

## Article

# Land Use and Lithology Drive Divergent Vertical Distribution and Stabilization of Soil Organic Carbon Fractions in Karst Agroecosystems

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## Abstract

In karst agroecosystems, the stability of soil organic carbon (SOC), which is essential for sustainable land management, is strongly influenced by the carbonate parent material. However, how land use and lithology interactively affect the vertical distribution and stabilization of SOC fractions remains unknown. This study investigated the vertical distribution (0–70 cm) of SOC fractions (particulate, POC; mineral-associated, MOC; recalcitrant, ROC; microbial biomass, MBC; dissolved, DOC; permanganate-oxidizable, LOC) in paddy and upland systems overlying limestone and dolomite. Results showed that land use and lithology jointly determined depth-dependent patterns of SOC fractions. The key finding was a depth-dependent reversal of land use effects: paddy soils had higher concentrations of SOC fractions (especially MBC and LOC) in the top 0–20 cm, whereas upland soils had higher concentrations of DOC, ROC, and POC below 50 cm. This pattern underscores an overlooked deep carbon sequestration potential in aerated uplands. Furthermore, lithology exerted fundamental control on SOC stability, with limestone soils exhibiting higher MOC/SOC and MOC/POC ratios than dolomite soils, indicating stronger organo-mineral association. Random Forest and Structural Equation Modeling revealed that soil aggregate stability is a key factor influencing the stability of SOC. These findings demonstrate that effective SOC management in karst agroecosystems must consider both surface land use decisions and deep lithological constraints. Specifically, promoting aggregate stability offers a more targeted pathway for long-term carbon stabilization in these ecologically fragile agricultural systems.

**Keywords:** soil organic carbon fractions; carbon stability; land use; lithology; karst agriculture; deep soil carbon

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## 1. Introduction

Globally, paddy fields and upland systems represent the most widespread agricultural ecosystems in which soil organic carbon (SOC) represents the most labile fraction of the terrestrial soil carbon pool, and its dynamics may exert profound impacts on both global carbon cycling and crop productivity [1]. Previous studies have demonstrated that increasing SOC by 1.0% would lead to an average cereal yield increase of 0.43 t ha<sup>-1</sup> (12.3%) and a 3.5% decline in yield variability [2]. The marked contrasts in vegetation types and land management regimes between paddy fields and upland croplands induce substantial

modifications in soil properties, particularly in carbon–cycling–related attributes including soil enzymatic activities, physicochemical properties, and aggregate structure dynamics [3,4]. Paddy soils typically exhibit higher organic carbon (OC) content and consequently greater carbon sequestration potential compared to upland soils [5,6]. To date, it remains unclear which specific fraction of SOC exhibits the most pronounced increase in paddy soils compared to upland soils.

Accumulating evidence from subtropical China reveals a pronounced vertical stratification of SOC, with surface layers exhibiting significantly higher concentrations than subsurface horizons across diverse agricultural systems, including both paddy and upland fields [7,8]. This consistent pattern underscores the critical role of surface processes in SOC sequestration, but deep subsoil layers contain substantial carbon stocks [9]. Deep soil horizons represent particularly stable carbon sinks due to their inherent buffering capacity against climate variability and anthropogenic disturbances such as land use and land management practices [10,11]. Consequently, understanding the vertical distribution patterns of soil organic carbon (SOC) fractions in both paddy and upland systems is of critical importance for global carbon cycling and agricultural sustainability.

SOC is a heterogeneous mixture of organic carbon compounds formed through the progressive breakdown of plant, animal, and microbial biomass, exhibiting varying chemical and physical properties [12]. To better understand its dynamics, SOC is often divided into operationally defined fractions, each with unique physicochemical properties and decomposition rates [13,14]. Particulate organic carbon (POC) and mineral-associated organic carbon (MOC) are physical fractionation components of SOC obtained through particle size separation methods. MOC represents the physically stabilized fraction of SOC [15]. Separation based on the chemical characteristics of organic carbon, such as solubility and oxidizability, yields distinct fractions, including dissolved organic carbon (DOC), permanganate-oxidizable organic carbon (LOC), and recalcitrant organic carbon (ROC). ROC is the acid hydrolysis residue obtained with 6 M hydrochloric acid (HCl) and is considered a recalcitrant carbon pool with chemical stability. Although present in relatively small quantities, microbial biomass carbon (MBC) plays a direct role in soil carbon cycling [16]. Collectively, these common SOC fractions represent a suite of critical carbon pools, where shifts in their proportional contributions reflect the complex dynamics and key processes governing soil carbon cycling. Consequently, they have been proposed as sensitive indicators for assessing the impacts of agricultural management practices.

Karst landscapes, covering ~15% of the world's land area, are characterized by soils derived from carbonate rocks such as limestone and dolomite [17]. These lithologies differ in weathering susceptibility and mineral composition, potentially influencing SOC storage and fractionation [1]. For instance, limestone, with its higher solubility, may promote stronger organo–mineral associations due to its abundant secondary calcium carbonates, whereas dolomite's slower weathering could lead to distinct SOC distribution patterns. Despite these differences, few studies have systematically compared SOC fractions between soils derived from these two lithologies, limiting our ability to predict SOC responses to land use in karst farmlands. Additionally, the drivers of SOC vertical distribution, such as aggregate structure, enzymatic activity, and soil physicochemical properties, may vary with lithology. To address these knowledge gaps, this study investigates: (1) vertical stratification of SOC fractions (labile vs. stable) in paddy soils and upland soils developed from limestone and dolomite and the key drivers governing SOC distribution, and (2) how land use– and lithology–driven changes in soil properties influence the vertical distribution of SOC fractions. We hypothesized that: (1) Dolomite-derived soils may possess higher labile SOC fraction concentrations, but limestone-derived soils, due to their pedogeochemical properties, will exhibit stronger organo–mineral associations (higher MOC/SOC and MOC/POC ratios), resulting in a more stable carbon pool. (2) Due

to vertical gradients in carbon input and environmental conditions, paddy management will enrich SOC fractions in the surface layer (0–20 cm), while upland systems may retain or accumulate more SOC fractions at greater depths (>50 cm). (3) Land use may not have altered the stability of SOC. The results of this study can facilitate a deeper understanding of the effects of land use and lithology on soil carbon cycling and accumulation in karst areas, and can offer mechanistic insights that inform the design of targeted management practices aimed at improving organic carbon stabilization in karst agroecosystems.

## 2. Materials and Methods

### 2.1. Study Region

This study was conducted in Huanjiang Maonan Autonomous County, Guangxi Zhuang Autonomous Region (24°44′–25°33′ N, 107°51′–108°43′ E), a representative karst region characterized by a subtropical monsoon climate. This climate features concurrent rainfall and heat, with a frost-free period of 290 days. The mean annual temperature is 17.9 °C, averaging 10.1 °C in January and 28.0 °C in July, and the historical minimum temperature recorded is −5.2 °C. Precipitation is concentrated from April to September, with an average annual rainfall of 1750 mm. Dominant soil types include brown and black calcareous soils, derived primarily from dolomitic and limestone parent materials, with minor occurrences of sandstone and shale.

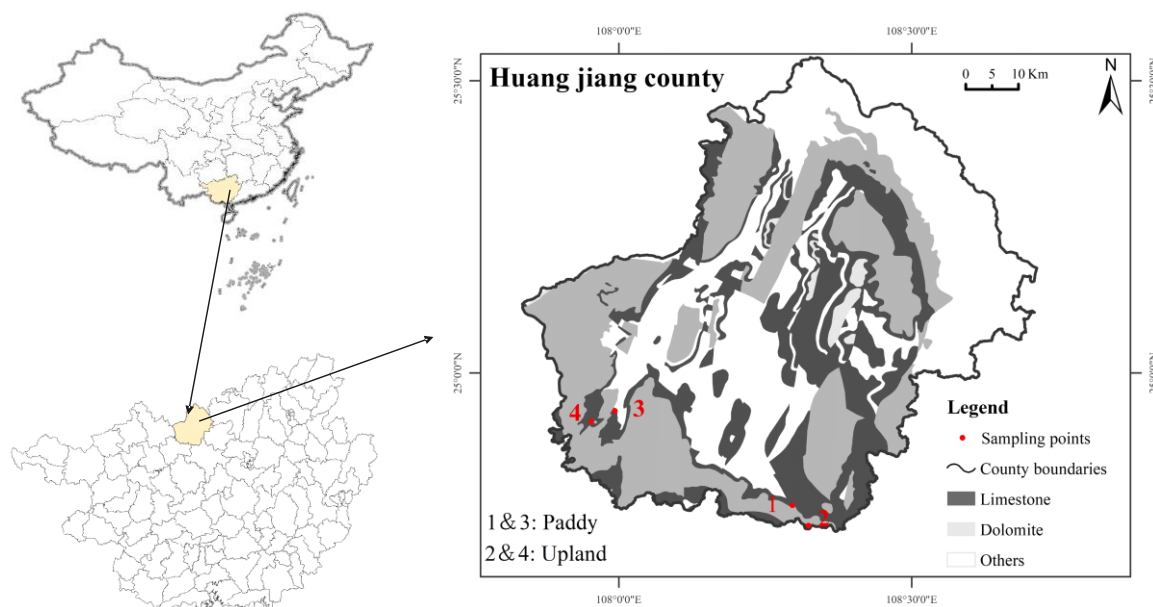
### 2.2. Field Sampling and Analyses

Based on field investigations, paddy and upland soils distributed over areas underlain by dolomite and limestone were selected. The study sites were primarily located within peak-cluster depression areas characterized by similar topographic features. All paddy fields were cultivated with rice under a double-cropping system, while upland soils followed a maize-soybean rotation. All sites had comparable agricultural histories and had maintained stable current land uses for at least 20 years. Sampling was conducted in November 2021 during dry periods when the fields remained fallow with no incorporation of fresh organic carbon inputs into the soil. Sampling points were established for each of the following categories: dolomite paddy fields, dolomite upland fields, limestone paddy fields, and limestone upland fields. At each sampling point, three independent replicate plots were set up, with a distance greater than 50 m between them. A total of 12 plots were established, each covering an area of 100 m<sup>2</sup> (10 m × 10 m). Lithological and sampling point distribution is shown in Figure 1, and the detailed information (including lithology, land use, and coordinates) for each site is presented in Table S1.

In each plot, after the removal of the soil surface litter and biomass, five subsamples were collected within each plot (plum blossom sampling method), and these subsamples were subsequently homogenized to obtain a single composite sample representing the plot, weighing approximately 1 kg. Five composite samples from five soil depths (0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm and 50–70 cm) were collected within each plot by using a soil auger. Consequently, each plot yielded five composite samples (one from each soil depth), giving a final total of 60 samples across all plots. Each composite sample was divided into two parts: one part was air-dried and sieved through 2 mm and 0.147 mm to determine the LOC, ROC and physicochemical properties, and another part was stored at 4 °C prior to measuring the MBC, DOC and soil enzyme activity.

Concurrently, a soil profile was established at the center of each plot, and undisturbed soil samples weighing approximately 1 kg were collected from five depths (0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm, and 50–70 cm) in each profile. A total of 60 undisturbed soil samples from 12 soil profiles were respectively collected and packed in hard plastic

boxes to prevent vibration and tipping. The bulk density (BD) of each soil layer was measured using a 100 cm<sup>3</sup> stainless steel cutting ring in each plot. The undisturbed soil samples were properly air-dried in the room, then broken into about 10 mm particles along the natural cracks, and finally air-dried. These air-dried samples were divided into two parts: One part was sieved through a 2 mm screen to determine POC and MOC. Another part was used to measure the mechanical stability of aggregates and the water stability of aggregates.



**Figure 1.** Spatial distribution of dolomite and limestone bedrock with sampling locations across the study area.

### 2.3. Determination of Soil Biotic and Abiotic Variables

MBC was determined by the chloroform fumigation extraction method using ethanol-free chloroform (>99.5%) for 24 h of vacuum fumigation, followed by extraction with 0.5 mol L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> at a soil-to-solution ratio of 1:4, and the carbon concentration in the extracts was measured using a TOC analyzer (Vario TOC, Germany, elemental) [18]. DOC was extracted with 0.5 mol L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> (soil:solution = 1:4) by shaking for 30 min, centrifuged at 4000 rpm for 10 min, filtered through 0.45 µm membrane filters, and determined directly using a TOC analyzer (Vario TOC, Germany, elemental) [19]. LOC was measured by oxidation with 333 mmol L<sup>-1</sup> KMnO<sub>4</sub> in the presence of 0.5 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> (2 mL) at 60 °C for 1 h in a water bath, and the absorbance of the diluted supernatant was read at 565 nm using a UV-Vis spectrophotometer; the amount of KMnO<sub>4</sub> consumed was used to calculate the LOC content (1 mmol KMnO<sub>4</sub> consumed corresponds to 9 mg C) [20]. POC and MOC were separated by dispersing the soil with 5 g L<sup>-1</sup> sodium hexametaphosphate (soil:solution = 1:10) and shaking for 18 h, followed by wet sieving through a 53 µm sieve; the fractions retained (>53 µm, POC) and passed (<53 µm, MOC) were both oxidized with 0.4 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> at 180 °C for 5 min, and the remaining dichromate was back-titrated with 0.2 mol L<sup>-1</sup> FeSO<sub>4</sub> using o-phenanthroline as an indicator, with organic carbon content calculated from the volume of FeSO<sub>4</sub> consumed [21]. ROC was determined by digesting the soil with 6 mol L<sup>-1</sup> HCl (1:10 solid-to-liquid ratio) through boiling for 2 h to remove labile fractions, then washing, drying at 60 °C, and measuring the residual carbon by the dichromate oxidation method (0.4 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-concentrated H<sub>2</sub>SO<sub>4</sub>,

180 °C, 5 min), with back-titration of excess dichromate by 0.2 mol L<sup>-1</sup> FeSO<sub>4</sub> using o-phenanthroline as an indicator [22].

The enzymes associated with the C-cycle, including β-D-cellobiosidase (CBH); β-1,4glucosidase (BG); and β-1,4-xylosidase (BX), were analyzed using the microplate fluorimetry method [23]. Briefly, 200 µL of 200 µmol L<sup>-1</sup> 4-MUB substrates (MUB-β-D-cellobioside for CBH, MUB-β-D-glucoside for BG, and MUB-β-D-xyloside for BX), 200 µL of 50 mmol L<sup>-1</sup> acetate buffer (pH 5.0), and 50 µL of soil suspension (1 g soil in 125 mL buffer) were incubated in black 96-well microplates at 25 °C in the dark for 4 h. The reaction was terminated by adding 10 µL of 1 mol L<sup>-1</sup> NaOH, and the fluorescence intensity was measured using a fluorescence microplate reader with excitation at 365 nm and emission at 450 nm.

Soil physicochemical properties were determined following the methods described by Lu [24]. SOC was measured using the dichromate oxidation method with concentrated H<sub>2</sub>SO<sub>4</sub> and 0.4 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> under external heating at 180 °C for 5 min, followed by back-titration with 0.2 mol L<sup>-1</sup> FeSO<sub>4</sub> using o-phenanthroline as an indicator with which carbonates were not determined. Total nitrogen (TN) was determined using the micro-Kjeldahl (8400 Danish FOSS) method with concentrated H<sub>2</sub>SO<sub>4</sub> digestion in the presence of a catalyst mixture (K<sub>2</sub>SO<sub>4</sub>:CuSO<sub>4</sub>:Se = 10:1:0.1). To determine total phosphorus (TP), briefly, soil samples were fused with NaOH at 720 °C and dissolved in dilute H<sub>2</sub>SO<sub>4</sub>, and the phosphate in the solution was determined colorimetrically at 700 nm (ultraviolet and visible spectrophotometer) using the molybdenum blue method. Cation exchange capacity (CEC) (alkaline) was determined using the sodium acetate flame photometry method; soil samples were leached with 1 mol L<sup>-1</sup> sodium acetate (pH 8.2), washed with ethanol, and the adsorbed NH<sub>4</sub><sup>+</sup> was displaced with KCl and determined by flame photometry. Exchangeable Ca was determined by the ammonium acetate exchange-atomic absorption spectrometry method; soil samples were extracted with 1 mol L<sup>-1</sup> ammonium acetate (pH 7.0), and the Ca concentration in the extract was measured using an atomic absorption spectrophotometer. Soil pH was measured in a 1:2.5 (w/v) soil-to-water suspension using a pH meter. BD was measured by collecting undisturbed soil cores with 100 cm<sup>3</sup> stainless steel cutting rings, oven-drying at 105 °C to a constant weight, and calculating dry mass per unit volume (g cm<sup>-3</sup>). The basic soil physicochemical properties are shown in Table S2.

For aggregate size distribution analysis, seven mechanically stable aggregate fractions (<0.25 mm, 0.25–0.5 mm, 0.5–1 mm, 1–2 mm, 2–5 mm, 5–8 mm, and >8 mm) were isolated through dry sieving according to Liu [25], and the mass percentage of each fraction was quantified. Based on these dry-sieving results, two 50.0 g replicates per sample were prepared. Water-stable aggregates were subsequently separated via wet sieving [25] into six size classes (<0.25 mm, 0.25–0.5 mm, 0.5–1 mm, 1–2 mm, 2–5 mm, and >5 mm). The mass percentage of water-stable aggregates was determined after oven-drying at 65 °C until a constant weight. The particle size distribution of mechanical aggregates and water-stable aggregates is shown in Figures S1 and S2. Aggregate stability indices, including the mean weight diameter (MWD, mm), the geometric mean diameter (GMD, mm), and the percentage of aggregate destruction (PAD, %), were calculated using formulas (1) to (3).

$$MWD = \sum_{i=1}^n \bar{R}_i \times M_i \quad (1)$$

$$GMD = \exp\left(\sum_{i=1}^n M_i \times \ln \bar{R}_i\right) \quad (2)$$

$$PAD = \frac{m_d - m_w}{m_d} \times 100 \quad (3)$$

where  $n$  is the number of different-sized soil aggregates;  $R_i$  is the mean diameter (mm) of soil aggregate  $i$ ;  $M_i$  is the content (%) of soil aggregate  $i$  in bulk soil;  $m_d$  is the content (%) of soil mechanical stability aggregate with size  $> 0.25$  mm; and  $m_w$  is the content (%) of soil water-stable aggregate with size  $> 0.25$  mm.

#### 2.4. Statistical Analyses

Statistical analyses were performed using R software (v4.4.2). Before analyses, all data were first tested for normality and homogeneity of variances, and the data were log-transformed prior to analysis if they were not normally distributed. A three-way analysis of variance (ANOVA) was conducted with lithology, land use, and soil depth as fixed factors to examine their main effects and interactions (including two-way and three-way interactions) on SOC fractions. In soil depth gradient studies, treating different soil layers as independent observations in multi-factor ANOVA is a widely adopted approach [26–28]. When significant interactions were detected, simple effects analyses were performed to interpret the interactions. One-way ANOVA was used as a supplementary tool to test the effects of lithology and land use on SOC fractions, aggregate stability indices, and enzymatic activities within specific soil depth subgroups when significant interactions were found in the three-way ANOVA, facilitating the interpretation of complex interaction patterns. Mean comparisons were performed using the least significant difference (LSD) test at the 0.05 significance level. Spearman's correlations were used to test for the relationships among SOC fractions, stability index of aggregate structures, enzymatic activity and other physicochemical variables. Random Forest (RF) was applied to further clarify the contribution of environmental factors to soil carbon fraction variation. Partial least squares path modeling (PLS-PM) was employed to explore how lithology and vegetation type affect SOC fractions.

### 3. Results

#### 3.1. Content and Distribution of SOC Fractions

Lithology, land use and soil depth exerted significant effects on the contents of various SOC fractions (Table 1, Figure 2). Soil depth exerted a dominant influence, with a highly significant effect ( $p < 0.001$ ) observed across all measured carbon fractions. Regardless of lithological differences or land uses, SOC fractions exhibited a consistent decreasing trend with soil depth. Additionally, soil depth showed significant two-way interactions with lithology and land use, respectively (Table 1). However, no significant interaction was detected for the lithology  $\times$  land use combination or for the three-way interaction.

Concerning the influence of lithology, dolomite-derived soils exhibit significantly higher concentrations of SOC fractions compared to limestone-derived soils. In the 0–30 cm soil layers, MBC, DOC, LOC, POC and ROC in dolomite soils were significantly higher than in limestone soils. The respective increases ranged from 52.40 to 95.45%, 28.43 to 65.91%, 99.49 to 172.45%, 213.21 to 308.70%, and 71.59 to 113.35% (Figure 2a–e). In addition, the contents of LOC, POC and ROC in the 30–50 cm soil layer were higher over dolomite than limestone. Notably, the contrasting effects between dolomite and limestone on SOC fractions became progressively less distinct with increasing depth, with the differences concentrated primarily in the upper 30 cm ( $>75\%$ ).

The effect of land use types on SOC fractions was primarily manifested in different soil layers (Figure 2g–l). Compared to upland soils, paddy soils showed significantly higher contents of SOC fractions in the 0–20 cm layer, as well as higher MBC and LOC in the 20–30 cm layer, with increases ranging from approximately 20% to 150% depending on the fraction. Conversely, the DOC, ROC and POC contents in upland soils at the 50–70 cm depth were 33.96%, 39.69% and 35.38% higher ( $p < 0.05$ ) than in paddy soils.

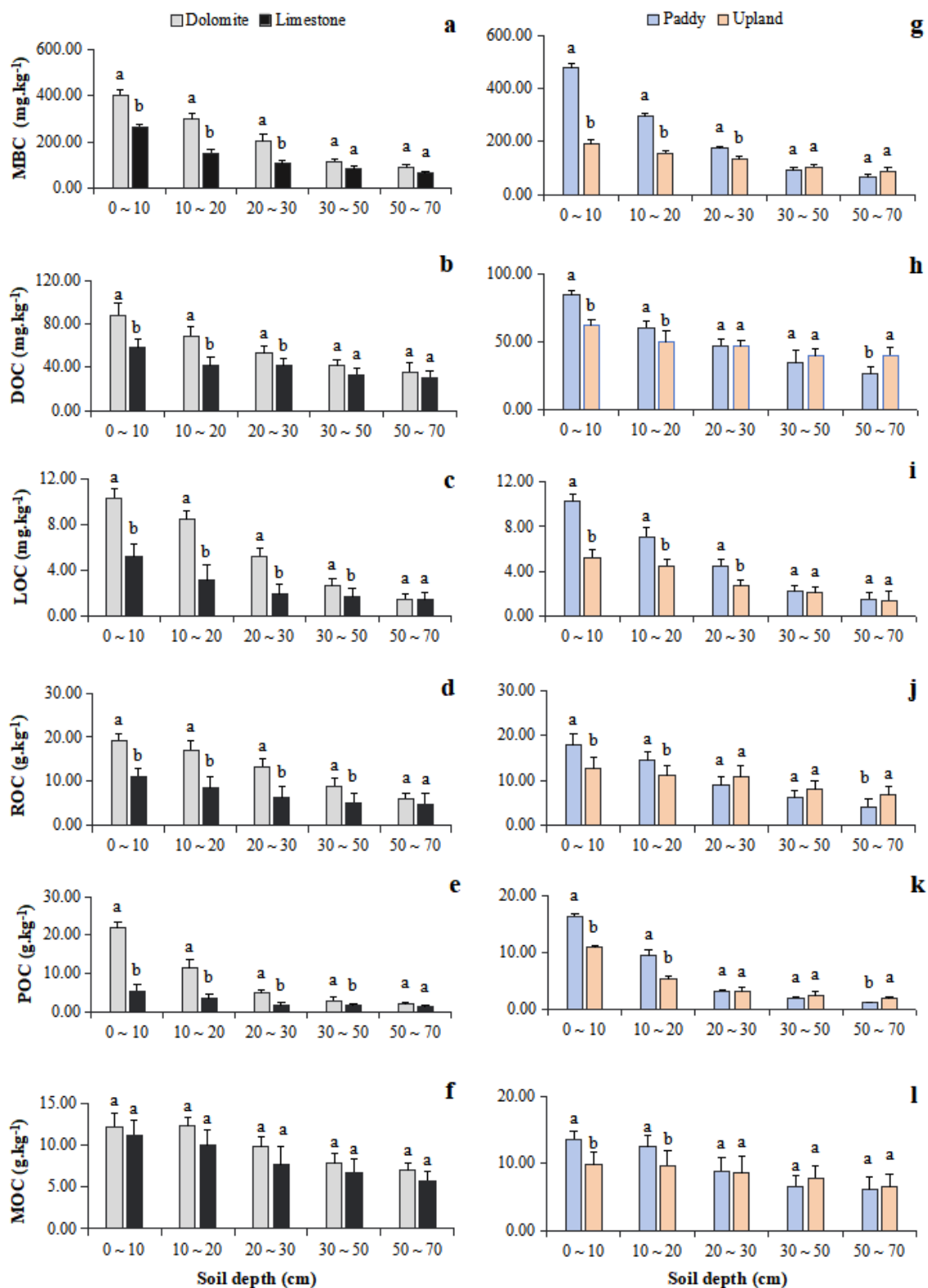
Stratified analysis by lithology revealed that land uses exerted significant impacts on both dolomite- and limestone-derived soils (Figure 3). The variation patterns of MBC, DOC, LOC and ROC contents under different land uses over dolomite were similar to those over limestone. Under dolomite parent material, no significant differences were observed in POC and MOC contents between paddy and upland soils across all soil layers. Moreover, paddy soils showed higher POC and MOC contents than those upland soils within the 0–20 cm layer over limestone, while the opposite pattern emerged in deeper soil horizons. Further analyses indicated that significant differences in SOC fractions between the two lithology types were found in both paddy and upland soils, with MBC, DOC, LOC, ROC and POC contents being significantly greater over dolomite. MOC contents in the 20–70 cm layers of paddy soil and 0–20 cm layers of upland soil were significantly higher over dolomite than over lithology.

**Table 1.** Results of three-way ANOVA for the effects of lithology (LT), land use (LU), soil depth (SD), and their interactions on SOC fractions.

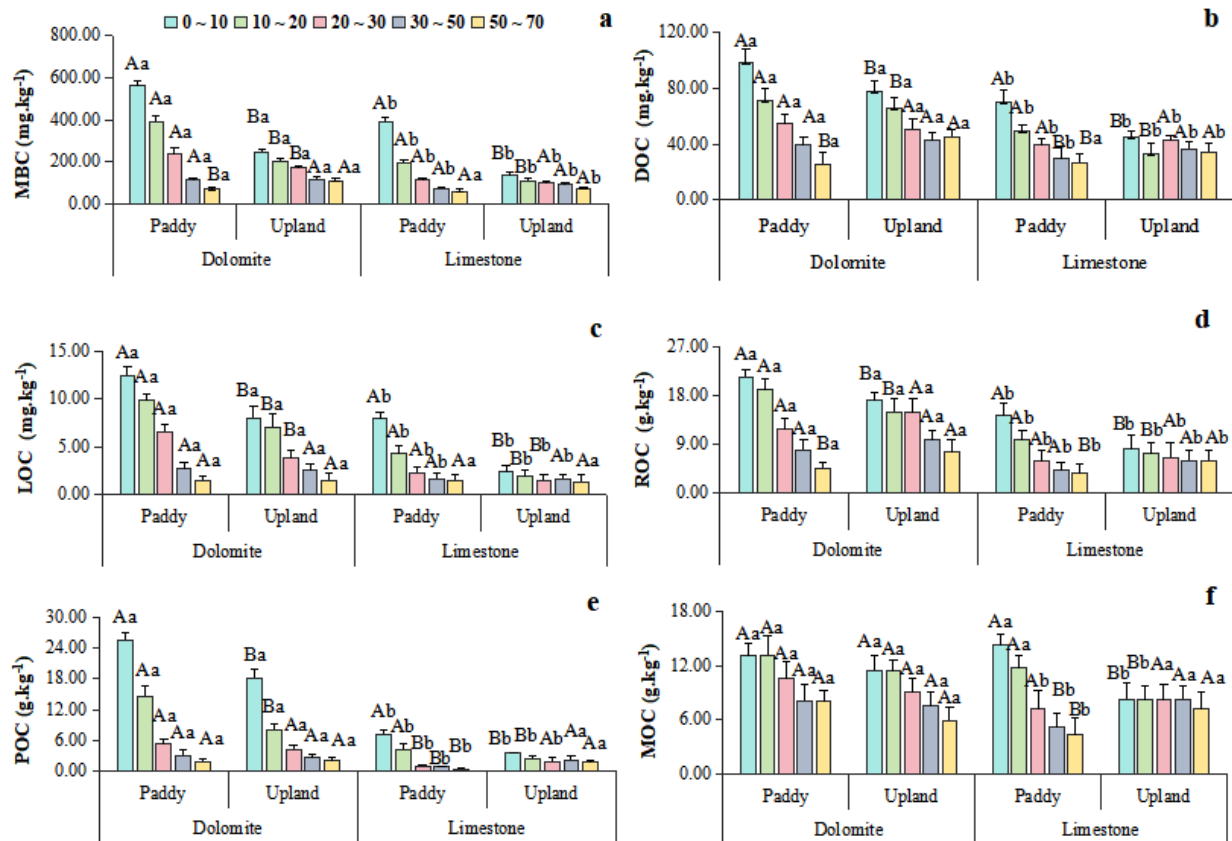
|              | MBC       | DOC      | LOC       | ROC       | POC       | MOC      |
|--------------|-----------|----------|-----------|-----------|-----------|----------|
| LT           | 68.58 *** | 11.72 ** | 52.13 *** | 46.54 *** | 19.51 *** | 3.68     |
| LU           | 65.44 *** | 0.36     | 21.82 *** | 0.25      | 1.61      | 1.58     |
| SD           | 78.22 *** | 8.67 *** | 31.86 *** | 18.21 *** | 10.99 *** | 6.72 *** |
| LT × LU      | 3.27      | 0.14     | 0.094     | 0.13      | 0.94      | 0.37     |
| LT × SD      | 5.89 ***  | 1.15     | 6.80 ***  | 2.78 *    | 4.64 **   | 0.11     |
| LU × SD      | 29.98 *** | 1.66     | 4.94 **   | 3.69 *    | 0.89      | 1.39     |
| LT × LU × SD | 1.15      | 0.156    | 0.37      | 0.18      | 0.056     | 1.19     |

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

Analysis of the proportional contribution of each fraction to total SOC demonstrated distinct stabilization mechanisms between the two lithologies or two land uses. Irrespective of lithology or land use, the same SOC fraction had the same change law with soil layers. The ratios of LOC/SOC in the 10–30 cm layers and POC/SOC in the 0–30 cm layers were significantly higher over dolomite than limestone, whereas the DOC/SOC and MOC/SOC ratios were higher in limestone soils (Figure 4). Land use types did not significantly affect the DOC/SOC, ROC/SOC, POC/SOC and MOC/SOC ratios, but the MBC/SOC and LOC/SOC ratios were higher in paddy soil.



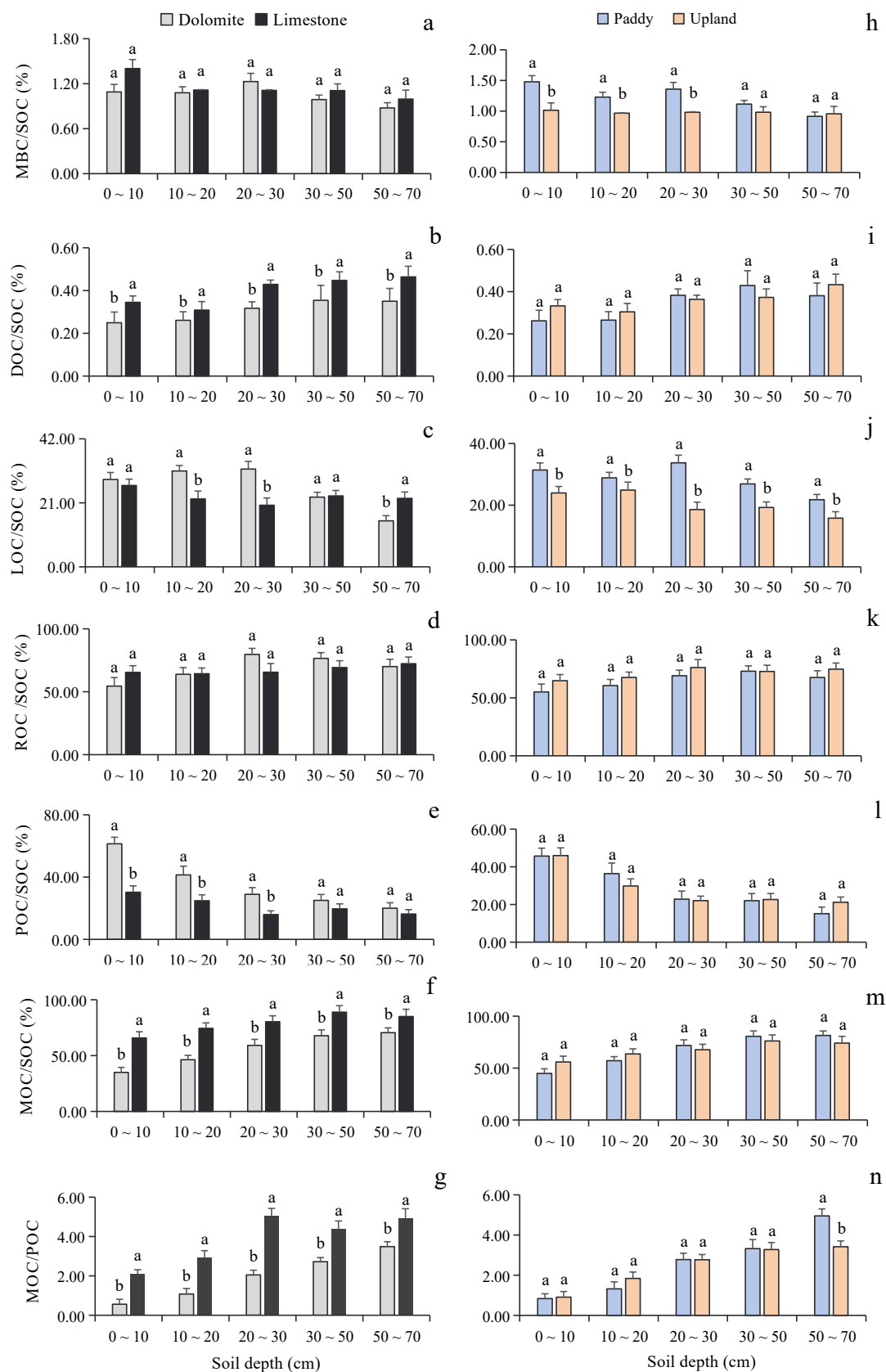
**Figure 2.** Contents of SOC fractions across five soil depths affected by lithology (dolomite vs. limestone) and land uses (paddy and upland). Panels (a–f) compare lithologies, while panels (g–l) compare land uses. Error bars represent standard error. Different lowercase letters indicate significant differences at  $p < 0.05$ .



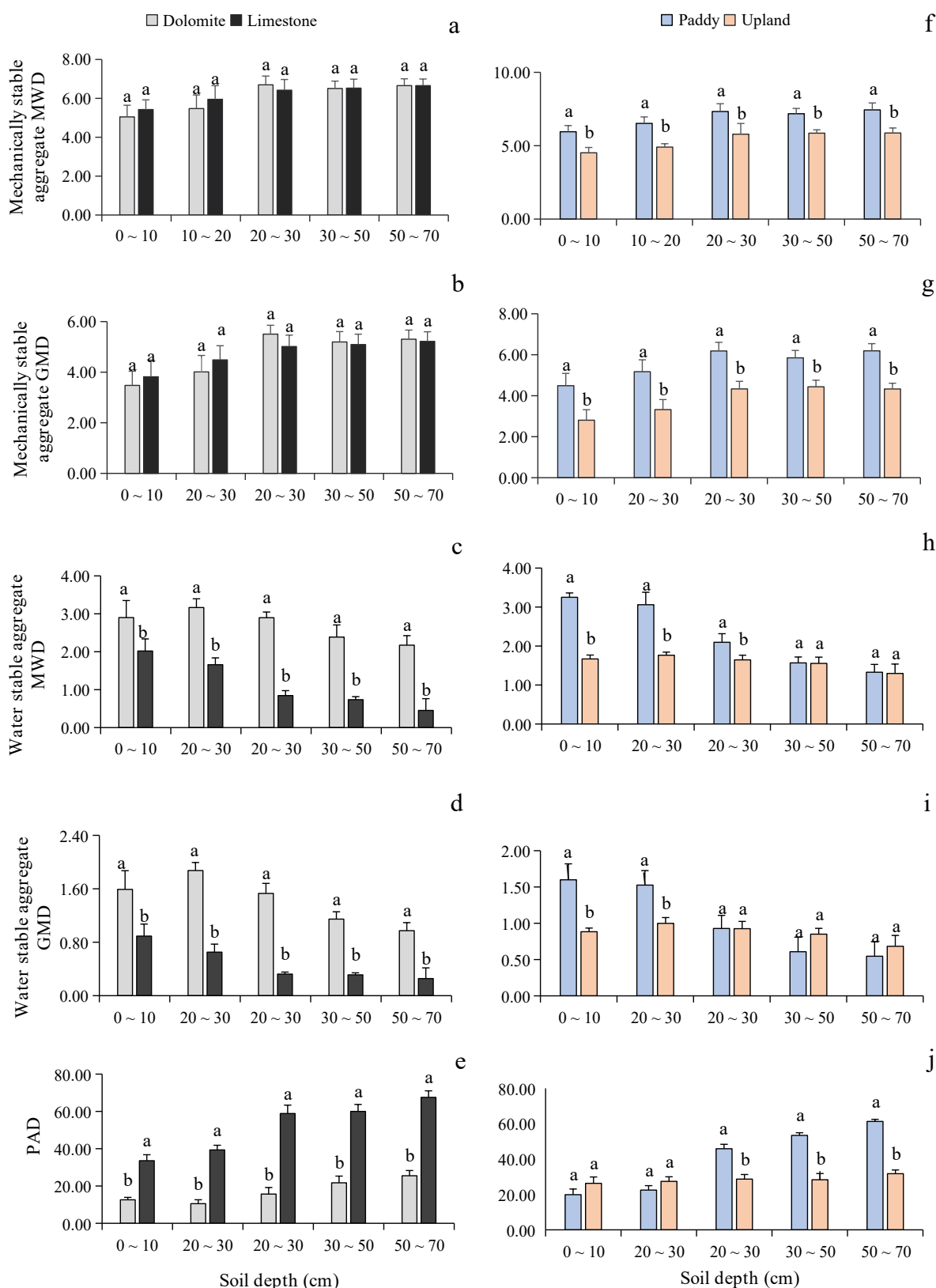
**Figure 3.** Effects of land use on contents of SOC fractions across five soil depths over dolomite and limestone, respectively. (a) MBC; (b) DOC; (c) LOC; (d) ROC; (e) POC; (f) MOC. Error bars represent standard error. Capital letters indicate differences between paddy and upland fields under the same lithology, while lowercase letters denote differences between dolomite and limestone under the same land use ( $p < 0.05$ ).

### 3.2. Stability of Soil Aggregates

The stability of water-stable aggregates was significantly greater in dolomite soils, as evidenced by significantly higher values for the mean weight diameter (WMWD) and geometric mean diameter (WGMD) of water-stable aggregates, along with a significantly lower PAD across all soil depths (Figure 5a–e). Aggregate stability was generally higher in paddy soils than in upland soils (Figure 5f–j), as indicated by the significantly elevated MMWD and MGMD of the mechanically stable aggregate across all depths and significantly greater WMWD and WGMD in the topsoil (0–20 cm). This pattern, however, was reversed in the 20–70 cm layer, where paddy soils showed a significantly increased PAD.



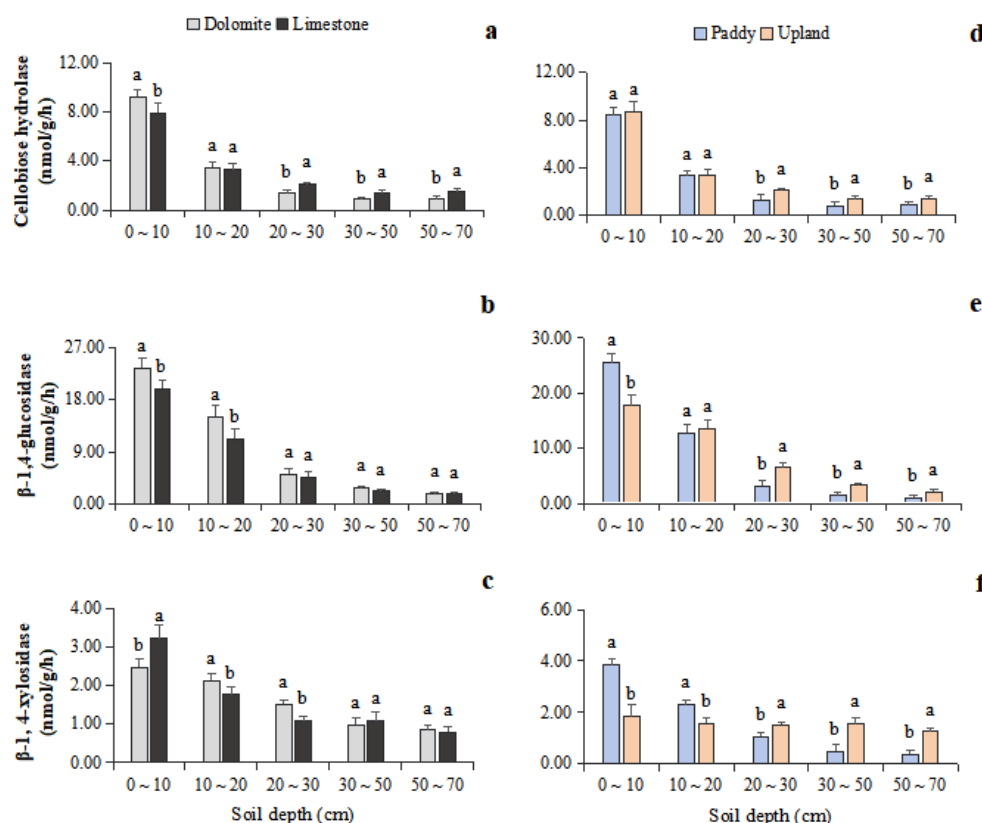
**Figure 4.** Proportional contribution of labile and stable fractions to total SOC and ratio of mineral-associated organic carbon to particulate organic carbon (MOC/POC) across five soil depths, as affected by lithology and land use. Panels (a–g) compare lithologies; panels (h–n) compare land uses. Different lowercase letters indicate significant differences at  $p < 0.05$ .



**Figure 5.** Aggregate stability indices across five soil depths as affected by lithology and land use. Panels (a–e) show comparisons between lithologies; panels (f–j) show comparisons between land uses. MWD and GMD are mean weight and geometric mean diameter for mechanically stable aggregates or water-stable aggregates; PAD is percentage of aggregate destruction. Different lower-case letters indicate significant differences at  $p < 0.05$ .

### 3.3. Characteristics of Soil Enzyme Activities

All three carbon-degrading hydrolases, including CBH, BG, and BX, exhibited significant decreasing activities with soil depth (Figure 6). The influence of lithology exhibited complex patterns: dolomite soils showed higher CBH and BG activities in the upper 0–20 cm and higher BX activity in the 10–30 cm layer, whereas limestone soils exhibited higher CBH activity in deeper layers (20–70 cm) and higher BX activity in the surface layer (0–10 cm). The effects of land use varied with soil depth: all three soil enzyme activities were consistently 58.35–225.52% higher in upland soils than in paddy soils throughout the 20–70 cm layers, while paddy soils showed significantly elevated activities of BG and BX.

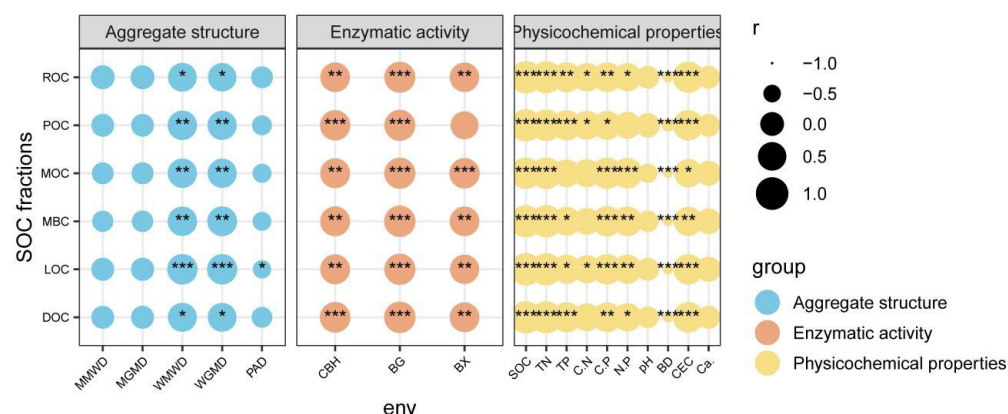


**Figure 6.** Activities of C-cycling enzymes across five soil depths as affected by lithology and land use. (a,d) Cellobiohydrolase (CBH), (b,e)  $\beta$ -1,4-glucosidase (BG), (c,f)  $\beta$ -1,4-xylosidase (BX). Different lowercase letters indicate significant differences at  $p < 0.05$ .

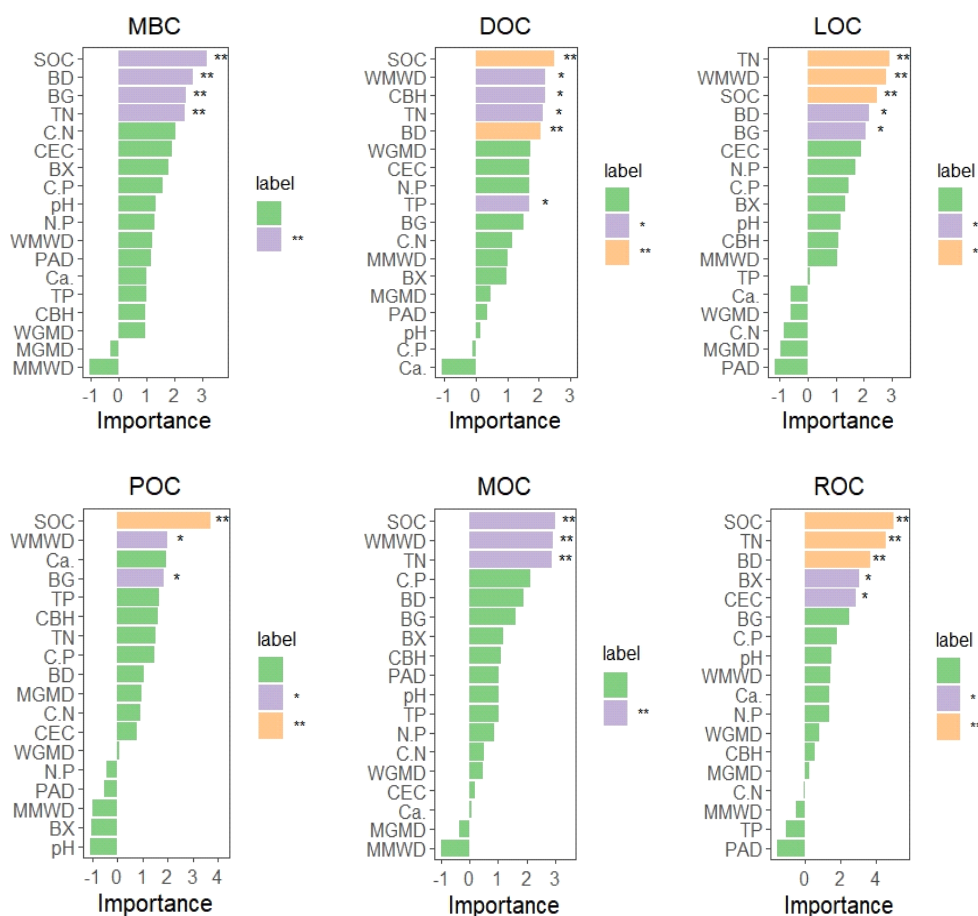
### 3.4. Relationships Between SOC Fractions and Environmental Factors

The correlation analysis bubble chart (Figure 7) shows the relationships between SOC fractions and aggregate structure, enzyme activities, and physicochemical properties. These carbon fractions were positively correlated with SOC, TN, TP, C/P ratio, N/P ratio, CEC, WMWD, WGMD, and enzyme activities (CBH, BG and BX). Conversely, they were negatively correlated with BD and PAD.

Random Forest analyses identified the key controlling factors governing the distribution patterns of soil carbon fractions (Figure 8). MBC was significantly influenced by SOC, BD, BG and TN. SOC and BD were the most important variables for predicting DOC, followed by WMWD, CBH, TN and TP. TN, WMWD, SOC, BD and BG were important factors affecting LOC. SOC, TN, BD, BX and CEC were the main causes of ROC variation. SOC was identified as the most important factor for explaining the variation in POC, followed by WMWD and BG. MOC was significantly influenced by SOC, WMWD and TN.



**Figure 7.** Spearman correlation heatmap showing relationships between soil carbon fractions and key environmental factors, including soil properties, aggregate stability indices, and enzyme activities. The size of the circles represent the magnitude and direction of the correlation coefficients. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . SOC fractions (particulate, POC; mineral-associated, MOC; recalcitrant, ROC; microbial biomass, MBC; dissolved, DOC; permanganate-oxidizable, LOC); MMWD and MGMD, weight and geometric mean diameter for mechanically stable aggregates; WMWD and WGMD, weight and geometric mean diameter for water-stable aggregates; PAD, percentage of aggregate destruction; CBH,  $\beta$ -D-cellobiosidase; BG,  $\beta$ -1,4-glucosidase; BX,  $\beta$ -1,4-xylosidase; TN, total nitrogen; TP, total phosphorus; BD, bulk density; CEC, cation exchange capacity; C.N, C/N; N.P, N/P; C.P, C/P; Ca., exchangeable Ca.

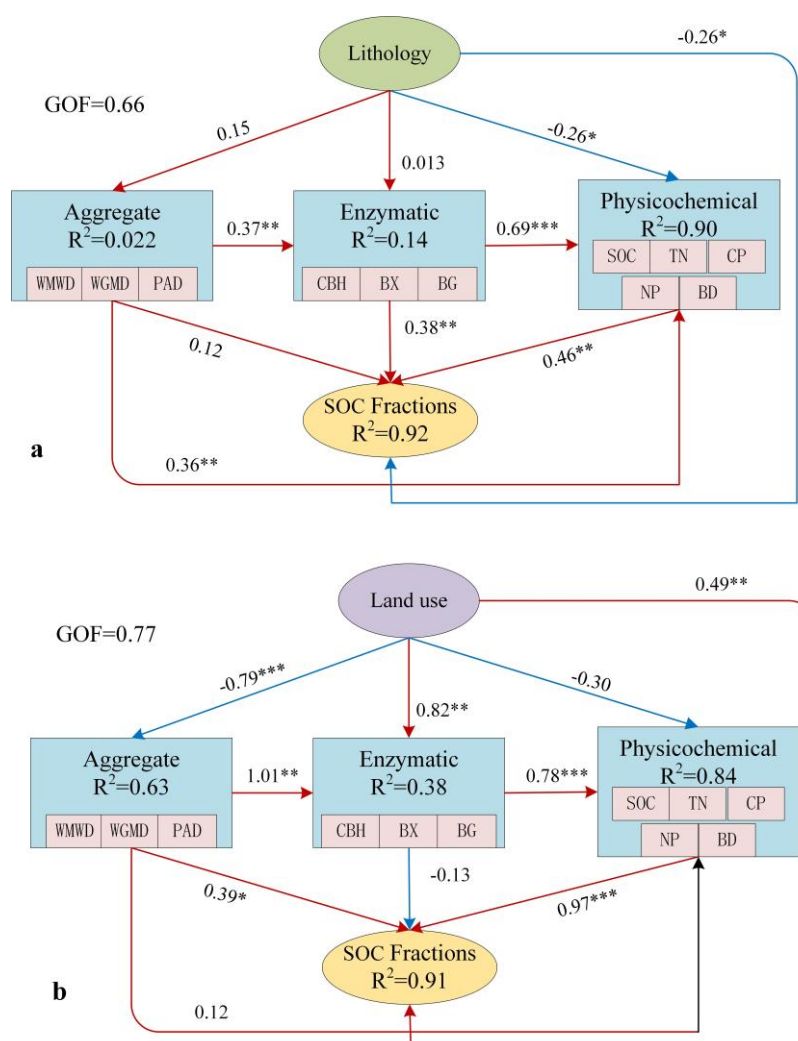


**Figure 8.** Relative importance of environmental variables in predicting soil organic carbon (SOC) and its fractions, determined by Random Forest models. SOC fractions (particulate, POC; mineral-

associated, MOC; recalcitrant, ROC; microbial biomass, MBC; dissolved, DOC; permanganate-oxidizable, LOC); MMWD and MGMD, weight and geometric mean diameter for mechanically stable aggregates; WMWD and WGMD, weight and geometric mean diameter for water-stable aggregates; PAD, percentage of aggregate destruction; CBH,  $\beta$ -D-cellobiosidase; BG,  $\beta$ -1,4-glucosidase; BX,  $\beta$ -1,4-xylosidase; TN, total nitrogen; TP, total phosphorus; BD, bulk density; CEC, cation exchange capacity; C.N, C/N; N.P, N/P; C.P, C/P; Ca., exchangeable Ca. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

### 3.5. Path Analysis of Lithology and Land Use on SOC Fractions

The partial least squares path modeling (PLS-PM) clearly delineated the direct and indirect pathways through which lithology and land use affected SOC fractions (Figure 9). GOF indices were 0.66 and 0.77, suggesting good fits for the used models. As demonstrated by the models, lithology had significantly indirect effects on SOC fractions via soil physicochemical properties, such as bulk density, nutrient content and stoichiometric ratio. Land use primarily influenced SOC fractions via indirect pathways. It first affected soil aggregate structure or enzyme activities, which in turn altered soil physicochemical properties, ultimately regulating the contents of the various carbon fractions. Additionally, both lithology and land use had significantly direct effects on SOC fractions. Overall, the models explained 92% to 91% of the variance in SOC fraction, indicating that the constructed pathways effectively elucidated the driving mechanisms of SOC fraction dynamics in the study area.



**Figure 9.** Partial least squares path modeling (PLS-PM) illustrating the direct and indirect effects of lithology (a) and land use (b) on SOC fractions. Solid arrows indicate positive paths, and dashed

arrows indicate negative paths. Path coefficients and significance levels (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ) are shown. SOC fractions (particulate, POC; mineral-associated, MOC; recalcitrant, ROC; microbial biomass, MBC; dissolved, DOC; permanganate-oxidizable, LOC); WMWD and WGMD, weight and geometric mean diameter for water-stable aggregates; PAD, percentage of aggregate destruction; CBH,  $\beta$ -D-cellobiosidase; BG,  $\beta$ -1,4-glucosidase; BX,  $\beta$ -1,4-xylosidase; TN, total nitrogen; BD, bulk density; N.P, N/P; C.P, C/P.

## 4. Discussion

### 4.1. Vertical Characteristics and Controlling Factors of SOC Fractions and Stability

Our results confirmed significant depth-dependent declines in all measured SOC fractions, consistent with well-established vertical stratification patterns in soil profiles. This was likely related to the natural distribution of organic carbon content in soil profiles [29], but was mainly caused by abundant plant residues increasing carbon input to the topsoil, and there being less vegetal roots and limited microorganism activity in the subsoil [30]. A large-scale study [31] highlighted the depth-dependent dichotomy in SOC controls—anthropogenic factors (land use/history) dominating in surface horizons versus geogenic factors (parent material/stratigraphy) prevailing in subsoils. In the agroecosystem, crop residues, manure, and chemical fertilizers applied for biomass and grain yield maximization were identified as critical factors regulating topsoil SOC content [32,33].

Our study revealed distinct stabilization mechanisms for different SOC fractions along the vertical profile. Labile SOC fractions exhibit greater sensitivity to environmental changes than bulk SOC and serve as dynamic indicators of SOC pool fluctuations [34]. In this study, the labile SOC pool was characterized by four operational fractions: MBC (0.88–1.40% of SOC), DOC (0.25–0.46%), LOC, (15.10–32.09%), and POC (12.29–61.39%). We found that the proportions of MBC, LOC, and POC in total SOC decreased significantly with increasing soil depth, indicating attenuated microbial processing capacity due to oxygen deficiency and reduced aggregate-mediated protection of organic carbon in subsurface layers [35]. Random Forest analysis suggested that this pattern may be attributed to the depth-dependent decreases in: (i) soil enzyme activities (particularly BG), (ii) aggregate stability (WMWD), and (iii) TN content. In contrast, the DOC/SOC ratio, as a key indicator of carbon availability for microorganisms [36], exhibited an increase with soil depth, indicating that DOC constitutes one of the primary sources of subsoil SOC [37]. Characterized by high lability and mobility within the SOC pool, DOC can be transported to most soil compartments through diffusion and convection processes. Therefore, the primary mechanism appears to be a two-stage process: vertical transport of surface-generated DOC via preferential flow paths, and subsequent stabilization through selective sorption reactions with reactive mineral surfaces in subsurface horizons [38].

Furthermore, the analysis of MOC and ROC fractions revealed crucial information about SOC stabilization mechanisms. The ratios of MOC/SOC and MOC/POC were calculated to analyze the physical stability of soil C, and higher MOC:SOC and MOC:POC are generally considered indicators of greater SOC stability [39,40]. Previous studies have demonstrated that MOC:SOC and MOC:POC are higher in the subsoil than topsoil in limestone soil [40], and our study showed similar results. This indicates that subsoil organic carbon exhibits a stronger mineral association compared to topsoil carbon. This phenomenon may be attributed to the higher proportion of microaggregates in subsoil horizons, where a significant fraction of carbon is strongly protected through physical occlusion. The ROC/SOC ratio increases with depth, indicating that as total organic matter content decreases, the residual fraction becomes increasingly enriched in chemically recalcitrant compounds, including lignin and other complex biomolecules [41,42]. Driven by increasing physical (MOC/SOC) and chemical (ROC/SOC) stability with depth, the subsoils

of these karst ecosystems form a critical carbon reservoir, securing it against decomposition through multiple mechanisms.

#### 4.2. Land Use Effects on SOC Fractions: Paddy Versus Upland Systems

Compared with upland soil, paddy soil exhibits a more pronounced vertical distribution of SOC fractions, primarily due to the greater accumulation of organic carbon in the surface layer of paddy soil. Similar findings were reported in fire-affected upland soils under rotational shifting cultivation, where high surface TOC and more negative  $\delta^{13}\text{C}$  values also reflected enhanced carbon inputs and limited surface decomposition [43]. Relevant studies indicate that organic carbon content in the surface layer of paddy soils is generally 1.5–1.8 times greater than that of upland soils [32]. Land use regulates the composition and turnover of SOC fractions primarily through biogeochemical mechanisms such as carbon input pathways, microbial metabolic efficiency, and organo-mineral stabilization processes [44,45]. The pronounced accumulation of all SOC fractions in the surface layer (0–20 cm) of paddy soil, especially the sharp increases in MBC and LOC, can be attributed to the anaerobic conditions characteristic of flooded environments. The particularly significant rise in MBC and LOC underscores a fundamental shift in microbial ecology: flooding creates a metabolic bottleneck where labile compounds accumulate due to the low efficiency of anaerobic respiration, thereby enlarging the pool of transient organic carbon [35]. Furthermore, the regular input of organic substrates from rice cultivation provides a constant supply of energy [46], which, under anaerobic conditions, is inefficiently utilized, leading to the disproportionate buildup of these labile pools [47]. In the 20–50 cm soil layer, significant differences are absent in most organic carbon fractions between paddy and upland soil. The subsoil receives minimal fresh carbon inputs from roots and no inputs from aboveground residues [48], and experiences long-term physical isolation of organic matter due to the absence of intensive tillage [32,49]. This means that land use-induced differences have been attenuated or obscured in subsoil. In contrast, below 50 cm depth, upland soils exhibited significantly higher SOC and SOC fractions (especially DOC, ROC, and POC) than paddy soils. This aligns with the findings of Wei et al. (2021) [32], who reported that carbon allocation declines with increasing soil depth, thereby resulting in a smaller surplus of C stocks in paddy subsoils than in upland subsoils. This discrepancy may be attributed to the contrasting root architectures: compared with shallow-rooted rice, upland crops such as maize are deep-rooted, and their root exudates and the decomposition of dead roots supply carbon inputs to the subsoil. These findings appear to indicate that contrasting land uses drive depth-dependent mechanistic shifts in carbon sequestration pathways.

Despite higher organic carbon concentrations in paddy surface soils, their stability shows no significant divergence from upland systems. Consistent with existing studies revealing negligible differences in C-mineral associations between cropping systems [50], our results further demonstrate comparable MOC/SOC ratios across land uses, collectively challenging the hypothesis of superior carbon stability in paddy soils and underscoring consistent mineral stabilization efficiency regardless of hydrological regimes. However, there is a significantly higher MOC/POC ratio in the 50–70 cm layer of paddy soils. This pattern may be attributed to the relatively higher POC content in deeper upland soils, where aerobic conditions and biological activity (e.g., root turnover and bioturbation) sustain greater POC accumulation. This further demonstrates that POC is more susceptible to environmental influences such as land uses and carbon input patterns than MOC [34].

Soil aggregation plays a pivotal role in agricultural carbon sequestration, as it simultaneously facilitates physical occlusion of particulate organic matter and enhances chemisorption at mineral surfaces [51]. Consequently, the significantly higher water-stable aggregate stability indices (WMWD and WGMD) in paddy surface soils compared to upland systems (Figure 5) serve as the dominant factor controlling the sequestration of their elevated organic carbon fractions, primarily through enhanced physical protection within aggregate architectures. In addition, land uses directly regulate soil enzyme activities, which subsequently modify soil physicochemical properties (such as BD, TN and nutrient stoichiometric ratio), ultimately shaping the composition and turnover of soil organic carbon fractions through microbially driven biochemical pathways. This is corroborated by a study on the conversion of upland soil to paddy fields in Northeast China, which found that land use change significantly altered the activity of carbon-cycling enzymes ( $\beta$ -glucosidase, xylosidase, and cellobiohydrolase) within soil aggregates, and these changes were closely correlated with shifts in soil organic carbon content [52]. The unique cultivation practices in paddy systems reconfigure soil aggregate structure and microbial community distribution [53]. Different aggregate classes develop distinct microenvironments and architectures that selectively host microbial populations with specialized functional traits, ultimately driving carbon utilization and storage through coupled biotic and abiotic interactions [54].

#### *4.3. Lithology-Controlled Vertical Stratification and Stability of SOC Fractions*

Our results demonstrate that upland soils on limestone display significantly attenuated vertical heterogeneity in SOC fractions compared to dolomitic uplands, implying lithology-specific modulation of organic matter transport and retention processes within the soil profile. We postulate that this homogeneity arises from stronger organo-mineral complexation driven by elevated pH and abundant  $\text{Ca}^{2+}$  ions throughout the soil profile [55], which promotes uniform carbon stabilization across depths. Conversely, the steeper decline in SOC with depth in dolomitic uplands suggests a stronger reliance on depth-dependent protective mechanisms that are less effective or consistent in the dolomite weathering environment. This divergence likely reflects the lower  $\text{Ca}^{2+}$  availability and higher insoluble residue content in dolomite, which promote a more stratified distribution of organic matter. However, paddy soils developed on both lithologies exhibit a convergent vertical zonation of SOC fractions, with a pronounced decline in concentration corresponding to increased soil depth. The reduced expression of underlying lithological differences is likely because the flooded paddy field environment exerts a strong overprinting effect.

Furthermore, this study found that lithology can directly or indirectly regulate SOC fractions, with a clear depth-dependent attenuation of lithological effects in deeper layers. Notably, dolomite-derived soils accumulated a larger SOC pool in the upper 30 cm, characterized by a greater proportion of labile carbon (MBC, DOC, LOC, POC) relative to limestone-derived soils, suggesting enhanced carbon sequestration potential. We attribute this phenomenon primarily to the mineralogical traits of dolomite. The core mechanism lies in the differences in the mineral chemical composition (mainly carbonate minerals) between the two lithologies and the variations in weathering processes and rates controlled by these compositions. Dolomite is characterized by the chemical component of calcium magnesium carbonate, whereas limestone predominantly consists of calcium carbonate [56]. The high solubility of calcium carbonate and its minimal insoluble residue content lead to slow pedogenesis. In contrast, dolomite exhibits a substantially higher proportion of insoluble residues, which provide the essential skeletal framework and serve as the fundamental matrix for soil aggregate formation. These differences, in turn, profoundly affect the physical, chemical, and biological environments of soil [57]. Specifically, these

insoluble residues (primarily clay minerals and silicates) promote the development of water-stable aggregates and reduce bulk density, while the magnesium-rich weathering environment favors nitrogen retention through enhanced cation-bridging with organic matter [58]. Consequently, dolomite soils exhibited significantly greater water-stable aggregate stability (WMWD), higher TN content, and lower bulk density (BD) (Table S2). Random Forest analyses further identified WMWD, TN and BD as critical predictors for SOC fractions, which is consistent with the well-documented tight coupling between soil N and C in various ecosystems [59], the negative correlation between BD and SOC content [60], and the pivotal role of aggregate stability and composition in SOC sequestration and dynamics [61]. Dolomite soils exhibited significantly greater water-stable aggregate stability and TN and lower BD (Table S2). We therefore propose that the higher labile SOC fractions in dolomite soils are driven by a combination of these mineralogically derived traits that collectively slow decomposition: greater aggregate stability physically shields organic matter, while higher TN content may stabilize C by improving the C:N balance for microbial retention. Concurrently, the looser soil structure likely creates a more aerated environment that, contrary to expectations in some contexts, may limit the persistence of anaerobic microsites and slow the rate of organic matter breakdown.

However, MOC/SOC and MOC/POC ratios in limestone soils are significantly higher than dolomite soils, revealing that a larger proportion of extant carbon in limestone soils is stabilized through complex mineral interactions. Previous studies have shown that in soils with relatively low carbon content, carbon storage is predominantly mediated by MOC [62]. This can be explained by the MOC saturation concept: before reaching saturation capacity, carbon accrual is dominated by MOC formation; beyond this threshold, POC accumulation becomes the primary pathway [63]. In our study, limestone soils, exhibiting lower SOC content and a diminished POC pool, likely remain below MOC saturation. This sub-saturation state promotes efficient stabilization of newly incorporated carbon onto reactive mineral surfaces. In contrast, dolomite soils experience higher carbon loading, potentially driving the system toward MOC saturation. Upon approaching this threshold, mineral-associated stabilization is compromised, redirecting subsequent carbon accumulation toward the formation of a labile POC pool. As a consequence, dolomite soils exhibit a higher total carbon storage capacity, but the carbon retained within limestone soils demonstrates a proportionally greater degree of stability. This is consistent with the view of Schmidt et al. [64], who emphasized that sequestration potential should be assessed not only by total SOC stocks, but also by the composition and stability of carbon pools.

Overall, these findings show that C management in karst agroecosystems should be site-specific. Lithology plays a dominant role in upland soils, but flooding in paddy systems reduces this lithological influence. Therefore, future management should map lithology and land use together to enhance C storage and persistence.

## 5. Conclusions

This study identified that the vertical distribution and stability of SOC fractions are co-determined by land use management and lithology controls in karst agroecosystems. Land use drives a depth-dependent reversal in concentrations of SOC fractions. The anaerobic environment of paddy fields promotes the accumulation of all SOC fractions (particularly MBC and LOC) in surface horizons, but well-aerated upland systems stabilize greater quantities of SOC fractions (DOC, ROC, POC) below 50 cm depth. This suggests that the carbon storage capacity of subsoils in upland systems may have been previously underestimated. Furthermore, there was no significant difference in soil organic carbon stability between paddy and upland soils. The inherent differences in weathering and soil properties between dolomite and limestone may lead to distinct pathways of soil organic

carbon stabilization, as reflected by the distribution of SOC fractions. Soils derived from dolomite exhibit higher concentrations of several SOC fractions, whereas soils derived from limestone contain a greater proportion of SOC stabilized through mineral association, resulting in a more stable carbon pool. Land uses primarily influence SOC fractions indirectly by altering soil structure. Sustainable strategies for enhancing carbon sequestration in karst regions must therefore integrate management practices that leverage the inherent mineralogical potential of the soil, with a specific focus on conserving and enhancing the stable deep carbon pool in upland systems. Future research should prioritize understanding the long-term fate of deep carbon and its vulnerability to land use change.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agriculture16141495/s1>: Figure S1: Distribution of soil mechanical stability aggregates; Figure S2: Distribution of soil water-stable aggregates; Table S1: Site characteristics of the sampling plots; Table S2: Soil physicochemical properties under different lithologies and land use across various soil depths. SOCS (SOC storage).

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