)]

Treatments[2)]	Depth (cm)	pH	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})
CK	0–10	5.83±0.10 Ab	7.07±0.61 Da	0.82±0.04 Ea	0.46±0.05 Ca	15.63±0.48 Aa
	10–20	6.12±0.03 Aa	5.14±0.70 Cb	0.67±0.07 Cb	0.27±0.01 Cb	13.69±0.45 Ab
	20–30	6.24±0.14 Aa	4.64±0.33 Cb	0.61±0.10 Bb	0.27±0.05 Cb	15.36±2.93 Ca
N	0–10	3.69±0.02 Dc	6.67±0.14 Da	0.96±0.01 Da	0.22±0.01 Da	15.12±0.74 Ba
	10–20	3.80±0.07 Eb	6.64±0.03 Ba	0.85±0.01 Bb	0.22±0.02 Ca	13.74±0.56 Aa
	20–30	3.97±0.01 Da	5.25±0.05 BCb	0.68±0.03 Bc	0.20±0.03 Da	17.03±1.24 Ca
NCa	0–10	5.61±0.18 Aa	8.06±0.87 Ca	1.07±0.01 Ca	0.24±0.01 Da	13.11±1.07 Ba
	10–20	5.73±0.06 Ba	7.65±0.25 ABa	0.97±0.02 Ab	0.23±0.01 Ca	12.62±0.71 Aa
	20–30	5.11±0.06 Bb	5.10±0.05 BCb	0.67±0.01 Bc	0.21±0.03 CDa	15.44±0.15 Ca
NPK	0–10	4.55±0.21 Cb	11.33±0.21 Aa	1.27±0.03 Aa	1.09±0.01 Ba	13.03±0.25 Aa
	10–20	4.59±0.19 Dab	8.81±0.04 Ab	1.02±0.01 Ab	0.85±0.03 Bb	12.55±0.49 Aa
	20–30	4.92±0.02 Ca	5.38±0.58 Bc	0.70±0.00 Bc	0.74±0.04 Bc	14.26±3.11 Aa
NPKCa	0–10	5.29±0.14 Ba	10.20±0.43 Ba	1.17±0.04 Ba	1.38±0.09 Aa	13.56±0.62 Aa
	10–20	4.90±0.05 Cb	8.60±1.23 Ab	1.02±0.07 Ab	1.09±0.09 Ab	16.10±0.77 Aa
	20–30	5.10±0.14 Cab	7.94±0.42 Ab	0.88±0.07 Ac	1.02±0.02 Ac	15.38±0.10 Ba

[1)] SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium.
[2)] CK, no fertilizer; N, chemical nitrogen fertilizer; NCa, chemical nitrogen fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime.
The data presented in the table represent the mean±standard error (n=3). Different capital letters indicate significant differences between treatments at the same soil depth, while different lowercase letters indicate significant differences across soil depths within the same treatment (P≤0.05).

Table 3 Soil acidity variables in different treatments[1)]

Treatments[2)]	Depth (cm)	Ex. acidity (cmol kg^{-1})	Ex. Al^{3+} (cmol kg^{-1})	Ex. H^{+} (cmol kg^{-1})	Ex. Ca^{2+} (cmol kg^{-1})	Ex. Mg^{2+} (cmol kg^{-1})
CK	0–10	0.14±0.00 Eab	0.05±0.03 Eb	0.07±0.07 Cab	11.44±0.27 Ba	7.16±0.21 Ba
	10–20	0.10±0.00 Eb	0.02±0.01 Eb	0.05±0.00 Db	12.13±0.44 Aa	7.69±0.34 Ba
	20–30	0.19±0.03 Da	0.12±0.03 Da	0.10±0.02 Da	11.89±1.16 Aa	7.51±0.91 Aa
N	0–10	6.01±0.04 Ab	5.50±0.07 Ab	0.52±0.03 Aa	4.23±0.18 Da	0.42±0.14 Da
	10–20	6.37±0.06 Aa	5.87±0.09 Aa	0.50±0.04 Aa	3.96±0.03 Db	0.22±0.03 Eb
	20–30	5.86±0.15 Ab	5.44±0.15 Ab	0.42±0.03 Ab	4.02±0.00 Db	0.26±0.00 Db
NCa	0–10	1.18±0.18 Db	1.00±0.16 Db	0.17±0.03 Cb	12.54±0.09 Ba	6.91±0.07 Ba
	10–20	1.25±0.30 Db	1.06±0.25 Db	0.19±0.05 Cb	10.29±0.16 Bb	5.15±0.12 Db
	20–30	3.08±0.12 BCa	2.80±0.11 BCa	0.28±0.02 Ba	7.04±0.06 Cc	2.62±0.05 Cc
NPK	0–10	4.21±0.25 Ba	3.90±0.16 Ba	0.31±010 Ba	7.36±0.17 Ca	5.74±0.13 Ca
	10–20	4.46±0.69 Ba	4.16±0.65 Ba	0.31±0.05 Ba	7.39±0.73 Ca	5.77±0.57 Ca
	20–30	3.85±0.94 BCa	3.59±0.87 BCa	0.27±0.07 BCa	6.73±1.22 Ca	5.24±0.95 Ba
NPKCa	0–10	1.68±0.03 Cb	1.50±0.02 Cb	0.17±0.04 Ca	12.20±0.25 Aa	9.52±0.20 Aa
	10–20	2.68±0.28 Ca	2.49±0.27 Ca	0.19±0.04 Ca	10.75±0.12 ABb	8.38±0.09 Ab
	20–30	2.36±0.40 Ca	2.15±0.43 Ca	0.20±0.03 Ca	9.65±0.30 Bc	7.53±0.24 Ac

[1)] Ex. acidity, exchangeable acidity; Ex. Al^{3+}, exchangeable aluminum; Ex. H^{+}, exchangeable hydrogen; Ex. Ca^{2+}, exchangeable calcium; Ex. Mg^{2+}, exchangeable magnesium.
[2)] CK, no fertilizer; N, chemical nitrogen fertilizer; NCa, chemical nitrogen fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime.
The data presented in the table represent the mean±standard error (n=3). Different capital letters indicate significant differences between treatments at the same soil depth, while different lowercase letters indicate significant differences across soil depths within the same treatment (P≤0.05).

concentrations were 4.45, 4.66, and 5.23 g kg^{-1}, while Fed remained elevated at 29.38, 29.48, and 28.63 g kg^{-1}. NPK fertilizer application further enhanced both Ald and Fed concentrations, even with lime application. In lime-treated soils, aluminum concentrations decreased compared to the control. Significant differences (P≤0.05) emerged for Alo and Feo fractions across all fertilization treatments N, NCa, NPK, and NPKCa (Figs. 1-B and 2-B), though no significant differences appeared between these treatments. For Alp (Fig. 1-C), concentrations were higher in the N treatment compared to NCa, with a significant difference observed only between NPK with and without lime at the 0–10 cm depth. Similarly, for Fep (Fig. 2-C), lime treatments, regardless of fertilizer integration, yielded higher concentrations than the control, which exhibited the lowest levels. A paired sample t-test evaluated the impact of lime application on aluminum and iron oxides in the N treatment (Fig. 3-A and B). However, lime application did not significantly affect aluminum oxides in the NPKCa treatment (Fig. 3-C and D). For iron oxides, significant effects appeared only in the Fed and Fec fractions.

3.3. Effect of lime and fertilizer application on carbon fractions and carbon sequestration

The effect of lime on SOC varied depending on the fertilizer regime (Table 2). At 0–10 cm depth, SOC increased by 20.84% under the NCa treatment compared to nitrogen alone, with values rising from 6.67 to 8.06 g kg^{-1}. Conversely,

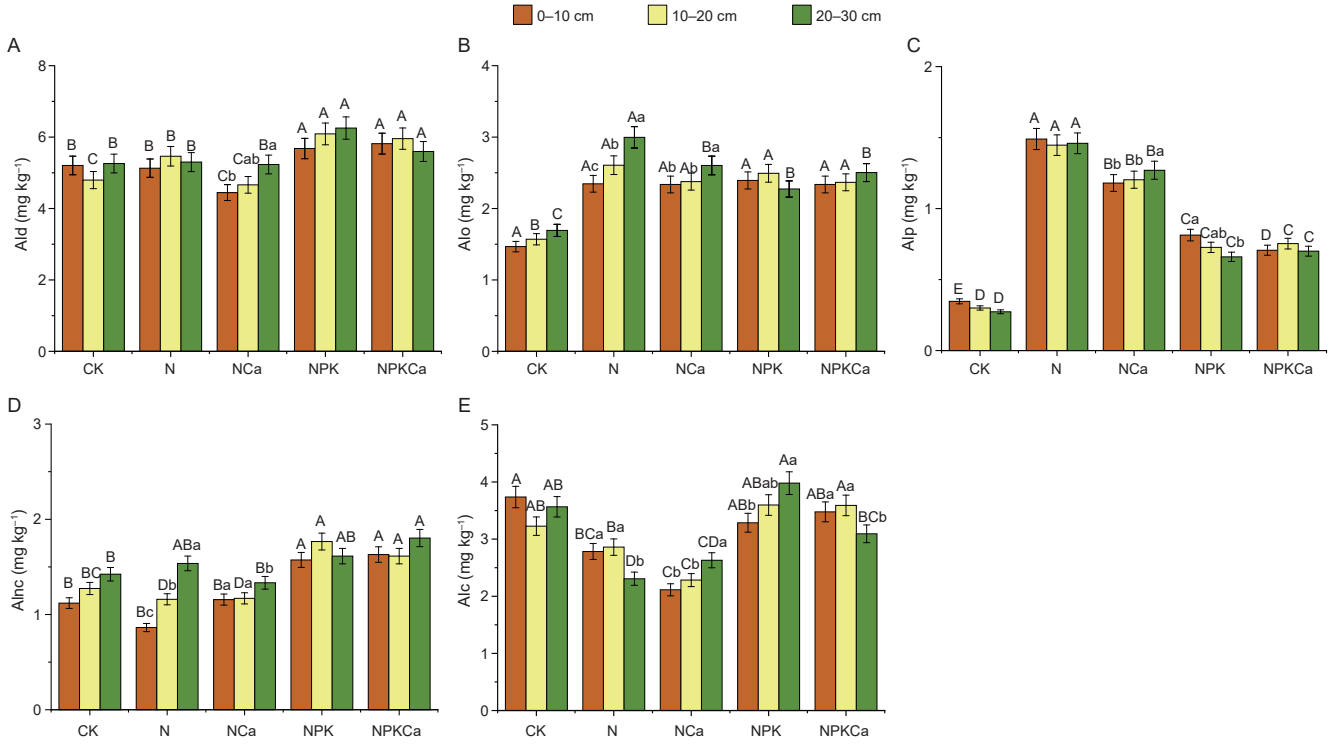


Fig. 1 Effect of long-term fertilization on aluminum fractions in 0–10, 10–20, and 20–30 cm soil depths. A, total free aluminum (Ald). B, reactive aluminum (Alo). C, organic bounded aluminum (Alp). D, non-crystalline aluminum (Alnc). E, crystalline aluminum (Alc). CK, no fertilizer; N, chemical nitrogen fertilizer; NCa, chemical nitrogen fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime. Different capital letters indicate significant differences between treatments at the same soil depth, while different lowercase letters indicate significant differences across soil depths within the same treatment ($P \leq 0.05$).

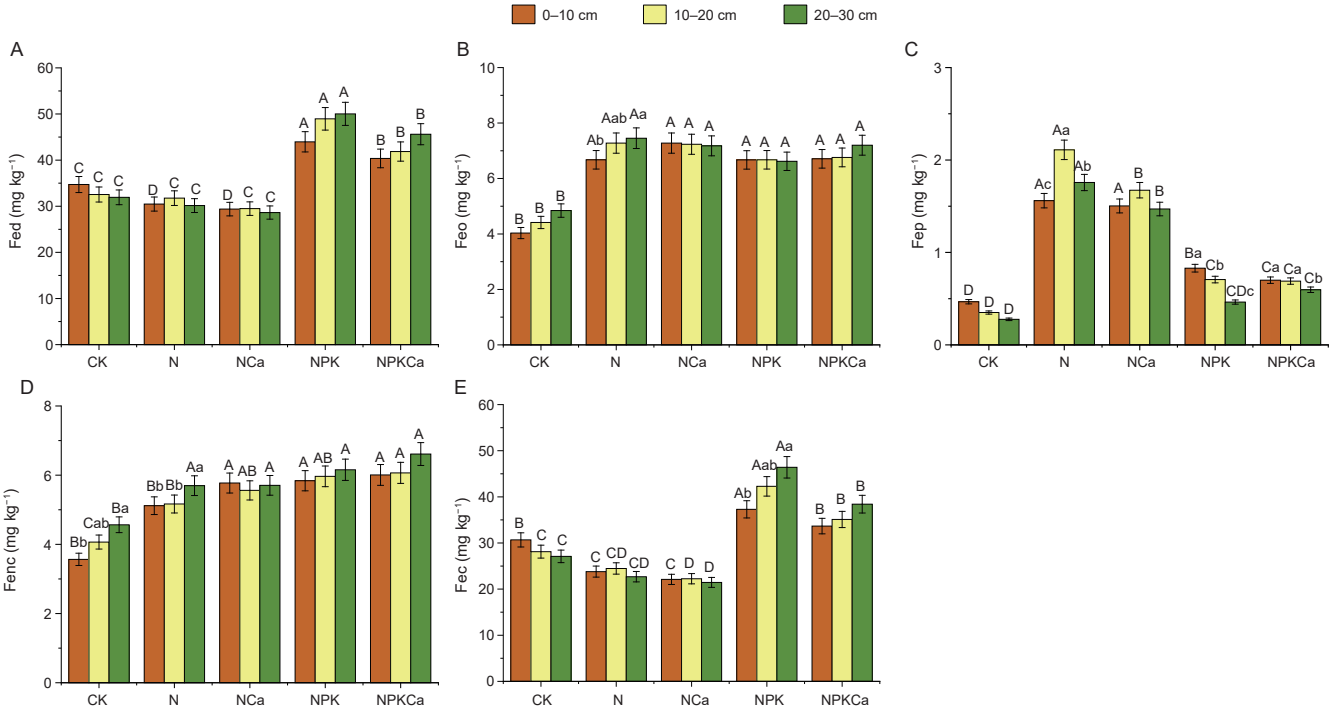


Fig. 2 Effect of long-term fertilization on iron fractions in 0–10, 10–20, and 20–30 cm soil depths. A, total free iron (Fed). B, reactive iron (Feo). C, organic bounded iron (Fep). D, non-crystalline iron (Fenc). E, crystalline iron (Fec). CK, no fertilizer; N, chemical nitrogen fertilizer; NCa, chemical nitrogen fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime. Different capital letters indicate significant differences between treatments at the same soil depth, while different lowercase letters indicate significant differences across soil depths within the same treatment ($P \leq 0.05$).

the NPKCa treatment led to a 9.97% decrease in SOC compared to NPK, with concentrations of 10.20 and 11.33 g kg^{-1}, respectively.

SOC fractions demonstrated significant changes following 13 years of lime application. DOC increased significantly compared to the control ($P \leq 0.05$) (Fig. 4-A), reaching concentrations of 410.51 mg kg^{-1} under NCa treatment and 372.83 mg kg^{-1} under NPKCa at 0–10 cm soil depth. MBC showed significant enhancement with full NPK fertilizer application, both with and without lime, measuring 264.77, 196.52, and 166.41 mg kg^{-1} at depths of 0–10, 10–20, and 20–30 cm, respectively. The NPKCa treatment yielded MBC values of 224.21, 166.94, and 157.35 mg kg^{-1} across these depths (Fig. 4-B). The N treatment produced the lowest MBC concentrations, measuring 51.93, 21.05, and 9.79 mg kg^{-1} at 0–10, 10–20, and 20–30 cm depths, respectively. For easily oxidizable carbon, while no significant differences emerged among CK, N, NPK, and NPKCa treatments (Fig. 4-C), NCa

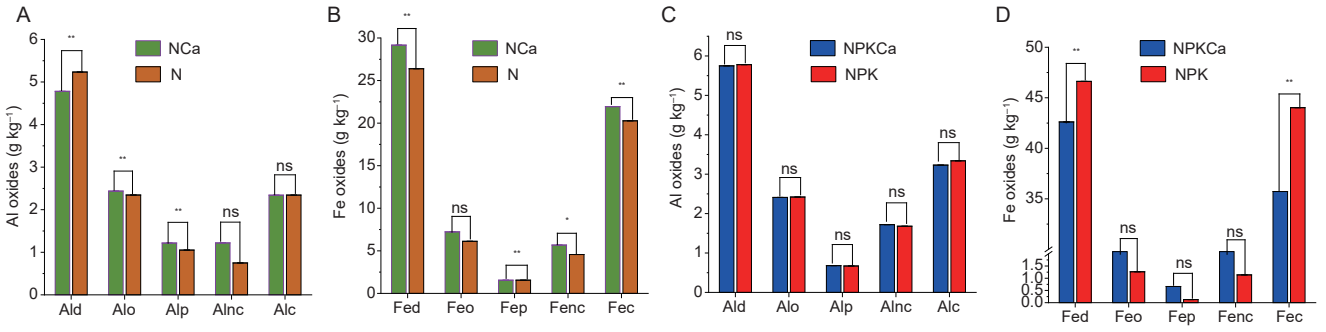


Fig. 3 Paired samples *t*-test analysis for the effects of lime application on Al and Fe oxides. A and B, effects of lime application on Al (A) and Fe (B) oxides under N treatment. C and D, effects of lime application on Al (C) and Fe (D) oxides under NPK treatment. NCa, chemical nitrogen fertilizer plus lime; N, chemical nitrogen fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer. Ald, total free aluminum; Alo, reactive aluminum; Alp, organically bound aluminum; Alnc, non-crystalline aluminum; Alc, crystalline aluminum; Fed, total free aluminum; Feo, reactive aluminum; Fep, organically bound aluminum; Fenc, non-crystalline aluminum; Fec, crystalline aluminum. The Fe and Al oxide contents are mean values across the 0–10, 10–20, and 20–30 cm soil layers. *, $P \leq 0.05$; **, $P \leq 0.01$; ns, no significance.

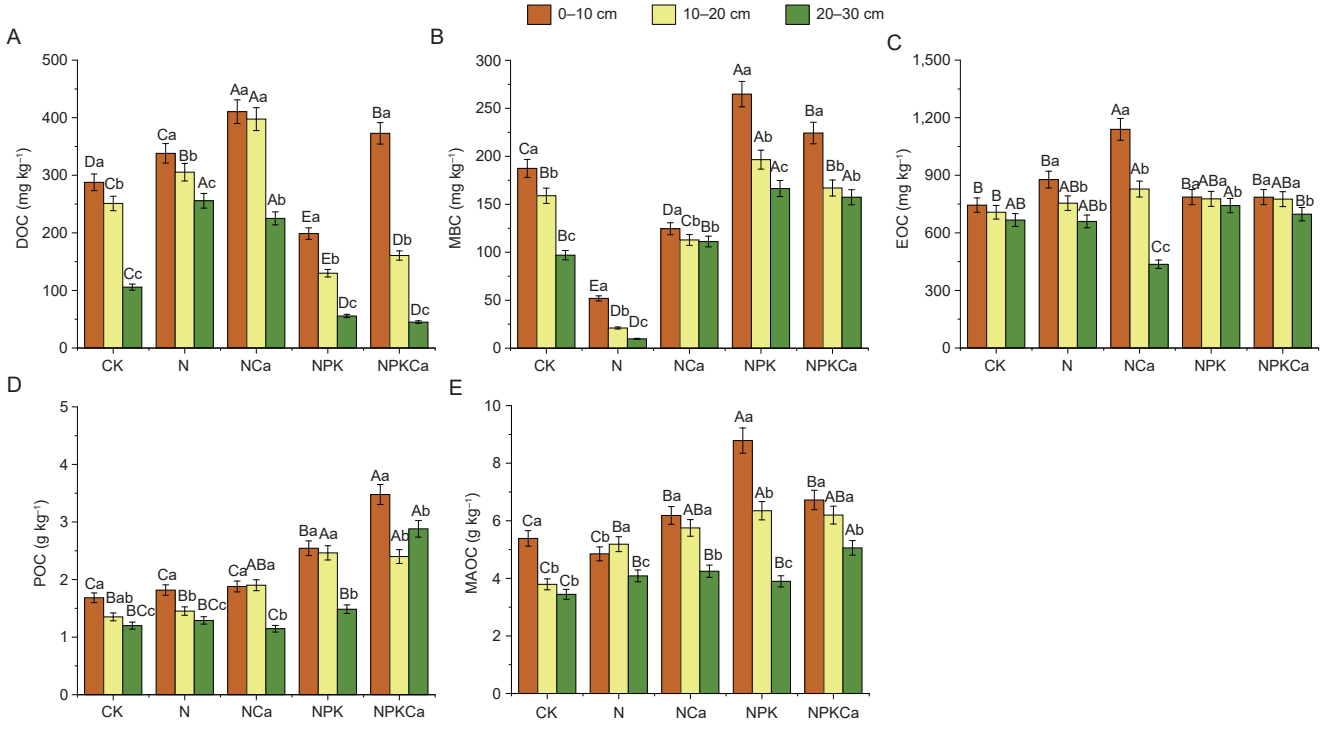


Fig. 4 Effect of long-term fertilization on soil organic carbon fractions in 0–10, 10–20, and 20–30 cm soil depths. A, dissolved organic carbon (DOC). B, microbial biomass carbon (MBC). C, easily oxidizable organic carbon (EOC). D, particulate organic carbon (POC). E, mineral-associated organic carbon (MAOC). CK, no fertilizer; N, chemical nitrogen fertilizer; NCa, chemical nitrogen fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime. Different capital letters indicate significant differences between treatments at the same soil depth, while different lowercase letters indicate significant differences across soil depths within the same treatment ($P \leq 0.05$).

significantly exceeded the CK, displaying peak values of 1,139.49 and 828.33 mg kg^{-1} at 0–10 and 10–20 cm depths, respectively. Both POC and MAOC levels increased under NPK treatments, regardless of lime application, across all examined soil depths (0–10, 10–20, and 20–30 cm) (Fig. 4-C and D).

Paired samples *t*-test analysis indicated significant variations in DOC, MBC, and MAOC under the N treatment with and without lime application (P≤0.05) (Fig. 5-A). Under NPK treatment, lime application significantly affected only MBC, with other carbon fractions showing no significant differences (Fig. 5-B). Regarding SOC stock, NPK treatments with and without lime increased SOC stock by 43.49 and 36.82%, respectively, relative to the control (Table 4). The N treatment increased SOC stock by 7.75 t ha^{-1} in the top 10 cm, while NCa enhanced it further by 28.26% to 9.94 t ha^{-1}. However, NPK treatments with and without lime showed no significant differences in SOC stock at this depth. NPK application achieved the highest CSR among all treatments, reaching 0.07 t ha^{-1} yr^{-1} in the 0–10 cm soil depth. Other treatments exhibited negative CSR values, indicating net carbon loss. While lime addition to NPK treatment reduced CSR, lime application alone enhanced carbon retention, with CSR values of −0.12 t ha^{-1} yr^{-1} for N and −0.01 t ha^{-1} yr^{-1} for NCa (Table 4). Carbon sequestration rates were negative at 10–20 and 20–30 cm depths for most treatments, except for NPKCa at 20–30 cm, which showed positive sequestration.

3.4. Relationship between SOC fraction and Al and Fe oxides

MAOC, as shown in Fig. 6-A, demonstrated a strong positive correlation with Alo and Feo. DOC exhibited significant positive relationships with both Alo and Alp, while EOC correlated positively with Fed and Feo. MBC showed positive associations with Alnc and Fep. Conversely, MAOC displayed a significant negative correlation with Alc. POC exhibited negative correlations with Alc and Fec. At

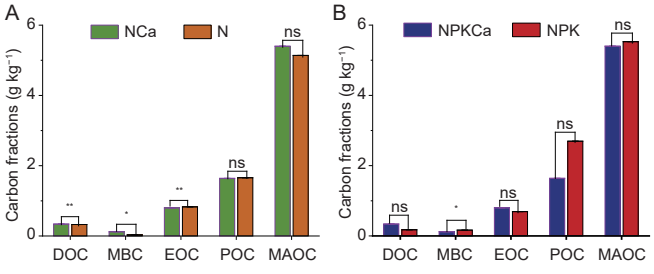


Fig. 5 Paired samples *t*-test analysis for the effects of lime application on soil organic carbon fractions. A, effects of lime application on soil organic carbon fractions under N treatment. B, effects of lime application on soil organic carbon fractions under NPK treatment. N, chemical nitrogen fertilizer; NCa, chemical nitrogen fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime. DOC, dissolved organic carbon; MBC, microbial biomass carbon; EOC, easily oxidizable organic carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon. The carbon content of different fractions are mean values across the 0–10, 10–20, and 20–30 cm soil layers.*, P≤0.05; **, P≤0.01; ns, no significance.

Table 4 Soil organic carbon sequestration rate and stock in different treatments[1)]

Treatments[2)]	Depth (cm)	SOC stock (t ha^{-1})	CSR (t ha^{-1} yr^{-1})
CK	0–10	9.18±0.96 BCa	–0.12±0.05 Cb
	10–20	6.85±1.24 Cb	–0.27±0.05 Ca
	20–30	6.88±0.32 Bb	–0.19±0.03 Cab
N	0–10	7.75±0.27 Ca	–0.12±0.01 Ca
	10–20	8.09±1.15 BCa	–0.12±0.00 ABa
	20–30	8.32±1.23 Ba	–0.09±0.01 Bb
NCa	0–10	9.94±1.42 Ba	–0.01±0.07 Bb
	10–20	9.94±0.41 ABa	–0.04±0.02 Ab
	20–30	7.92±0.44 Ba	–0.09±0.01 Ba
NPK	0–10	13.17±0.55 Aa	0.07±0.02 Ab
	10–20	10.73±1.47 Ab	–0.12±0.00 ABa
	20–30	8.29±1.14 Bc	–0.13±0.04 Ba
NPKCa	0–10	12.56±1.26 Aa	–0.02±0.03 Bb
	10–20	11.20±1.90 Aa	–0.14±0.09 Ba
	20–30	11.65±0.48 Aa	0.07±0.03 Bb

[1)] SOC, soil organic carbon; CSR, carbon sequestration rate.
[2)] CK, control; N, chemical nitrogen fertilizer; NCa, chemical nitrogen fertilizer plus lime; NPK, chemical nitrogen, phosphorus and potassium fertilizer; NPKCa, chemical nitrogen, phosphorus and potassium fertilizer plus lime.
Different capital letters indicate significant differences between treatments at the same soil depth, while different lowercase letters indicate significant differences across soil depths within the same treatment (P≤0.05).

10–20 cm soil depth (Fig. 6-B), these correlation patterns remained consistent. MBC maintained positive correlation with Fep and Alnc, while POC continued to show negative correlations with Alc and Fec, matching observations at 0–10 cm depth. At 20–30 cm depth (Fig. 6-C), similar trends persisted. MAOC maintained strong positive correlations with Alo and significant correlations with Feo, while DOC retained positive associations with Alo and Alp. EOC preserved its positive relationships with Fed and Feo. The upward trends of Alnc and Fep corresponded to positive correlations with MBC, while POC maintained negative associations with Alc and Fec fractions.

4. Discussion

4.1. Response of pH, exchangeable ion, and SOC to lime and chemical fertilizer application

Long-term lime application influences soil properties differently depending on the fertilizer type utilized. As shown in Table 2, nitrogen fertilizer decreased soil pH in both the N and NPK treatments compared to the control. However, lime application significantly increased soil pH and altered the concentrations of Ex. Mg^{2+}, Ex. Mg^{2+}, Ex. H^{+}, and Ex. Al^{3+}, which function as key regulators of soil acidity. Notably, Ex. H^{+} and Ex. Al^{3+}concentrations increased as soil pH decreased, aligning with findings reported by Daba *et al.* (2021) and Shen *et al.* (2024).

4.2. Response of Al and Fe oxides to lime and chemical fertilizer application

The results demonstrated that fertilizer application significantly influences the distribution of aluminum and iron oxides in red acidic soil, confirming findings by Wang *et al.* (2019).

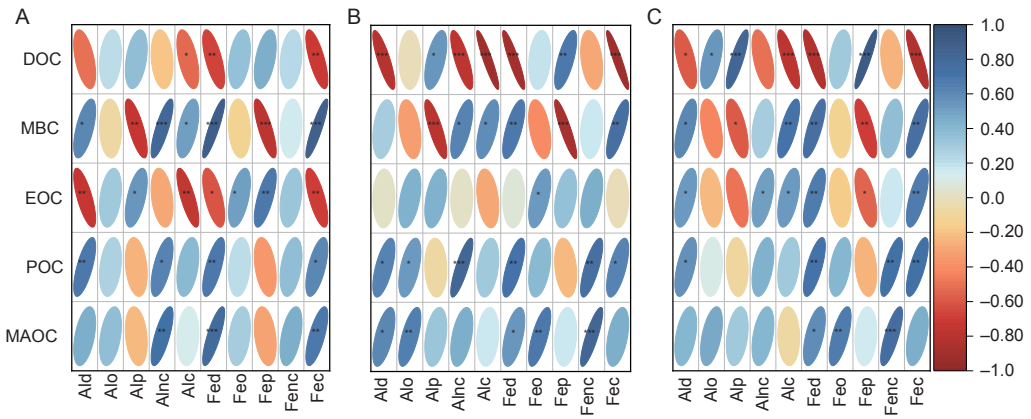


Fig. 6 Pearson's correlation analysis of soil organic carbon fractions and Al and Fe oxides in different soil depths. A, 0–10 cm. B, 10–20 cm. C, 20–30 cm. Ald, total free aluminum; Alo, reactive aluminum; Alp, organically bound aluminum; Alnc, non-crystalline aluminum; Alc, crystalline aluminum; Fed, total free aluminum; Feo, reactive aluminum; Fep, organically bound aluminum; Fenc, non-crystalline aluminum; Fec, crystalline aluminum. DOC, dissolved organic carbon; MBC, microbial biomass carbon; EOC, easily oxidizable organic carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Concentrations of Ald and Fed increased under NPK and NPKCa treatments, whereas they decreased under N and NCa treatments (Figs. 1-A and 2-A). Multiple mechanisms may explain these observations. NPK fertilizers enhance soil phosphorus availability, facilitating Al and Fe oxide mobilization within the soil profile. Under acidic conditions, phosphorus accelerates mineral dissolution, increasing Al and Fe release into the soil solution (Liu *et al.* 2007; Yi *et al.* 2023). Lime application elevates soil pH, altering Al and Fe mineral solubility dynamics, typically reducing their solubility. However, elevated phosphorus levels from NPK fertilizers can partially offset this effect by increasing soil solution ionic strength and promoting Ald and Fed mobility (Ifansyah 2014; Anthonio *et al.* 2022). Additionally, He *et al.* (2019) reported that NPK fertilizers enhance microbial activity, accelerating mineral decomposition and subsequent Al and Fe release. In contrast, N treatment demonstrates lower nutrient exchange efficiency than NPK fertilizers, resulting in reduced free Al and Fe availability (Zhao and Shen 2018; Liu H *et al.* 2023). The absence of phosphorus in N treatment limits microbial decomposition of organic residues, reducing mineral mobility. Phosphorus from NPK fertilizers competes with Al and Fe for organic matter binding sites, diminishing organic matter's metal retention capacity. As phosphorus occupies these sites, Al and Fe concentrations decrease (Yi *et al.* 2023; Priya *et al.* 2024). Furthermore, phosphorus forms stable complexes with iron oxides, further reducing Al and Fe availability for organic matter binding (Liu H *et al.* 2023; Priya *et al.* 2024). Moreover, NPK fertilization may alter microbial community composition, favoring organisms that preferentially utilize phosphorus over nitrogen or carbon. This shift could reduce the production of Al and Fe-stabilizing organic compounds, contributing to decreased Alp and Fep concentrations (Liu H *et al.* 2023; Yi *et al.* 2023).

4.3. Response of SOC fractions to lime and chemical fertilizer application

Our results revealed that lime addition enhanced SOC content in N treatment, while reducing it in NPK treatments. This contrasting effect relates to increased nutrient availability, particularly phosphorus and potassium in NPK treatments, which stimulate microbial activity (Wang *et al.* 2023). Microorganisms decompose organic matter for energy, consuming carbon to support growth (Soong *et al.* 2020). This accelerated decomposition leads to elevated CO_2 emissions, resulting in carbon loss and reduced total organic carbon (Raza *et al.* 2021).

Fig. 4-A illustrates that lime application significantly increased DOC under both N and NPK treatments in the topsoil layer (0–10 cm). As soil pH increases, acidic functional groups on organic molecules, particularly carboxylic acids, lose protons, enhancing their solubility and mobility in soil solution (Zhao *et al.* 2018). Additionally, improved soil conditions promote bacterial growth, accelerating organic matter decomposition and releasing more soluble organic compounds (Li Y *et al.* 2022). Increased microbial biomass generates higher quantities of microbial exudates and metabolites, further elevating DOC concentrations. The observed decline in microbial activity following NPK treatment stems from enhanced microbial mineralization. NPK fertilizers provide readily available nutrients, especially nitrogen, stimulating microbial activity. An expanding microbial population rapidly converts organic matter to inorganic forms such as CO_2, leading to carbon loss through CO_2 emissions and reducing DOC levels in soil solution (Tiefenbacher *et al.* 2020; Khan *et al.* 2024). The inclusion of phosphorus and potassium in NPK treatments intensifies microbial activity, accelerating carbon mineralization and limiting EOC accumulation. Conversely, NCa treatment appears to promote EOC retention. By providing optimal nitrogen and lime quantities, microbial activity can be regulated more effectively than with NPKCa treatment alone (Ma *et al.* 2018). In NCa treatment, enhanced microbial decomposition improves nutrient cycling, increasing mineral nitrogen and phosphorus availability (Mkhonza *et al.* 2020). Moreover, lime application can modify bacterial community composition, for instance, increasing Actinobacteria diversity

and abundance while reducing Bacteroidetes populations and promoting other bacterial groups (Ding *et al.* 2023).

Liming establishes a more favorable pH environment, enhancing microbial growth and activity, which influences both POC and MAOC pools (Ejigu *et al.* 2023). Additionally, liming decreases exchangeable aluminum toxicity in acidic soils, improving microbial habitat conditions and potentially modifying organic matter dynamics (Qaswar *et al.* 2020). NPK fertilization promotes plant growth, resulting in increased organic matter inputs into soil, which beneficially affects both POC and MAOC pools (Yahaya *et al.* 2023). The enhanced nutrient availability under NPK fertilization accelerates microbial activity, affecting organic matter decomposition and stabilization processes (Adhikari and Bhattacharyya 2015). Subsequently, NPKCa treatment increases POC through enhanced plant biomass and residue incorporation (Mahapatra *et al.* 2018). However, N treatment causes soil acidification, evidenced by a significant reduction in soil pH. Most soil microorganisms function optimally under neutral to slightly alkaline conditions; therefore, acidification inhibits microbial growth and activity. Acidic conditions reduce microbial processes and biomass due to unfavorable environmental conditions (Li *et al.* 2016; He *et al.* 2024). Decreased soil pH also facilitates the release of toxic soluble aluminum ions, which suppress microbial growth and function (Niu *et al.* 2020; Liu L *et al.* 2023). This toxicity diminishes microbial diversity and biomass, leading to reduced MBC levels.

Excessive nitrogen disrupts soil nutrient balance, limiting available carbon and constraining microbial growth (Naz *et al.* 2022). In conditions where nitrogen is abundant but carbon sources are limited, microbes lack sufficient organic substrates for growth, resulting in decreased MBC. While nitrogen addition may enhance certain microbial activities, it frequently leads to increased mineralization of existing SOC, resulting in net carbon loss. Phosphorus and potassium influence microbial processes but demonstrate less effectiveness than lime in stabilizing organic carbon fractions. Research indicates that these nutrient amendments produce a relatively weaker response in soil carbon dynamics (Aula *et al.* 2016; Guo *et al.* 2019). Furthermore, liming amplifies the beneficial effects of nitrogen application by supporting plant growth and increasing organic matter inputs, which promote accumulation of easily oxidizable carbon. Conversely, NPK fertilization alone may not achieve comparable organic matter inputs due to nutrient imbalances or plant health constraints caused by low soil pH (Coonan *et al.* 2019; Buthelezi and Buthelezi-Dube 2022).

4.4. Interactions of Al and Fe oxides and carbon sequestration under lime and chemical fertilizer application

Pearson's correlation analysis examined the relationships between SOC fractions and Al and Fe oxides (Fig. 6). The analysis revealed a strong positive correlation between Feo, Alo, and MAOC. This observation emphasizes the essential role of reactive and non-crystalline fractions in maintaining SOC stability (Fukumasu *et al.* 2021; Kirsten *et al.* 2021). Specifically, Alo and Feo play crucial roles in forming organo-mineral complexes that enhance carbon persistence in red acidic soils. These findings align with previous research demonstrating that reactive Fe and Al fractions substantially contribute to SOC stabilization due to their high reactivity and strong affinity for organic molecules (Cloy *et al.* 2014; Rani 2021). Moreover, the positive correlations between DOC and both Alo and Alp indicate that DOC functions as a precursor in SOC stabilization mechanisms. Labile DOC interacts with Alo and Alp, facilitating the formation of stable organic-metal complexes (Boča and Van Miegroet 2017; Peter 2017; Sun *et al.* 2020). This highlights the fundamental role of DOC in organic carbon cycling and stability, particularly in soils under long-term fertilization (Babur *et al.* 2021). MBC demonstrated a positive association with AInc and Fep, suggesting these reactive phases provide bioavailable organic carbon supporting microbial metabolism under extended fertilization regimes (Chen *et al.* 2021). Conversely, POC showed a negative correlation with Alc and Fec across all soil depths. This indicates that crystalline Fe and Al oxides exhibit lower effectiveness in stabilizing coarse organic matter fractions due to reduced surface reactivity and limited capacity to bind larger particulate organic matter (Kleber *et al.* 2005; Chen *et al.* 2014). This inverse relationship challenges conventional expectations and emphasizes the distinct functions of non-crystalline and crystalline Fe/Al fractions in organic carbon dynamics (Franks *et al.* 2021; Li H *et al.* 2022; Chen *et al.* 2023).

The persistent negative relationship between MAOC and Alc further demonstrates that elevated concentrations of crystalline aluminum may impede the stabilization of MAOC, primarily due to crystalline phases exhibiting reduced reactivity compared to amorphous or non-crystalline forms. Similarly, the inverse correlations between POC and both Alc and Fec support the understanding that crystalline Fe/Al phases are less effective in stabilizing coarse organic carbon fractions (Doydora *et al.* 2017; da Silva *et al.* 2020). Table 2 presents the results for CSR and SOC stock. To evaluate the influence of Al and Fe oxides on CSR and SOC stock, a relative importance analysis was conducted (Fig. 7). Under lime application, DOC emerged as the predominant factor, contributing approximately 35 and 25% of the variation in CSR and SOC stock, respectively (Fig. 7-A and B). This aligns with the understanding that increasing pH leads to deprotonation of organic functional groups such as carboxylic acids, enhancing their solubility and mobility in soil solution (Zhao *et al.* 2018). The observed positive correlations between DOC and both Alo and Alp further substantiate the role of DOC as a key precursor in SOC stabilization processes through its interaction with reactive aluminum phases (Boča and Van Miegroet 2017; Peter 2017; Sun *et al.* 2020). Total nitrogen, total phosphorus, and total potassium significantly influenced CSR variability, suggesting that nutrient availability promotes carbon sequestration by enhancing plant productivity and microbial activity (Chen *et al.* 2024). Soil pH and EOC demonstrated moderate effects, reflecting how lime-induced pH modifications facilitate carbon stabilization (Coonan *et al.* 2019; Buthelezi and Buthelezi-Dube 2022). Reactive iron, Fep and Alp contributed to a lesser extent, highlighting their role in stabilizing labile carbon

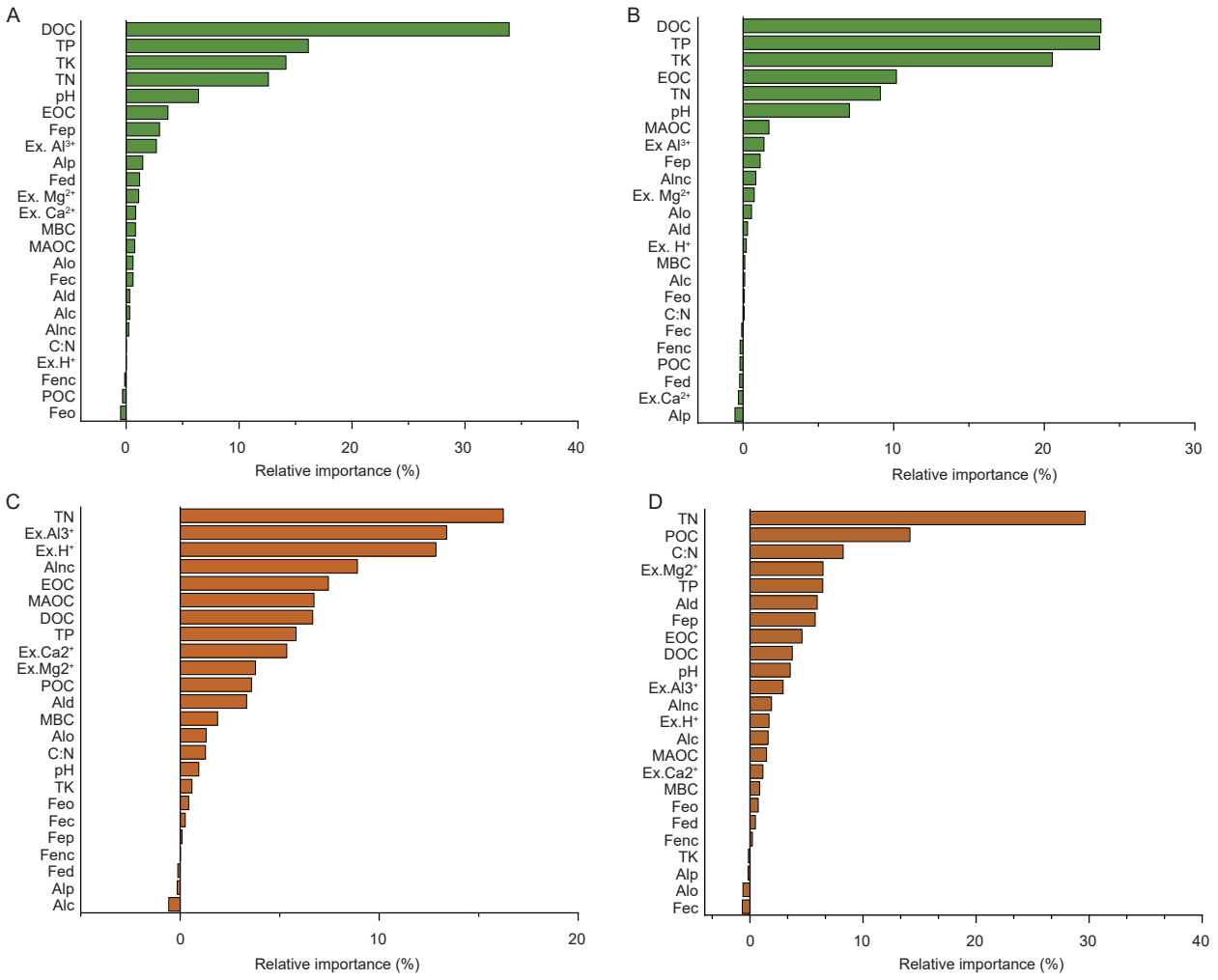


Fig. 7 Relative importance of soil properties in the boosted regression tree (BRT) models for soil organic carbon (SOC) stock and soil organic carbon sequestration rate (CSR). A and C, relative importance of soil properties for SOC stock in treatments without lime (N and NPK) (A) and with lime (NCa and NPKCa) (C). B and D, relative importance of soil properties for CSR in treatments without lime (N and NPK) (B) and with lime (NCa and NPKCa) (D). N, nitrogen; TP, total phosphorus; TK, total potassium; TN, total nitrogen; C:N, the ratio of carbon to nitrogen; Ex. H^+, exchangeable hydrogen; Ex. Al^{3+}, exchangeable aluminum; Ex. Ca^{2+}, exchangeable calcium; Ex. Mg^{2+}, exchangeable magnesium; Ald, total free aluminum; Alo, reactive aluminum; Alp, organically bound aluminum; Alnc, non-crystalline aluminum; Alc, crystalline aluminum; Fed, total free aluminum; Feo, reactive aluminum; Fep, organically bound aluminum; Fenc, non-crystalline aluminum; Fec, crystalline aluminum. DOC, dissolved organic carbon); MBC, microbial biomass carbon; EOC, easily oxidizable organic carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon.

fractions (Cloy *et al.* 2014; Rani 2021). In the absence of lime application (Fig. 7-C and D), TN was the most influential factor, accounting for 20–30% of the variation in CSR and SOC stock. This emphasizes nitrogen's critical role in supporting microbial and plant processes that drive SOC sequestration (Li C *et al.* 2022; Ngaba *et al.* 2023). Other significant contributors included exchangeable aluminum, exchangeable hydrogen, and non-crystalline aluminum, highlighting the substantial impact of aluminum toxicity and soil acidity on carbon cycling in acidic soils without liming. EOC, MAOC, and DOC remained essential components in SOC stabilization and turnover under these conditions (Franks *et al.* 2021; Li C *et al.* 2022; Chen *et al.* 2023).

A PLS-SEM was employed to examine the effects of lime addition on soil pH and its cascading impacts on Al and Fe oxides, organic carbon fractions, and ultimately soil carbon sequestration rate and stock (Fig. 8). Under lime application (Fig. 8-A), Ald exhibited a strong negative effect on both CSR (path coefficient: –0.78) and SOC stock (path coefficient: –0.49), indicating that Ald inhibits carbon sequestration processes. Conversely, Fed demonstrated a positive, though weaker, direct effect on SOC stock (path coefficient: 0.69) with no significant effect on CSR. DOC negatively influenced CSR (–0.21) but contributed indirectly to SOC stock *via* CSR. The model explained 76% of the variance in CSR (R^2=0.76) and 91% in SOC stock (R^2=0.91), with excellent fit indices (SRMR=0.00, NFI=1.00). Lime-induced pH increases mitigated Ald's negative impacts on CSR and SOC stock, fostering a more favorable environment for carbon stabilization. While Ald inhibited carbon sequestration, its effects were attenuated under limed conditions. The strong positive influence of Fe

on SOC stock aligns with previous findings supporting Fe's role in stabilizing organic matter through mineral interactions (Thakuria *et al.* 2016; Aye 2017; Mosharrof *et al.* 2021). Without lime (Fig. 8-B), Ald unexpectedly exhibited a positive effect on CSR (path coefficient: 0.17) and a strong positive effect on SOC stock (path coefficient: 0.89), contrasting with its inhibitory role under limed conditions. Fed also strongly and positively influenced SOC stock (path coefficient: 0.95) and indirectly affected CSR through SOC stock. DOC showed no significant impact on CSR, while EOC negatively affected CSR directly (–0.41). This model accounted for less variance in CSR (R^2=0.17) and SOC stock (R^2=0.38) but maintained excellent fit (SRMR=0.00, NFI=1.00). These results indicate that in acidic soils without lime, Al and Fed dominate SOC stock dynamics and serve as primary drivers of carbon stabilization. However, the negative effect of EOC on CSR suggests enhanced microbial decomposition of labile carbon, potentially offsetting carbon sequestration gains (Bailey *et al.* 2019; Kirsten *et al.* 2021). In summary, lime application mitigated the negative impact of aluminum on carbon sequestration and enhanced the positive role of iron in stabilizing SOC. In contrast, under acidic conditions without lime, both aluminum and iron contributed positively to SOC stock, but with reduced explanatory power and increased carbon loss due to microbial decomposition of labile carbon. The findings highlight the complex interplay between phosphorus, iron, and aluminum oxides in controlling carbon sequestration and stabilization processes, emphasizing the need for further research on their interactions and combined effects on soil carbon dynamics.

5. Conclusion

This study establishes a theoretical framework for understanding how the combined application of chemical nitrogen fertilization and lime enhances SOC sequestration. The research reveals the intricate relationships between fertilization practices, soil amendments, and soil chemical processes. NPK applications increase nutrient availability and enhance microbial activity, subsequently mobilizing aluminum and iron oxides within the soil. The minimal effects of nitrogen-only (N) treatments demonstrate the significance of balanced fertilization in nutrient cycling and Fe and Al oxide behavior in red acidic soils. Additionally, lime application enhances the effects of DOC, total phosphorus, and total potassium on carbon sequestration by optimizing soil pH, increasing nutrient availability, and stabilizing SOC. Conversely, without lime application, factors including total nitrogen, exchangeable acidity, and non-crystalline aluminum demonstrate greater influence over carbon dynamics, illustrating how soil acidity limits SOC mobility. The distinctive functions of labile carbon fractions such as DOC and easily oxidizable carbon, along with aluminum and iron oxides, emphasize the importance of lime amendments for effective carbon sequestration in red acidic soils. These findings confirm the essential role of Fe and Al oxides, together with labile organic carbon pools, in controlling SOC stabilization. Therefore, integrating strategic mineral fertilization with pH adjustment *via* liming offers an effective strategy to enhance soil carbon storage and mitigate carbon losses in acidic red soils.

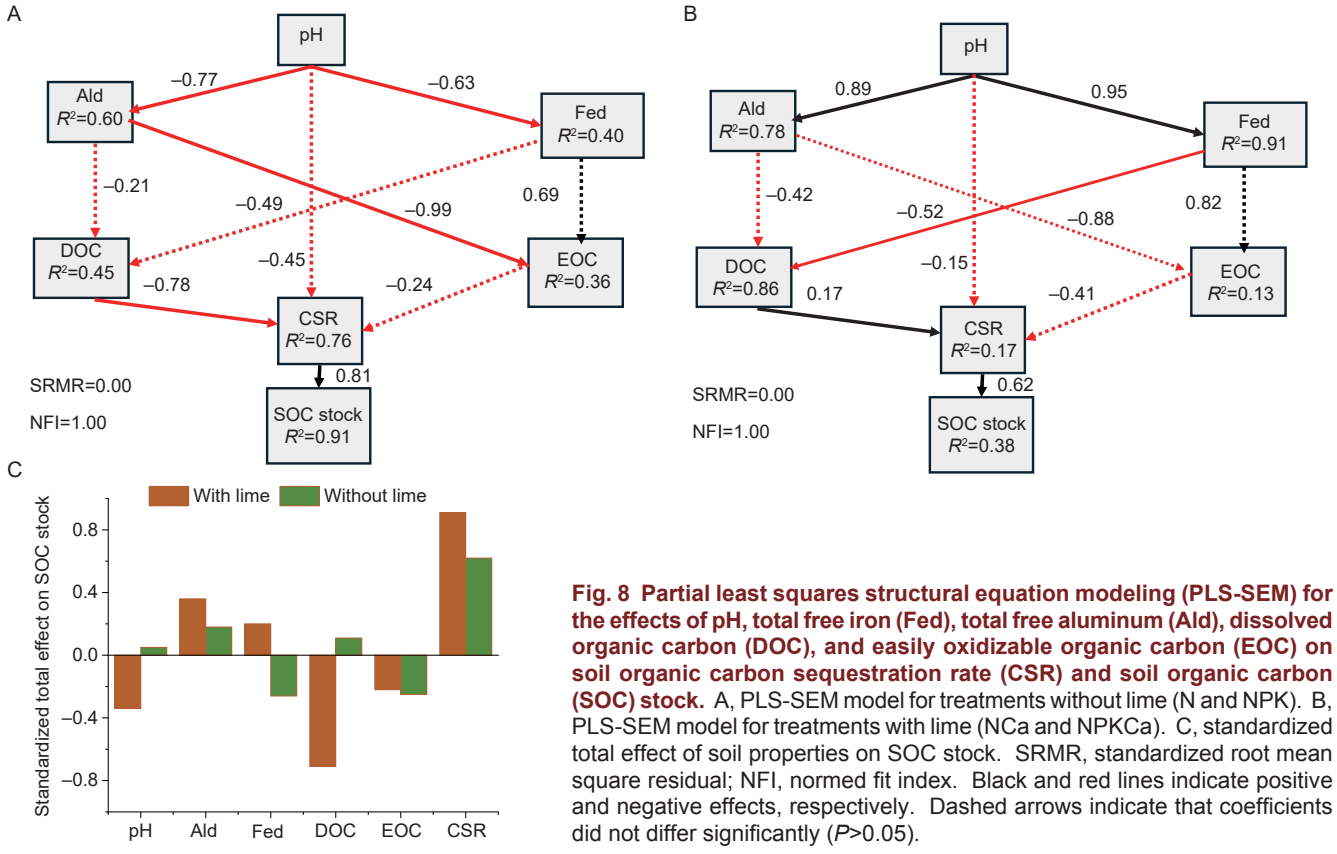


Fig. 8 Partial least squares structural equation modeling (PLS-SEM) for the effects of pH, total free iron (Fed), total free aluminum (Ald), dissolved organic carbon (DOC), and easily oxidizable organic carbon (EOC) on soil organic carbon sequestration rate (CSR) and soil organic carbon (SOC) stock. A, PLS-SEM model for treatments without lime (N and NPK). B, PLS-SEM model for treatments with lime (NCa and NPKCa). C, standardized total effect of soil properties on SOC stock. SRMR, standardized root mean square residual; NFI, normed fit index. Black and red lines indicate positive and negative effects, respectively. Dashed arrows indicate that coefficients did not differ significantly (P>0.05).

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT 4.0 to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

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