

Original Research

# Soil Carbon Stock is Modulated by Nutrients Contents and Enzyme Activities in a Subalpine, Southwest China

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## Abstract

Little is known about the land use and vertical patterns of the soil organic carbon (SOC) and soil inorganic carbon (SIC) pools in the subalpine grassland of the Guizhou Plateau with experienced extensive landuse changes. We investigated Unused natural grassland (UG), Buffalo grazing grassland (BG), Mosaic grassland (MG), Shrubland (SL), Afforest land (AL), Secondary forest land (SF) and Cropland (CL) to depict the land use- and depth-related patterns of SOC and SIC in the upper half-meter soils. The results displayed that MG had the highest SOC stocks (16.21 kg m<sup>-2</sup>), but BG had the lowest SOC stocks (3.62 kg m<sup>-2</sup>). BG had the highest SIC stock (2.01 kg m<sup>-2</sup>), but SL had the lowest SIC stock (0.93 kg m<sup>-2</sup>). SOC stock was more affected by land use and SIC stock was more affected by soil depth. In addition, land use impacted the SOC stock within specific soil chemical ranges, land use indirectly changed the SIC stock via its influence on soil chemical properties. This study provided interesting snapshots on the effects of land use and soil depth on soil physicochemical properties, enzyme activities and SOC and SIC stocks with the regulations of soil factors.

**Keywords:** organic carbon, inorganic carbon, land use, conditional inference tree, path model

## Introduction

Serving as the largest terrestrial carbon (C) pool [1-4], the soil C pool has received considerable attention [5-7]. Its loss can increase the global atmospheric CO<sub>2</sub> content [8-9], enhance global warming [10-11], and lower soil quality [12] and productivity [12], thereby threatening food security [12]. Therefore, it is

advantageous and urgent to maintain and increase soil C stocks [4, 12].

As the second largest source of anthropogenic greenhouse gas [1, 13], land use change significantly modifies soil organic and inorganic carbon stocks [7, 14]. Therefore, SOC and SIC stocks is crucial for depicting the land use patterns of soil C [15-16]. In fact, land use alters soil C via series of cascade processes [17]. Land use change first transforms the type, biomass, cover, and quality of plants [18-19] and these processes further reshape the inputs and depth distributions of the plant C [20-21], soil respiration [18-19], runoff

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[22-23], soil erosion [7, 9] and soil leaching [15]. In addition, the above changes and vegetation intake affect the physicochemical (including SOC and SIC) [9, 21] and biological properties [9] of the soil, which in turn affect the input of vegetative C [24], dissolution and precipitation of SIC [7, 15] and soil respiration [18-19]. Furthermore, the changes in runoff, soil erosion and soil leaching affect the transfers of soil C [9, 15]. Ultimately, land uses shape soil C distribution patterns and stocks [24-25].

Inevitably, the effect of land use on soil C stock involves the physical, chemical, and biotic properties of the soil. A study in the Qinghai-Tibetan Plateau found that land-use-induced redistribution patterns of SOC and SIC stocks were primarily driven by soil nitrogen and pH [7]. In addition, a study in the Loess Plateau found that the SIC stock in the topsoil was adversely affected by the SOC stock and root mass density, but in the subsoil, it was positively influenced by silt and negatively influenced by sand contents [10]. Moreover, a study in the Yellow River Delta found that 80% of the total changes of SOC stock were caused by soil pH, silt+clay, water content, saline concentration, and their interaction [20]. Further, the soil enzymes that are secreted by microbiota and roots [26] decompose complex organic material into simpler forms and acquire resources for their producer [27]. For instance, b-1, 4-glucosidase (BGC) hydrolyses cellulose, b-1,4-N acetylglucosaminidase (NAG) catalyses chitin and peptidoglycan degradation, leucine aminopeptidase (LAP) catalyses proteolysis, and acid phosphatase (ACP) hydrolyses phosphate from phosphosaccharides and phospholipids [28]. Studies also found that soil C was increased by the suppression and enhancement of special soil enzyme activities [26]. Therefore, both soil physicochemical and enzymes activities may be the crucial modulators of soil C stock. In general, SOC stocks are constrained by direct plant-derived C input [21, 25] and are significantly affected by the soil nutrition supply, especially soil nitrogen (N) and phosphorus (P) in subtropical regions [29]. Thus, the SOC fluctuation is usually considered as a biological process. However, the fluctuation of SIC stock with land use is primarily associated with soil pH [4, 16], calcium ions, magnesium ions ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) [16, 30], and leaching [15]. Therefore, it is universally acknowledged that SIC fluctuation is a physical and chemical process [21], and this process is also described by several chemical reaction equations in substantial studies [21, 30].

The factors that impact SIC have been considered to be distinct from the factors that impact SOC [7]. However, a growing number of studies have reported a close relationship between SOC and SIC stocks [30, 31], suggesting that underlying mechanisms link SOC and SIC stocks [7]. The recent study concluded that SIC was formed from its precipitation with Ca that is released from shrub litter [21]. Further, aboveground and underground biomass also impact SIC [7, 31],

increasing SOC through organic amendments that enhance the accumulation of SIC accumulation [23]. A recent study even found that soil enzymes such as carbonic anhydrase can mediate the precipitation of SIC [32]. Therefore, we speculate that the regulators of SOC stock, e.g., the physical, chemical, and biotic properties of the soil, may regulate the SIC stock [1, 33].

In China, most studies of C stock under land uses have been performed in the Loess Plateau [9, 21], the Qinghai-Tibetan Plateau [7, 34], and the Mongolian plateau [34], but few investigations have been conducted in the Guizhou plateau. It has experienced extensive and drastic land use changes over the past decades due to the cultivation of native grassland, the intense grazing, afforestation, and nature recovery practices that occur on deforested land and grassland may significantly reshape the soil C pool. The few studies that have been conducted in this ecoregion found that land uses changed the soil physical, chemistry and biochemistry characteristics [35], which in turn significantly affected the accumulation of SOC [35]. However, SIC under different land uses remains to be clarified in the Guizhou plateau.

The objects of this study are (1) to characterize the vertical distribution of SOC and SIC stocks under seven major land uses in Guizhou plateau, (2) to test the role of land use, soil depth, soil physicochemical and enzymatic properties in shaping the distribution of SOC and SIC stocks, and (3) to determine the implications for land and soil C management.

## Material and Methods

### Study Site

The study was conducted in the natural grassland district of Longli County in the Guizhou plateau in SW China in early October of 2017. This district is a typical mid-subtropical humid region (code R16) according to the regional division of China's terrestrial ecosystems reported by Xu et al. [24]. The mean annual temperature is 14.7°C, ranging from -3 °C in January to 35°C in July. The mean annual precipitation is approximately 1160 mm, mostly falling from May to September.

### Experimental Design and Sampling

We chose seven land uses, including Buffalo grazing land (BG), Unused natural grassland (UG), Mosaic grassland (MG), Shrubland (SL), Afforest land (AL), Secondary forest land (SF) and Cropland (CL). All of the studied sites had the same soil parent materials and climate. Similar to many studies [7, 10], to minimize the variations from non-land use sources, the sites were all located on the mountain slope with little difference among the sites in altitude (1450-1620 m), gradient and topographical condition. The zonal soil was Haplic

Alisols [36]. Detailed information is available in <https://github.com/dlltargeting/land-use-and-soil-carbon>.

The soil was sampled in the upper 50 cm depth due to the universally shallow soil depth in this region. A plot (1 × 1 m) for grass community and/or a plot (5 × 5 m) for shrub community and/or a 10 × 10 m plot for forest community were used for surveys of vegetation species. Within each site, soil cores were collected using the methodologies outlined by Ding [36, 37]. All of the samples were immediately transported under ice bags and stored at 4°C until the subsequent assay. In total, we obtained 210 samples.

### Soil Physicochemical Properties Assay and Enzymatic Activities Assay

Assay methods we used to analyse soil physicochemical properties and extracellular enzyme activities follow studies of Ding et al. [36, 38].

### Statistical Analysis

ANOVA was used to analyse the effects of land use and soil depth and their interaction on the soil factors with reporting the partial eta squared ( $\eta^2$ ) to rank the effect sizes of land use and soil depth and their interaction [37] (IBM SPSS version 25.0, IBM Corp., NY, USA). Spearman correlation was applied to examine the relationships between SOC and SIC

with reporting of Holm corrected  $p$ -values. Regression analysis was used to explore the relationships between C and soil factors via using the function “lm” in R. The variance inflation factor (VIF) of variables are commonly recommended to be lower than 5.0 to avoid collinearity [39]. Conditional inference tree analysis is used to identify the thresholds for the effects of land uses, soil depth, STPc, LAP, etc. on SOC and SIC pools [17] by using the “partykit” package in R. Path analysis was implemented to examine the potential pathways that can account for how land use and soil depth alter the SOC stock or the SIC stock by using the “lavaan” package in R. A model was acceptable at Bollen-Stine Bootstrap  $p > 0.05$ ,  $0.97 \leq$  Comparative Fit Index (CFI)  $\leq 1$ ,  $0 \leq$  Root Mean Square Error of Approximation (RMSEA)  $\leq 0.05$  and  $0 \leq$  Standardized Root Mean Square Residual (SRMR)  $\leq 0.05$  [40].

## Results and Discussion

### Soil Physicochemical and Enzymatic Properties under Different Land Uses

Soil factors, including soil physicochemical and enzymatic properties, changed with land uses and soil depths (Fig. 1). Land use significantly impacted the soil physicochemical and biological properties (ANOVA,  $p < 0.05$ ), whereas soil depth significantly impacted

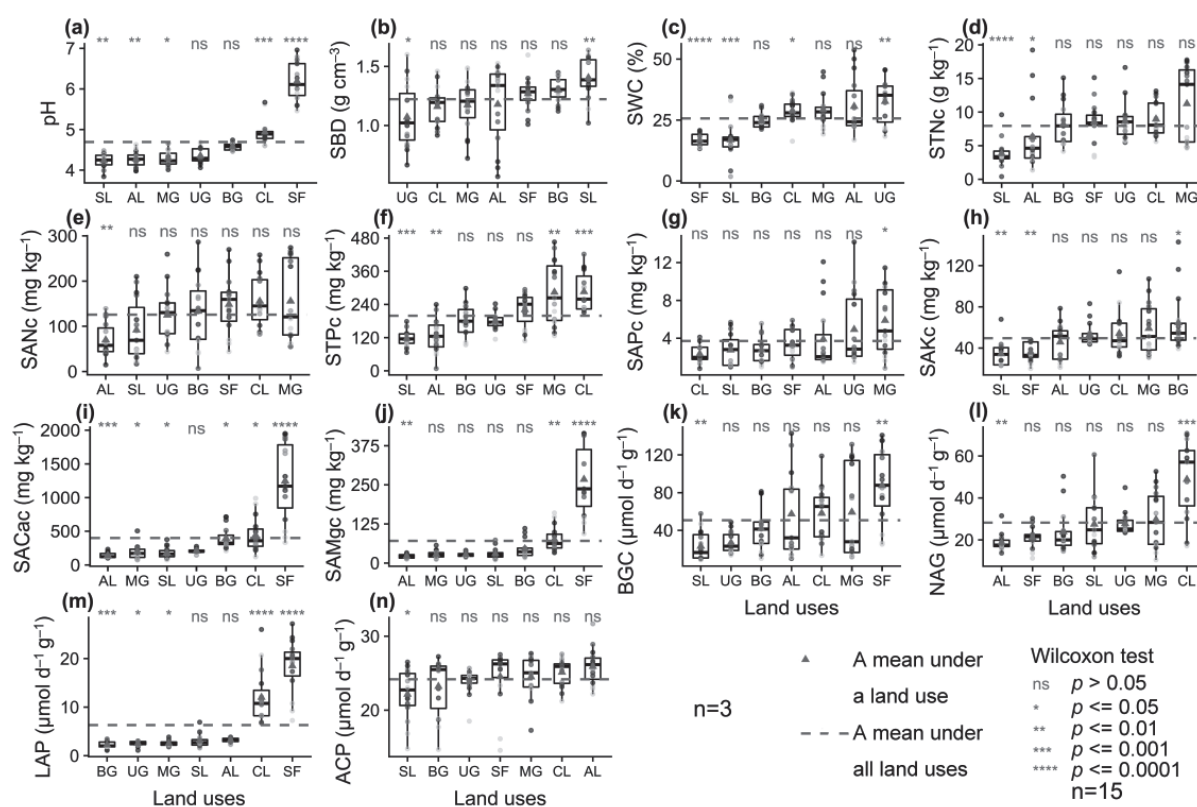


Fig. 1. Soil factors (soil physicochemical properties and enzyme activity) under seven land uses. Box plots illustrate upper and lower quartiles, error bars indicate maxima and minima. The abbreviations are listed in the method section.

the soil physicochemical and biological properties except for pH (ANOVA,  $p < 0.05$ ). Land use is more important than soil depth in changing the pH, SWC, the STP, SACa and SAMg contents and the BGC, NAG, and LAP activities (ANOVA,  $p < 0.05$ ), but soil depth is more important than land use in changing SBD, the STN, SAN, SAP, and SAK contents, and the ACP activity (ANOVA,  $p < 0.05$ ) (Table 1).

#### Distribution of SOC and SIC under Different Land Uses

As expected, SOC stocks varied with soil depth, and the vertical distributions of SOC stock were different among the seven land uses, but the vertical distributions of SIC stock were rarely different among the seven land uses (Fig. 2). At the vertical level, the SIC stock increased with soil depth under UG, CL and AL. Extremely weak changes in the SIC stock with soil depth appeared under SF and SL. The SOC stocks under MG ( $16.21 \text{ kg m}^{-2}$ ) were significantly higher than those under UG ( $9.69 \text{ kg m}^{-2}$ ), CL ( $9.52 \text{ kg m}^{-2}$ ),

SF ( $9.81 \text{ kg m}^{-2}$ ), SL ( $4.53 \text{ kg m}^{-2}$ ) and AL ( $3.62 \text{ kg m}^{-2}$ ) at the entire 0-50 cm soil depth (Duncan's post hoc test,  $p < 0.05$ ). The SOC stocks under BG, UG, CL, SF were significantly higher than those under SL and AL at the entire 0-50 cm soil depth (Duncan's post hoc test,  $p < 0.05$ ); the SIC stocks under MG ( $1.99 \text{ kg m}^{-2}$ ), BG ( $2.01 \text{ kg m}^{-2}$ ), UG ( $1.52 \text{ kg m}^{-2}$ ), and CL ( $1.61 \text{ kg m}^{-2}$ ) were significantly higher than those under SF ( $1.13 \text{ kg m}^{-2}$ ), SL ( $0.93 \text{ kg m}^{-2}$ ) and AL ( $1.19 \text{ kg m}^{-2}$ ) at the entire 0-50 cm soil depth (Duncan's post hoc test,  $p < 0.05$ ) (Fig. 2). Interestingly, however, the contribution of SOC stock to the STC stock did not significantly differ among the seven land uses, with averages of 84.4% (ANOVA,  $p > 0.05$ ). Land use is more important than soil depth in changing the SOC stock (ANOVA,  $p < 0.05$ ), but soil depth is more important than land use in changing the SIC stock (ANOVA,  $p < 0.05$ ) (Table 1).

#### Soil Factors Regulated the Effect of Land Use and Soil Depth on SOC Stock

Clearly, the conditional inference tree analysis (Fig. 3a) indicated that the primary patterns in the SOC stock were associated with STP content, land use, and LAP activity with specific thresholds, demonstrating significant groupings of SOC stock. In soils with high STP content ( $>195.61 \text{ mg kg}^{-1}$ ), there were generally moderate to high stock of SOC ( $0.5074\text{--}8.5037 \text{ kg m}^{-2}$ ). In soils with low STP content ( $\leq 195.61 \text{ mg kg}^{-1}$ ), there were generally low to moderate ( $0.0347\text{--}4.0819 \text{ kg m}^{-2}$ ) amounts of SOC across many land uses. Within the large primary grouping of soils with  $\leq 195.61 \text{ mg kg}^{-1}$  STP content, the lowest SOC stocks ( $0.0347\text{--}0.6479 \text{ kg m}^{-2}$ ) were associated with an STP content  $\leq 95.33 \text{ mg kg}^{-1}$ . Land use did play a role in differentiating SOC stocks when the STP content was  $\leq 195.61 \text{ mg kg}^{-1}$ . Within the grouping of soils with AL and SL, LAP activity did play a role in differentiating the SOC stocks with an activity threshold of  $3.57 \mu\text{mol d}^{-1}\text{g}^{-1}$  dry soil. Further, path models (Fig. 4) showed that land use, soil depth, and soil chemical and biological properties had significant effects ( $p < 0.05$ ) on SOC stock changes, together accounting for 29-47% of the variance. Interestingly, in the models containing soil N or P (Fig. 4a,b), the standardized direct path coefficients for land use, soil depth, and soil chemical and biological properties to changes in SOC content were ordered as soil factor > soil depth > land use, indicating that soil factor was the most important factor influencing the effect of land use and soil depth on SOC stock. In the models containing SAK or ACP (Fig. 4d,e), that order was land use > soil factor  $\geq$  soil depth, indicating that land use was the most important factor influencing SOC stock.

Differences in the SOC stock were observed with land use and soil depth. Three potential mechanisms might be responsible for this.

First, the SOC stock is controlled by OC inputs (mainly the plant-derived C input) [21, 25] and outputs (leaching, decomposition, emission, and erosion) [17],

Table 1. Partial eta squared ( $\eta^2$ ) and Significance for the effects of land use and soil depth on the soil variables on Guizhou plateau, SW China ( $n = 105$ ). The higher values of partial eta squared ( $\eta^2$ ) indicate the greater relative importance of a factor or interaction. ANOVA; ns,  $p > 0.05$ ; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.001$ . The abbreviations are listed in the method section.

Soil variables	Land use	Soil depth	Land use $\times$ Soil depth
pH	0.922****	0.050 <sup>ns</sup>	0.422**
SWC	0.694****	0.430****	0.588****
STPc	0.729****	0.535****	0.397*
SACac	0.887****	0.443****	0.648****
SAMgc	0.927****	0.448****	0.680****
BGC	0.521****	0.487****	0.418**
NAG	0.571****	0.310****	0.291 <sup>ns</sup>
LAP	0.918****	0.310****	0.580****
STCs	0.459****	0.127*	0.416**
SOCs	0.440****	0.087 <sup>ns</sup>	0.391*
SOCC	0.278***	0.250***	0.238 <sup>ns</sup>
SBD	0.466****	0.604****	0.535****
STNc	0.610****	0.701****	0.505****
SANc	0.502****	0.690****	0.524****
SAPc	0.415****	0.571****	0.485****
SAKc	0.590****	0.701****	0.553****
ACP	0.324****	0.594****	0.392*
SICs	0.188*	0.296****	0.215 <sup>ns</sup>



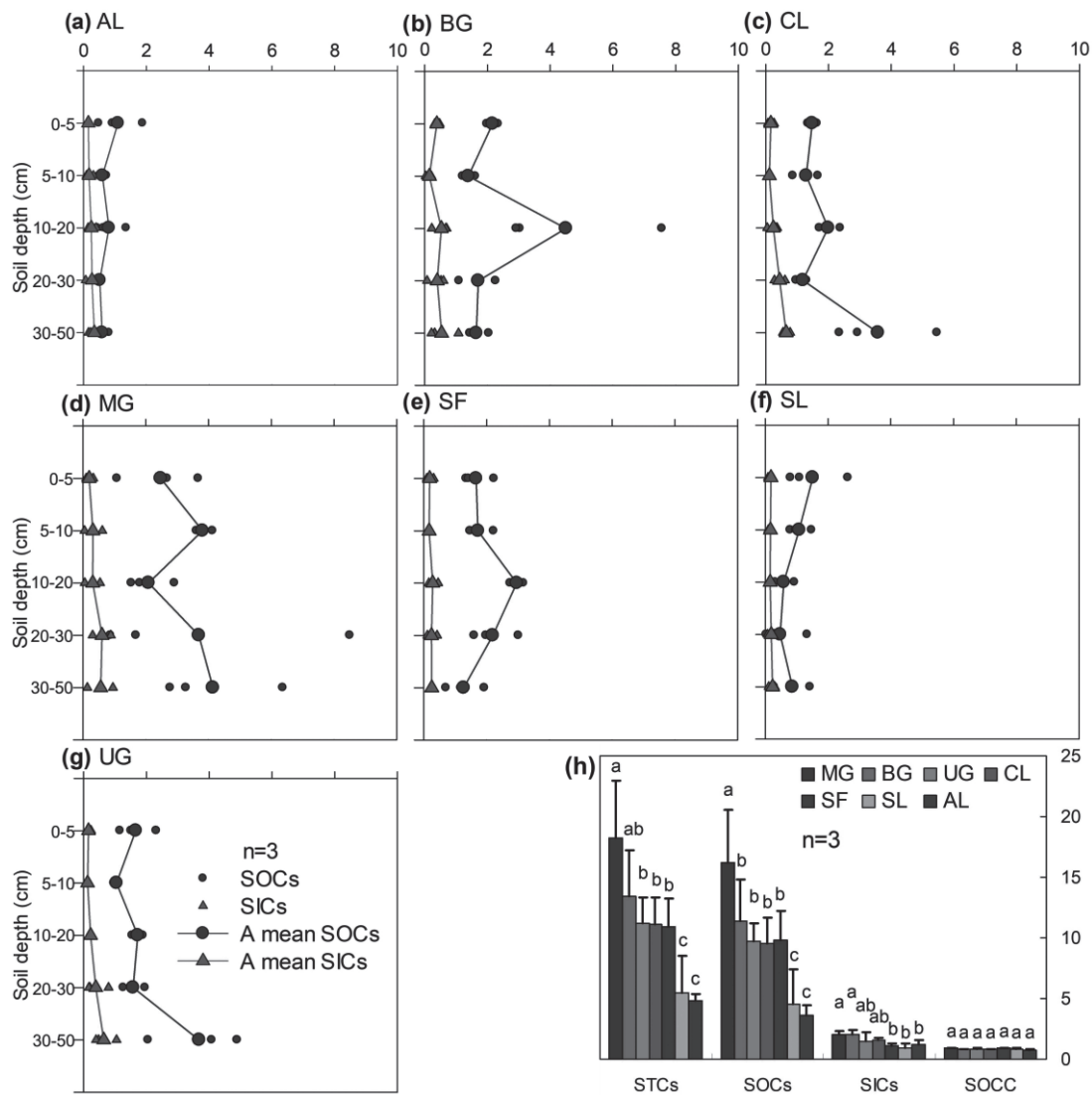


Fig. 2. Vertical distributions of soil organic carbon (SOCs) and inorganic carbon (SICs) stocks and soil organic carbon (SOC) contribution to soil total carbon (STC) under land uses. Bars indicate mean, error bars indicate standard deviation. Different lowercase letters indicate significant differences (Duncan's post hoc test,  $p < 0.05$ ). UG, Unused natural subalpine grassland, BG: Buffalo grazing improved grassland, MG, Mosaic grassland; SL, Shrubland; AL, Afforestation land; SF, Secondary forest land; CL, Cropland.

which are both determined by plant types and human disturbance [19]. The land uses in our study were mainly characterized by differences in plant functional types and human interference. Plant functional types, through differences in shoot/root allocations, significantly affected the SOC distributions along with the soil depth [12, 20]. Therefore, variability in the depth distribution of SOC was found to mainly depend on litter inputs and the vertical distribution of the roots [21, 25]. Human interference, i.e., buffalo grazing in BG, non-use in UG, weeding, harvesting grain and burning straw in CL, and understory vegetation removal and thinning in AL [41] could change the plant-derived C input and soil C loss. For the BG, grazing enhanced the allocation of underground C [6], thus increased the SOC stock compared to that of the unused grassland (Fig. 2h). For the AL, understory vegetation removal and thinning commonly decreased the SOC by reducing litter inputs

into the soil and accelerating decomposition rates due to changes in the microclimate [42], and increasing C emissions [8, 41], which possibly increased the leaching and erosion of SOC due to direct exposure to rainfall and varied the degree of change in the SOC among different soil depths [41]. Therefore, afforestation with *Pinus* caused an decrease in the SOC (Fig. 2h) [43]. For the CL, tillage and ridging increased the soil aeration and temperatures [41] and increased C emissions [44]. The bare soil caused by weeding increased the leaching and erosion of SOC. Weeding, harvesting grain and burning crop residues dramatically reduced the root- and litter-derived C, thereby resulted in the present SOC stock in the CL.

Second, SOC stock was also indirectly affected by the mediation of soil physicochemical factors. It is generally recognized that soil inorganic nutrients or nutrient availability limit C sequestration [2, 31].

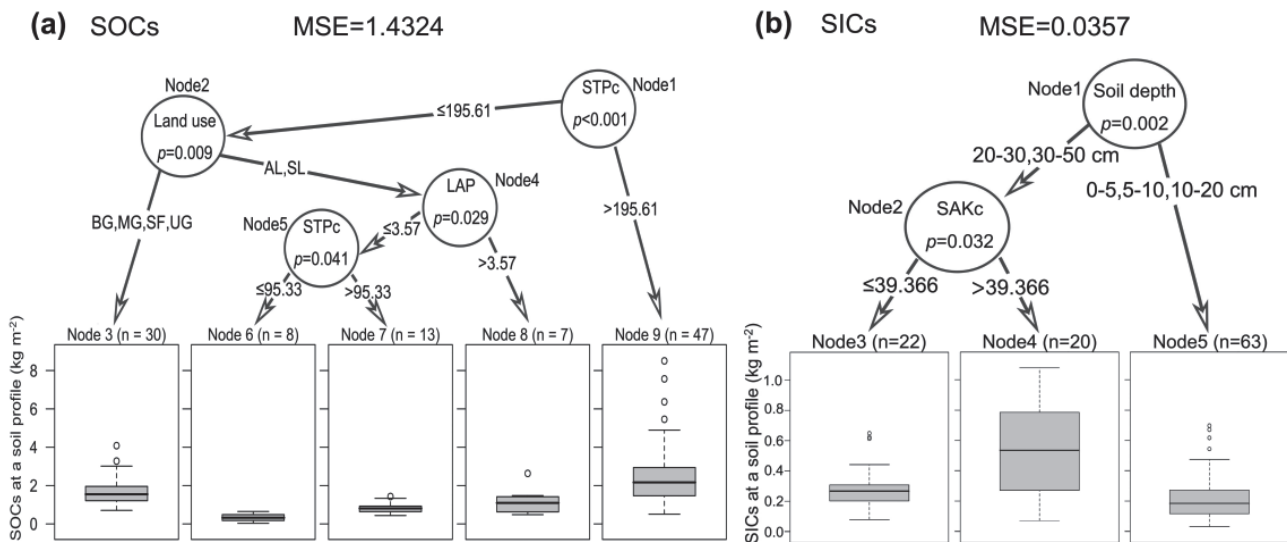


Fig. 3. Conditional inference tree analysis showing significant splits in soil organic carbon stocks (SOCs) and inorganic carbon stocks (SICs). The box plot represents the mean values of soil carbon stock in specific level of a variable, the level of significance of each split is shown inside the ovals. MSE, Mean Square Error; STPc, soil total phosphorus content; AL, Afforest land; SL, Shrubland; LCP, the activity of leucine aminopeptidase; SAKc, available potassium content.

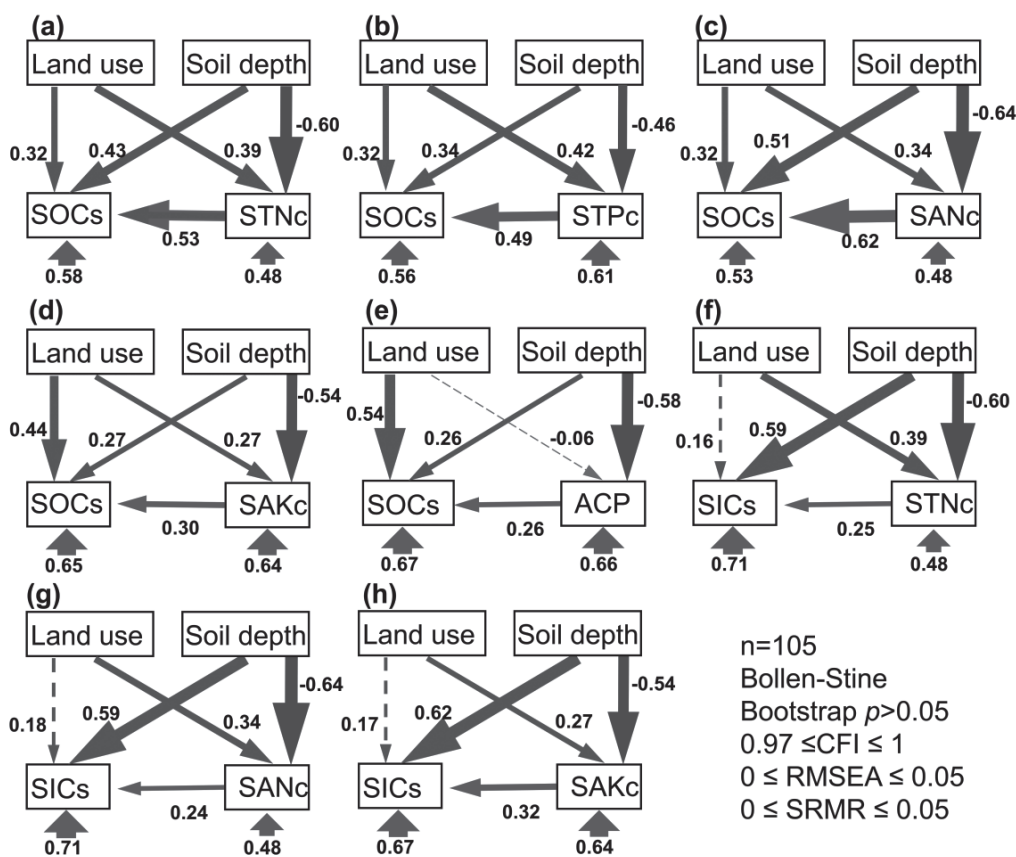


Fig. 4. Path models showing hypothetical pathways of effects of land use and soil depth on soil organic carbon stocks (SOCs) and inorganic carbon stocks (SICs). The models attained an acceptable fit. Solid lines show significant pathways ( $p < 0.05$ ), dotted lines indicate non-significant pathways ( $p > 0.05$ ), with line width representing effect size; green indicates positive coefficient; red indicates negative coefficient; and arrows indicate directionality. The numbers adjacent to the arrows represent standardized path coefficients, analogous to regression weights. The numbers at the originating end of the arrow are variance explained by error variables. STNc, soil total nitrogen content; STPc, soil total phosphorus content; SANc, soil available nitrogen content; SAKc, soil available potassium content; ACP, the activity of acid phosphatase; CFI, Comparative Fit Index; RMSEA, Root Mean Square Error of Approximation; SRMR, Standardized Root Mean Square Residual.

Synchronous changes in SOC vs. STN, STP, SAN and SAK were observed in our study (Fig. 5, Table 2), illustrating that SOC in this area was affected by soil N, P and K. Buffalo excrement in the BG and fertilization in CL could positively change the soil N, P and K, thereby changing the SOC. Specifically, significant decreases in the STN, SAN, STP, SAK, SACa, and SAMg contents and in pH (by 21%, 45%, 38%, 7.5%, 64%, 69% and 0.4 units, respectively) under AL were also observed compared with average levels under all land uses in this study. The degrees by which they decreased exceeded the decline in degrees (by 20% N, 29% SACa, 52% SAMg and 0.3 units pH) of the global average level, except for SAK (decrease by 23%) [43]. Berthrong [43] posited that more of the soil cations (Ca, Mg, and K) that are taken up by afforested plantations tend to be replaced by protons ( $H^+$ ) than plants in non-afforestation areas, thereby resulting in lower cation and pH levels in afforestation soil than non-afforestation soil (Fig. 2a,i-j);

they also pointed out that differences in the distribution and decomposability of plantation biomass C and C loss from soil induced the lower SOC in afforestation soil compared to grassland soil. Additionally, compared to no thinning, thinning significantly reduced the SOC and N stocks by 25% and 27%, respectively, and most of these losses occurred below a 20 cm soil depth [45]. However, MG that was characterized by patchy shrubs interspaced by grass showed increased the contents of STN, SAN, STP, SAP, and SAK (by 41%, 24%, 40%, 59% and 19%, respectively) compared with the average levels under all land uses in this study. High contents of soil N, P and K (Fig. 1d-h) could thus induce high SOC stock (Fig. 2h, Fig. 5).

Thirds, soil biological factors regulated the effect of land use and soil depth on SOC stock. LAP is produced by microorganisms and plant roots and plays an important role in acquiring N for the enzyme producers [27] and SOC mineralization [46]. LAP can

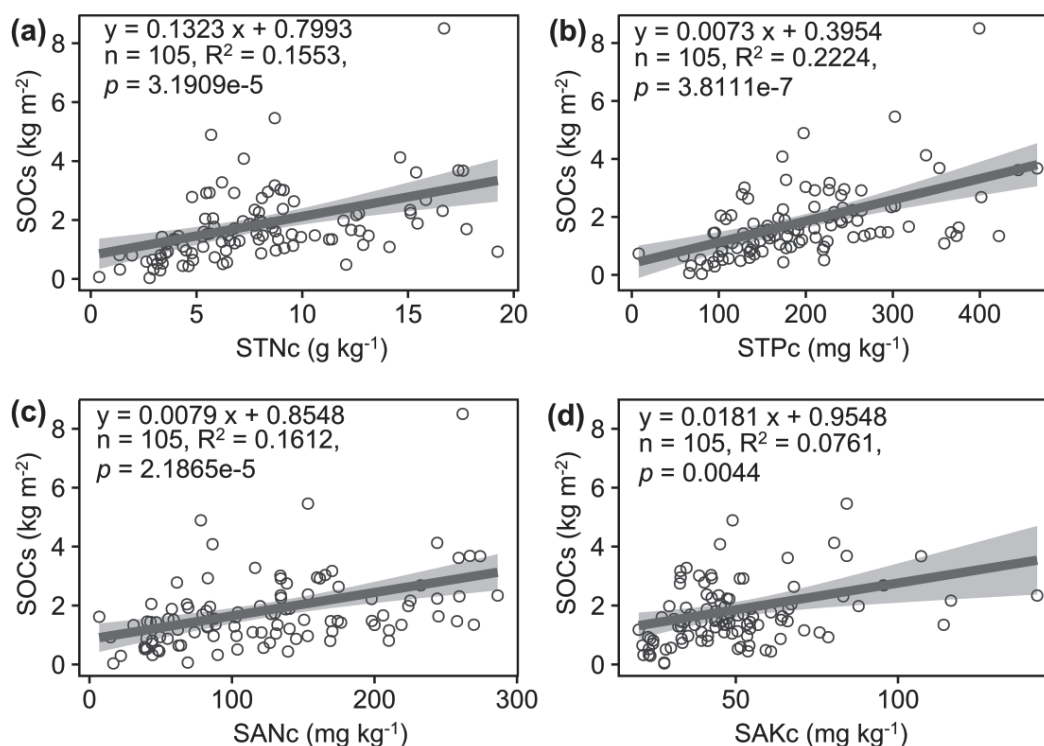


Fig. 5. Relationships between the soil organic carbon stocks (SOCs) and the soil total nitrogen (STNc), soil total phosphorus (STPc), soil available nitrogen (SANc) and available potassium (SAKc) contents.

Table 2. Regression models relating soil C with land use, soil depth, soil physicochemical and biological properties. \*\*\*\*,  $p < 0.001$ . STNc, soil total nitrogen content; STPc, soil total phosphorus content; SANc, soil available nitrogen content; SBD, soil bulk density; BGC, the activity of N-acetyl glucose aminidase; ACP, the activity of acid phosphatase; SAKc, soil available potassium content.

Regression models (n = 105)	R <sup>2</sup>
$SOCs = -6.2100 + 0.1505Land\ use + 0.6463Soil\ depth + 0.1633STNc + 0.0023STPc + 0.0075SANc - 0.2324pH + 1.3442SBD - 0.0064BGC + 0.1071ACP$	0.6215****
$SICs = -0.3198 + 0.0124Land\ use + 0.1144Soil\ depth + 0.0043STNc + 0.0004SANc + 0.0027SAKc$	0.3404****

hydrolyse leucine and other hydrophobic amino acids from the N-terminus of polypeptides by proteolysis [28] in plants and microorganisms. Our results showed that a high SOC stock (Fig. 3a) was therefore linked to high LAP activity (Fig. 3a) under SL and AL where nitrogen was scarce (Fig. 1d, e). ACP that is produced by microorganisms and plant roots plays an important role in acquiring P for the enzyme producers [27] and SOC mineralization [46]. ACP can hydrolyse phosphate from phosphosaccharides and phospholipids [28]. Therefore, high ACP activity was likely beneficial in the increase of the SOC stock in the subtropics where soil phosphorus was a generally limiting factor [46] (Fig. 1g). These findings suggested that the soil chemical and biological properties limited any effects of land use on SOC stock. This was consistent with previous studies in Eastern Australia [17] and in the Qinghai-Tibetan Plateau [7].

As expected, soil factors controlled the SOC stock [17]. Further, an increasing number of studies have concluded that the SOC stock was strongly controlled by soil environmental factors [8] including the physical [7-8], geochemical [7, 17] and biotic [9, 35] factors of the soil. In fact, many studies have confirmed that the most important factor changing SOC stock was the soil factor [17, 20]. The evidence from the conditional inference tree analysis in our study indicated that the primary patterns in the SOC stock were associated with STP content, land use, and LAP activity with specific thresholds, demonstrating significant groupings of SOC stock. Moreover, evidence from the path models showed that the STN, STP, SAN and SAK contents and the ACP activity regulated the significant effect of land use and soil depth on SOC stock. In this respect, the effects of land use and soil depth on SOC stock were highly dependent on soil chemical and biological conditions. Thus, land use had an effect on SOC stock within specific soil chemical and biological ranges, but the soil chemical and biological index significantly regulated the amount of SOC in Longli grassland area, suggesting that soil chemical and biological conditions needs to be considered under soil C management policies of land use reversion to increase SOC stock (e.g., land uses and fertilization).

#### Soil Factors Regulated the Effect of Land Use and Soil Depth on SIC Stock

The conditional inference tree analysis clearly indicated that the primary patterns in SIC stock were associated with soil depth and SAK content (Fig. 3b). In the topsoil (0-20 cm) there was generally a low stock of SIC (0.0306-0.6971 kg m<sup>-2</sup>), but within the grouping of subsoils (20-50 cm), SAK content did play a role in differentiating the SIC stocks with a content threshold of 39.366 mg kg<sup>-1</sup> dry soil, and high SIC stocks (0.0703-1.0787 kg m<sup>-2</sup>) were associated with an SAK content  $\geq$ 39.366. Further, the path models (Fig. 4) showed that soil depth and soil chemical properties rather than land use had significant effects (p<0.05) on SIC stock

changes, and land use significantly and indirectly changed the SIC stock via its significant influence on soil chemical properties rather than by directly influencing them (p<0.05); together, this accounted for 29-33% of the variance. In the models consisting of soil N or K (Fig. 4f,h), the standardized direct path coefficients for soil depth and soil chemical properties to changes in SIC stock were ordered as soil depth>soil factor>land use, indicating that soil depth was the most important factor influencing SIC stock and that soil factor was the most important factor influencing the effect of land use and soil depth on SIC stock.

The distribution patterns of SIC stock with land use changes were likely explained by the following reasons.

First, SIC exists primarily as carbonates of calcium (calcite, CaCO<sub>3</sub>) and magnesium (dolomite, MgCO<sub>3</sub>) [15]. According to the chemical reaction equilibria of dissolution and precipitation [15], the external carbonate (CO<sub>3</sub><sup>2-</sup>) and bicarbonate (HCO<sub>3</sub><sup>-</sup>) originating from autotrophic and heterotrophic respiration into soil water and the external Ca<sup>2+</sup> and Mg<sup>2+</sup> originating from the dissolution of Ca/Mg-silicates [10, 15], rain [15], dust [15], fertilizer [15, 23] and the decomposed organic matter [21] could result in a net increase in SIC [15], and the increase of H<sup>+</sup> [16] and the reduction of soil CO<sub>3</sub><sup>2-</sup>, HCO<sub>3</sub><sup>-</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> could result in a net decrease in SIC [47]. As mentioned above, AL resulted in lower cation and pH levels, thereby resulted in a lower SIC stock.

Second, from the perspective of translocation, leaching [15], redistribution [3, 15], runoff and erosion could directly shape the stock and vertical distribution of SIC. Therefore, land use-induced differences in the strength of those processes could cause the present stock and vertical distribution of SIC. This was used to explain the SIC profile in afforestation soil compared with cropland soil [15].

Third, SIC stock generally increased linearly with soil depths [33], but soil factors regulated this pattern with varying degrees of dependence on land use and soil depth (Fig. 3, Fig. 4). It is generally recognized that the formation and decomposition of SOC is a biological process while the dissolution and precipitation of SIC is a chemical reaction process. Therefore, the factors that impact SIC have been thought to be much different from the factors that impact SOC [7]. However, strong relationships were found between SIC and soil N, P, and K and biological properties [7, 33] (Table 1, Fig. 4), namely, the factors that influence one of the two C forms may simultaneously impact the other one [7]. We therefore explored whether soil factors were also the main control for SIC stock. The evidence from the conditional inference tree analysis in our study indicated that the primary patterns in SIC stock were associated with soil depth and SAK content. And the path models further showed that soil depth and soil chemical properties rather than land use had significant effects on SIC stock changes, and land use significantly and indirectly changed the SIC stock via its significant



Table 3. Spearman correlation between SOC and SIC stocks under a land use, soil depth (cm) and the entire study area. UG, Unused natural subalpine grassland, BG: Buffalo grazing improved grassland, MG, Mosaic grassland; SL, Shrubland; AL, Afforest land; SF, Secondary forest land; CL, Cropland. Entire, across all land uses and soil depths.

	Spearman correlation r	Holm corrected p
0-5	0.30	0.1805
5-10	0.27	0.2340
10-20	0.33	0.1442
20-30	0.47	0.0331
30-50	0.52	0.0158
AL	-0.45	0.0895
BG	0.68	0.0054
CL	0.35	0.2059
MG	0.13	0.6571
SF	0.02	0.9396
SL	0.28	0.3212
UG	0.65	0.0092
Entire	0.36	0.0001

influence on soil chemical properties rather than by directly influencing them.

### Relationship between SOC and SIC Stocks

Some studies argue that the formation of secondary carbonates is influenced by the presence of SOC [8, 13], so increasing the SOC stock could lead to an increase in the SIC stock [30-31]. This study found that significantly positive relationships between SIC and SOC were found at the 20-30 and the 30-50 cm soil depths and under the BG and UG with different correlation strengths (Spearman, Holm corrected  $p < 0.05$ ) (Table 3), while there was a significantly positive relationship between SIC and SOC at the entire level (Spearman, Holm corrected  $p < 0.05$ ), but insignificant relationships were also found (Spearman, Holm corrected  $p > 0.05$ ) (Table 3), indicating that both land use and soil depth changed the relationship between the SOC and SIC stocks. These inconsistent findings imply the complex relationship between SOC and SIC stocks [23].

Detailed interpretations on this relationship were limited [23, 30], but two potential factors could be responsible for this relationship. First, the transformations between SOC and SIC varied with the changes of land use. Li et al. [7] discussed this in detail.

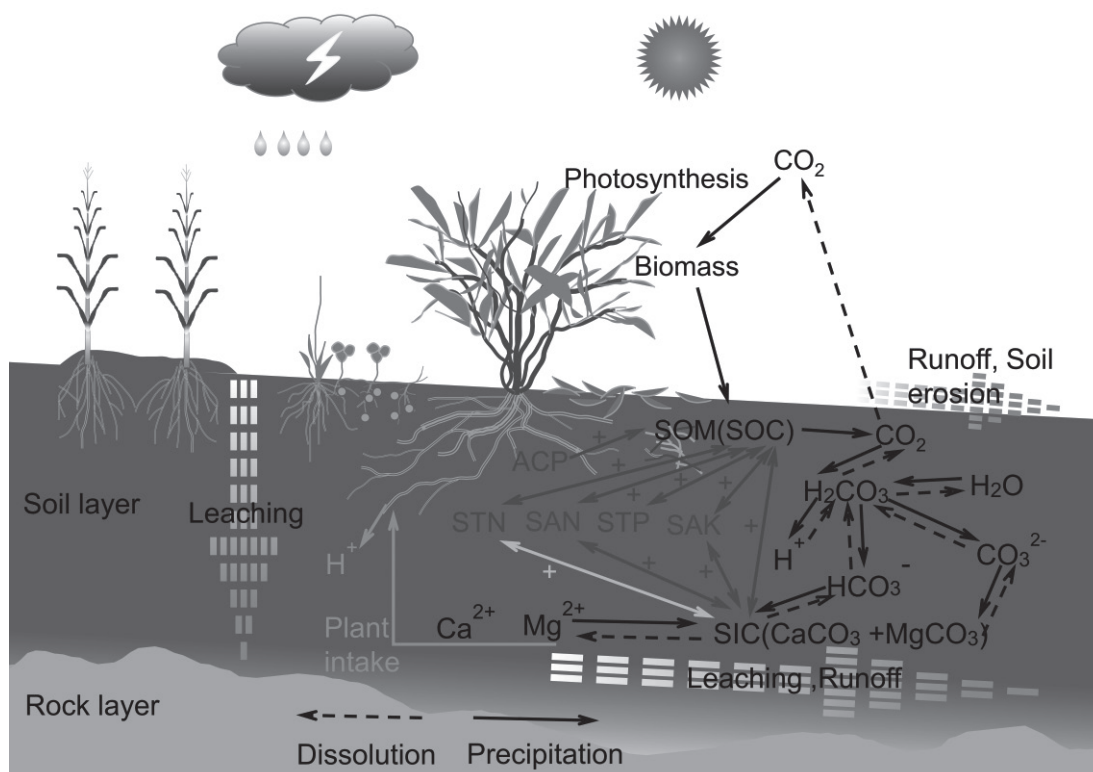


Fig. 6. The key factors for the carbon flow model in the land ecosystems of Guizhou plateau, China. The black part is cited from Li et al. [7]; the green and yellow parts have been found in other studies and the green were in line with our study but the yellow was not; the gray part has been found in other studies; and the red part shows new results obtained in our study. The "+" with a double arrow line indicates a significant positive correlation between two variables ( $p < 0.05$ ). SOM, soil organic matter. SOC, soil organic carbon; SIC, soil inorganic carbon; STN, soil total nitrogen; STPc, soil total phosphorus; SAN, soil available nitrogen; SAK, available potassium content; ACP, the activity of acid phosphatase;  $\text{CO}_2$ , carbon dioxide;  $\text{H}^+$ , hydron;  $\text{CO}_3^{2-}$ , carbonate;  $\text{HCO}_3^-$ , bicarbonate;  $\text{H}_2\text{CO}_3$ , carbonic acid;  $\text{CaCO}_3$ , calcite;  $\text{MgCO}_3$ , dolomite.

Undoubtedly, soil C conversion involves the fate of soil CO<sub>2</sub>. Briefly, as shown in Fig. 6, SOC mainly comes from photosynthetic C of plants and can be directly mineralized and stored in the soil as SIC [7]. SOC decomposition increases the soil CO<sub>2</sub> [31]. Additionally, acidic conditions can lead to the dissolution of SIC [3, 30] and its release as soil CO<sub>2</sub> [3]. Some of the CO<sub>2</sub> that is released from the soil is released to atmosphere at high partial pressure of soil CO<sub>2</sub>, but other soil CO<sub>2</sub> is precipitated as SIC. Consequently, the C that is lost from the organic form is partly sequestered as the inorganic form [7]. In this respect, the factors influencing this process (Fig. 6), including land use-induced differences in plant intake, soil physicochemical and biological properties, horizontal and vertical leaching and runoff, and soil erosion and surface runoff [3], could shape not only the stock and distribution of SOC and SIC but also the relationship between SOC and SIC. Second, SIC can change the stability of SOC via its impacts on the stability of aggregates and its physical protection for SOC [48]. Rowley et al. [48] discussed these views in detail. A simulative experiment with the use of stable isotope techniques [42] found that plant biomass was produced via using soil-derived inorganic C. This finding provided evidence that the C flows from SIC to plant C (Fig. 6). However, the existing data in this snapshot study made it difficult to clearly determine where the missing SOC and SIC had gone.

Compared with previous studies, we have made some new discoveries. As shown in our and previous studies (Fig. 6), the contents of STN [41], SAN [41], STP [41], and SAK [41] and the activity of ACP positively affected SOC stock, and SOC stock positively affected SIC stock [23, 30] (Fig. 4). Contrary to previous findings showing that STN content negatively affected SIC stock, our data supported the positive effect of STN content (Fig. 4). Further, our study was the first to report that the SAN and SAK contents positively affected SIC stock. These findings could be reasonable in these soils, which are characterized by the deficiencies in phosphorus [29] and potassium, and the middle level of nitrogen.

## Conclusions

This study provided interesting snapshot insights into the effects of land use and soil depth on SOC and SIC stocks with the regulations of soil factors, also implied that the importance of soil chemical and biological conditions of the soil C stock and soil chemical and biological conditions need to be considered when making land use reversion policies for land use and soil C management to increase the soil C stock.

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## Conflict of Interest

The authors declare no conflict of interest.

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