

Article

Response of Soil Organic Carbon in Citrus Orchards at Different Slope Positions to Citrus Peel Biochar and Field Snail Shell Powder

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Abstract

Soil organic carbon (SOC) loss in sloping farmland is a critical challenge for agricultural sustainability. This study investigated how citrus peel biochar (CPB), field snail shell powder (SSP), and their composite (CPB + SSP) differentially regulate SOC dynamics across slope positions (upper, middle, lower) in Guangxi's citrus orchards. Key findings revealed: CPB significantly increased SOC content (up to 5.5 g·kg⁻¹ at lower slopes) via high carbon input but suppressed mineralization amount in lower slope position (reduction of 17.9%) due to its high C/N ratio. SSP neutralized soil acidity (pH 3.95 to 7.5), stimulating microbial activity and raising mineralization rates by 58.95% (lower slope), yet minimally enhanced SOC (only +0.7 g·kg⁻¹). CPB + SSP effectively balanced carbon stability and active release: dissolved organic carbon (DOC) and readily oxidizable organic carbon (ROC) increased by 14.4 mg·kg⁻¹ and 0.22 g·kg⁻¹ (middle slope), while SOC rose significantly (e.g., +2.2 g·kg⁻¹ at lower slope). Slope position effects strongly influenced outcomes: the lower slope (highest initial SOC) responded most strongly to CPB for carbon stabilization, while middle slopes benefited from CPB + SSP to reconcile carbon loss with fertility. These results provide slope-specific strategies for SOC management by integrating amendment synergy and machine learning-driven insights in citrus orchards.

Keywords: sloping land; machine learning prediction; SOC; PLS-SEM; random forest model



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1. Introduction

Soil organic carbon (SOC) constitutes the most extensive active carbon reservoir within terrestrial ecosystems, with its dynamic equilibrium intricately associated with soil health, food security, and the global carbon cycle [1,2]. Nevertheless, under prolonged intensive agricultural practices and natural erosion, the rates of mineralization and loss of global soil organic carbon have significantly surpassed its natural accumulation capacity, resulting in a persistent degradation of soil carbon pools [3,4]. This challenge is especially pronounced in sloping farmland ecosystems. For instance, in citrus orchards in Guangxi, China, sloping farmland comprises up to 63.7% of the total area, and these regions are

consistently confronted with issues such as soil acidification, diminished organic matter content, and inadequate water and nutrient retention [5–7]. Even more critically, slope topography intensifies vertical carbon loss and inter-slope position effects through processes of runoff and sedimentation, thereby posing a substantial threat to the sustainability of regional agriculture [8,9]. Citrus growth requires a variety of mineral elements and organic nutrients such as N, P, K, Ca, Mg, etc. [10]. Planting citrus on slopes has certain challenges, including soil erosion, nutrient loss, and water stress [11].

In recent years, biochar and lime-based materials have emerged as highly promising soil amendments, attracting considerable attention. Biochar, characterized by its high carbon content and porous structure, has been demonstrated to significantly enhance SOC sequestration through mechanisms of physical adsorption and chemical bonding [12]. For instance, manganese-modified biochar has been shown to increase SOC content by 25.61% [13], while potassium-doped biochar further amplifies carbon sequestration potential by 45% [14]; biochar has high chemical stability and can resist microbial decomposition, thereby prolonging the retention time of carbon in soil [15]; additionally, biochar has been proven to mitigate CO₂ emissions [16]. Biochar can affect the decomposition and transformation of soil organic carbon, such as the mineralization of organic carbon by changing soil enzyme activity [17]. Biochar can reduce nutrient leaching and improve the utilization rate of soil nutrients [18]. Especially for the loss of nutrients such as nitrogen and phosphorus, the adsorption of biochar can effectively reduce its loss [19]. Citrus peel, a major byproduct of citrus processing, is produced in vast quantities annually but is frequently discarded; however, biochar derived from its pyrolysis exhibits unique potential for ameliorating acidic soils and enhancing carbon sequestration [20,21]. Snail shell powder, a calcium-rich material, effectively neutralizes soil acidity through calcium carbonate dissolution, although its high calcium input may accelerate the mineralization of active carbon [22]. Additionally, increased calcium content can induce a sensitivity reaction in some citrus rootstocks, which should be taken into consideration when applying calcium-rich amendments [23,24].

While previous studies have demonstrated the potential of biochar in enhancing SOC sequestration through its high porosity and alkaline nature, the synergistic effects of citrus peel-derived biochar (CPB) and snail shell powder (SSP) under slope-specific conditions remain unexplored [25]. Although machine learning approaches (e.g., Random Forest) have been used to predict biochar effects in homogeneous soils, their application in deciphering the interactive effects of biochar–calcium composites under slope position effects is limited [26,27]. To address these gaps, this study examines Ultisols in citrus orchards in Guangxi at distinct slope positions (upper, middle and lower slopes). By combining slope-position experiments with machine learning (Random Forest) and structural equation modeling (PLS-SEM), we test three hypotheses:

CPB + SSP would optimize SOC stability by synergizing biochar’s physical protection and snail shell powder’s pH-driven microbial activation, surpassing individual amendments.

Slope position would modulate amendment effects, with lower slopes exhibiting maximal SOC sequestration due to higher initial nutrient gradients.

Machine learning models would identify MBC and ROC as key predictors of CO₂ emissions, mediated by soil enzyme activities.

2. Materials and Methods

2.1. Sample Collection

The research area is situated in Putao Town, Yangshuo County, Guilin City, Guangxi Zhuang Autonomous Region. Nestled within a low-latitude region, it falls under the subtropical monsoon climate regime. Throughout the year, the region experiences a mild

climate accompanied by ample precipitation. The annual mean temperature spans from 17 °C to 25 °C, the annual mean precipitation amounts to 2500 mm, and the annual mean evaporation approximates 2300 mm, exhibiting the characteristic climate features of the tropical-subtropical transition zone. The experimental soil was sourced from the slope section of a citrus orchard (planted with *Citrus japonica* (Guijingan No. 1) on *Citrus X aurantii*. root stock), within the citrus industry demonstration area in Yangshuo County, Guangxi Zhuang Autonomous Region, China. The sampling site is positioned at the uppermost and steepest part of the slope (24°47'33" N, 110°23'41" E). In the study area, the slope measures approximately 150 m in length and 40 m in width, with an average slope angle of 20°. The upper slope is characterized as a barren mountain, while the lower slope is utilized as sloping farmland, thereby creating a typical slope land-use gradient. The soil was classified as Ultisols (USDA Soil Taxonomy), and the citrus grove has a 5-year stand age, presenting typical soil attributes of the subtropical hilly region. During soil sampling at 0–20 cm depth, five random points were sampled and combined to form one composite sample per slope position. Surface weeds and other interfering materials were initially eliminated. The collected soil specimens were uniformly mixed via the quartering method, placed in a sealed container, and transported to the laboratory. In the laboratory, following the removal of animal and plant remnants and gravel from the soil samples, the soil was naturally air-dried and stored in a dry setting for subsequent experimentation. The basic physicochemical properties of the tested soil are presented in Table 1.

Table 1. Basic physical and chemical properties of the tested soil.

Soil Slope Position	pH	Volume Weight of Soil (g·cm ^{−3})	SOC (g·kg ^{−1})	AP (Available Phosphorus) (mg·kg ^{−1})	AK (Available Potassium) (mg·kg ^{−1})	AN (Alkali-Hydrolyzable Nitrogen) (mg·kg ^{−1})
Upper	3.98 ± 0.07	1.20 ± 0.03	4.15 ± 0.03	22.70 ± 0.83	30.72 ± 4.09	47.83 ± 2.38
Middle	4.02 ± 0.04	0.95 ± 0.03	3.46 ± 0.06	10.98 ± 0.88	48.56 ± 1.64	40.25 ± 4.70
Lower	3.95 ± 0.05	0.86 ± 0.02	4.00 ± 0.03	9.07 ± 0.27	38.03 ± 1.1	34.76 ± 4.22

2.2. Material Preparation

For this experiment, fresh citrus peels were chosen as the raw materials. Initially, they were meticulously washed with deionized water to eliminate surface contaminants, followed by natural air-drying at ambient temperature. The air-dried citrus peels were then placed in an oven (Model: 202-1AB, Bangxi Instrument Technology Co., Ltd., Shanghai, China) and subjected to drying at 60 °C for a duration of 72 h to guarantee the thorough dryness of the samples. Subsequently, the dried citrus peels were comminuted using a laboratory crusher to yield a homogenous granular sample. This crushed sample was transferred to a muffle furnace (Model: Kusite K-XR1200-20, Guangzhou, China) and underwent pyrolysis at 500 °C for 2 h under oxygen-restricted conditions to fabricate citrus peel biochar. Once the pyrolysis process was completed, the biochar was cooled to room temperature for future utilization. Fresh snail shells were gathered, washed with deionized water to remove surface adherents, and then naturally air-dried at ambient temperature. The air-dried snail shells were placed in an oven and dried at 60 °C for 72 h to ensure the samples were completely dry. The dried snail shells were then pulverized using a laboratory crusher to obtain a uniform powdery sample. The crushed field snail shell powder was passed through a 60-mesh sieve to ensure a consistent particle size for subsequent use. The prepared citrus peel biochar and field snail shell powder were individually placed into sealed bags and stored in a desiccator to prevent moisture absorption for the subsequent experimental procedures.

2.3. Experimental Design

The experimental design is presented in Table 2. A completely randomized factorial experiment was designed with two factors: slope position (upper, middle, lower) and soil amendment (four types). In total, twelve treatments (3 slopes $\times$ 4 amendments) were arranged, with three replicates for each treatment. Additionally, based on the previous work of our research group, four application ratios were defined [28]. In total, twelve treatments were arranged, with three replicates for each treatment. For the incubation experiment, 100 g of soil was used. The amendments were thoroughly mixed with air-dried soil samples and incubated under controlled laboratory conditions for 100 days at 25 ± 1 °C and 60% water-holding capacity to simulate subtropical field moisture levels. Soil samples were periodically destructively sampled for analysis.

Table 2. Experimental design table.

Soil Slope Position	Treatment Group	Type of Improver	Add Proportion (<i>w/w</i>)
Upper	CK	Untreated soil with no amendment addition	0%
	CPB	citrus peel biochar	4%
	CPB + SSP	citrus peel biochar + field snail shell powder	2% + 2%
	SSP	field snail shell powder	4%
Middle	CK	Untreated soil with no amendment addition	0%
	CPB	citrus peel biochar	4%
	CPB + SSP	citrus peel biochar + field snail shell powder	2% + 2%
	SSP	field snail shell powder	4%
Lower	CK	Untreated soil with no amendment addition	0%
	CPB	citrus peel biochar	4%
	CPB + SSP	citrus peel biochar + field snail shell powder	2% + 2%
	SSP	field snail shell powder	4%

Note: *w/w*: weight by weight; citrus peel biochar (CPB), field snail shell powder (SSP), and their composite amendment (CPB + SSP), Control Check (CK).

Mineralization experiment: Thirty-six 500 mL polyethylene bottles were prepared. Fifty grams of soil samples from different slope positions were added to each bottle, and the soil was treated identically to that in the soil incubation experiment. A 10 mL beaker filled with 10 mL of $0.5 \text{ mol} \cdot \text{L}^{-1}$ NaOH absorption solution was placed inside each 500 mL incubation bottle. Subsequently, the incubation bottles were sealed with lids and incubated in a 25 °C constant-temperature incubator. The beakers were retrieved on days 1, 3, 5, 10, 15, 20, 30, 40, 60, 80, and 100.

2.4. Measurement Methods

Soil organic carbon (SOC) was determined using the potassium dichromate-concentrated sulfuric acid volumetric method [29]. Soil microbial biomass carbon (MBC) analysis employed the fumigation-extraction method: ten grams of fresh soil underwent 24 h fumigation followed by extraction with $0.5 \text{ mol} \cdot \text{L}^{-1}$ K_2SO_4 at a 1:5 (soil:solution) ratio during one hour of shaking. The resulting extract was quantified using a Multi N/C 3100 total organic carbon analyzer (Jena, Germany) [30]. For soil dissolved organic carbon (DOC), samples were extracted with pure water at a 1:5 (soil: solution) ratio during one hour of shaking, followed by the addition of $0.5 \text{ mol} \cdot \text{L}^{-1}$ K_2SO_4 at a 1:5 ratio. DOC concentration was then measured using the total organic carbon analyzer [31].

Soil readily oxidizable organic carbon (ROC) was determined via the $333 \text{ mmol} \cdot \text{L}^{-1}$ potassium permanganate oxidation method. This involved mixing five grams of air-dried soil with 25 mL of $333 \text{ mmol} \cdot \text{L}^{-1}$ KMnO_4 , shaking for one hour, centrifuging for five minutes, diluting the supernatant, and measuring its absorbance using a 752N UV-Vis spectrophotometer (Shanghai Analytical Instrument Co., Shanghai, China) [32].

Soil urease activity was assessed using the indophenol blue colorimetric method. Five grams of air-dried soil were treated with one milliliter of toluene. After 15 min,

10 mL of 10% urea solution and 20 mL of citrate buffer solution (pH 6.7) were added, and the mixture was incubated at 37 °C for 24 h. Following filtration, 0.1 mL of filtrate was transferred to a 50 mL colorimetric tube. Subsequently, 4 mL of sodium phenolate solution and 3 mL of sodium hypochlorite solution were added. After 20 min for color development, the volume was adjusted and absorbance measured with the UV-Vis spectrophotometer (UV-1200, Mapada, Shanghai, China) [33].

Soil catalase activity was measured by the potassium permanganate titration method. Five grams of air-dried soil were combined with 40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide (including a soil-free control setup). Following 30 min of shaking, 5 mL of 1.5 mol·L⁻¹ H₂SO₄ was added, and the mixture was filtered. 25 mL of the filtrate was then titrated with 0.002 mol·L⁻¹ potassium permanganate [34].

Soil sucrase activity was determined using the 3,5-dinitrosalicylic acid (DNS) colorimetric method. A mixture containing five grams of air-dried soil, 15 mL of 8% sucrose solution, 5 mL of phosphate buffer solution (pH 5.5), and 0.25 mL of toluene was incubated at 37 °C for 24 h. After filtration, 0.1 mL of filtrate was combined with 3 mL of DNS reagent. This mixture was heated in a water bath (HH-2, Changzhou Instrument Manufacturing Co., Changzhou, China) for five minutes, cooled in an ice bath for three minutes, diluted, and subjected to colorimetric analysis [35].

Soil CO₂ emissions were quantified using the alkali absorption method [36].

2.5. Data Analysis and Statistics

Data are presented as mean ± standard deviation ($n = 3$). Microsoft Excel 2016 is used to process the data. Prior to analysis, the normality of distribution and homogeneity of variance were tested. Graphs were plotted using Origin 2024. A two-way ANOVA was conducted using SPSS 22.0 to assess main effects (slope, amendment) and their interaction. Post hoc Duncan tests ($p < 0.05$) compared means. R (version 4.3.3), A random forest (RF) model was developed using the “random Forest” package to assess the impact of various environmental factors on soil CO₂ emissions. Additionally, a partial least squares structural equation model (PLS-SEM) was constructed with the “plsmp” package to identify potential direct and indirect pathways influencing soil CO₂ emissions.

3. Results

3.1. Characterization of Citrus Peel Biochar and Snail Shell Powder

As depicted in Table 3 regarding the fundamental properties and elemental compositions of citrus peel biochar and field snail shell powder, citrus peel biochar is characterized as a material of high alkalinity and elevated carbon content, with a carbon proportion of 77%. Field snail shell powder, similarly, exhibits high alkalinity and is endowed with a substantial cation-exchange capacity. Given that its principal constituent is calcium carbonate, its carbon content stands at 12.65%.

Table 3. Basic properties and elemental contents of citrus peel biochar and field snail shell powder.

	pH	CEC (cmol·kg ⁻¹)	C/N	C/H	Elemental Content (%)		
					C	H	N
citrus peel biochar	9.6 ± 0.02	30.96 ± 0.14	38.59 ± 0.15	14.95 ± 0.13	77.81 ± 5.20	5.2 ± 0.04	2.02 ± 0.01
field snail shell powder	8.53 ± 0.04	440.3 ± 0.22	31.40 ± 0.35	112.53 ± 0.91	12.65 ± 0.1	0.11 ± 0.0	0.40 ± 0.02

CEC: Cation Exchange Capacity.

As illustrated by the XRD patterns of citrus peel biochar and field snail shell powder presented in Figure 1, the citrus peel biochar exhibits no distinct peaks; instead, it displays a broad diffraction peak within the 20–30° range. This phenomenon implies the presence

of an amorphous and low-ash-content carbonaceous matrix in the material [37]. Regarding the field snail shell powder, BaMg₂As was detected at 25.72°, CaHBr at 32.64° and 52.02°, and CaCO₃ at 35.46°, 37.34°, 42.32°, 47.82°, and 49.72°. These findings suggest that CaCO₃ is the primary crystalline component in the snail shell [38].

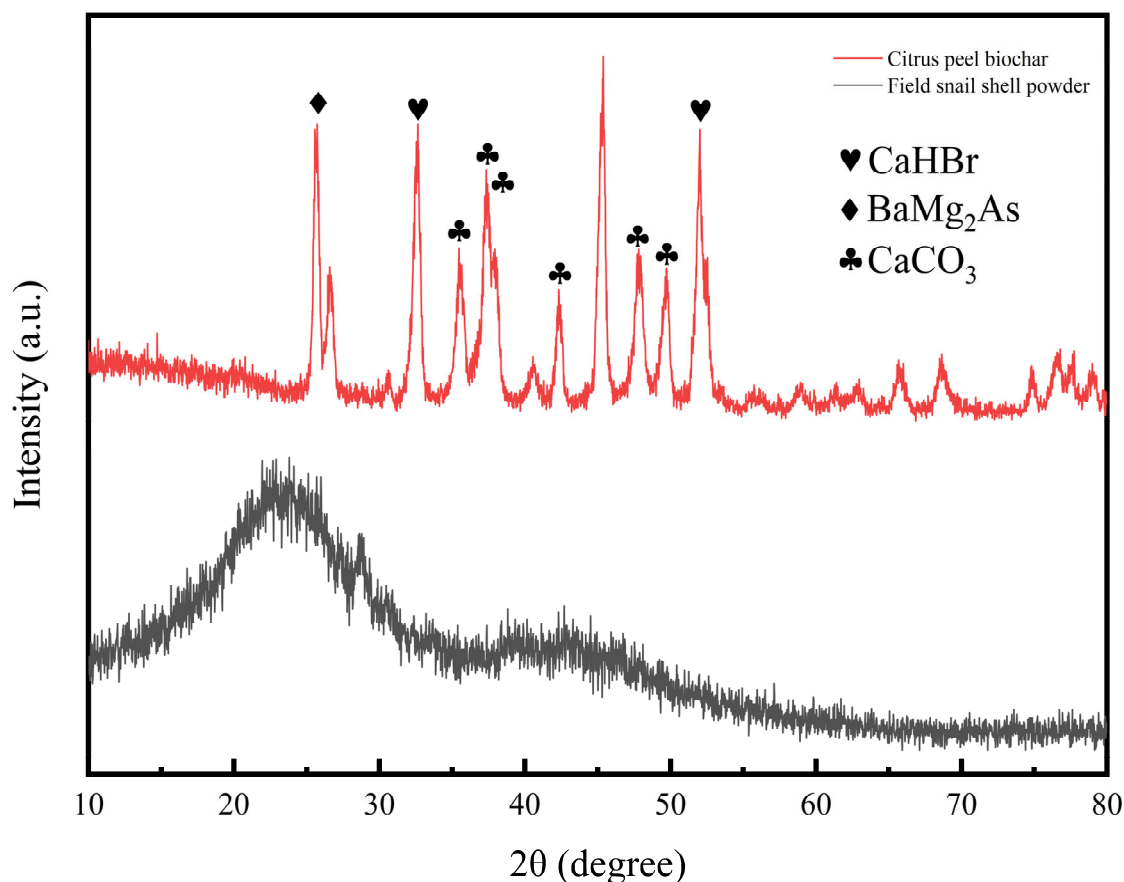


Figure 1. XRD patterns of citrus peel biochar and field snail shell powder, The red line represents Citrus peel biochar black line represents Field snail shell powder.

3.2. Effects of Different Amendments on Soil Physical and Chemical Properties

As shown in Figure 2a,b,g, the effects of the three treatment methods on soil pH and CEC in different slope positions are quite similar. The efficacy of the CPB + SSP and SSP treatments surpassed that of the CPB treatment. The CPB treatment elevated the soil pH to approximately 5.5, while the CPB + SSP and SSP treatments raised it to around 7.5. All three treatments augmented the soil CEC content at different slope positions. The CPB treatment resulted in a maximum increase of 2.8 cmol·kg^{−1}, whereas the CPB + SSP and SSP treatments led to increases of 8.9 and 8.62 cmol·kg^{−1}, respectively. The CPB treatment decreased the soil alkaline-hydrolyzable nitrogen content at all three slope positions. In the upper slope position, the CPB + SSP and SSP treatments reduced the available phosphorus content by 10.3 mg·kg^{−1} and 13.3 mg·kg^{−1}, respectively. The CPB treatment substantially enhanced the soil available potassium content, with the increase being significantly larger at lower slope positions (approximately 900 mg·kg^{−1}). The CPB + SSP and SSP treatments significantly boosted the sucrase activity in the upper slope position by 9.6 mg·g^{−1}·h^{−1}, while there was scarcely any response in the middle slope position. The CPB treatment had a negligible impact on the urease activity at all three slope positions, while the CPB + SSP and SSP treatments enhanced the urease activity by 0.27 and 0.23 mg·g^{−1}·h^{−1}, respectively. The CPB + SSP treatment was more effective in enhancing the catalase activity in the middle slope position than the CPB treatment.

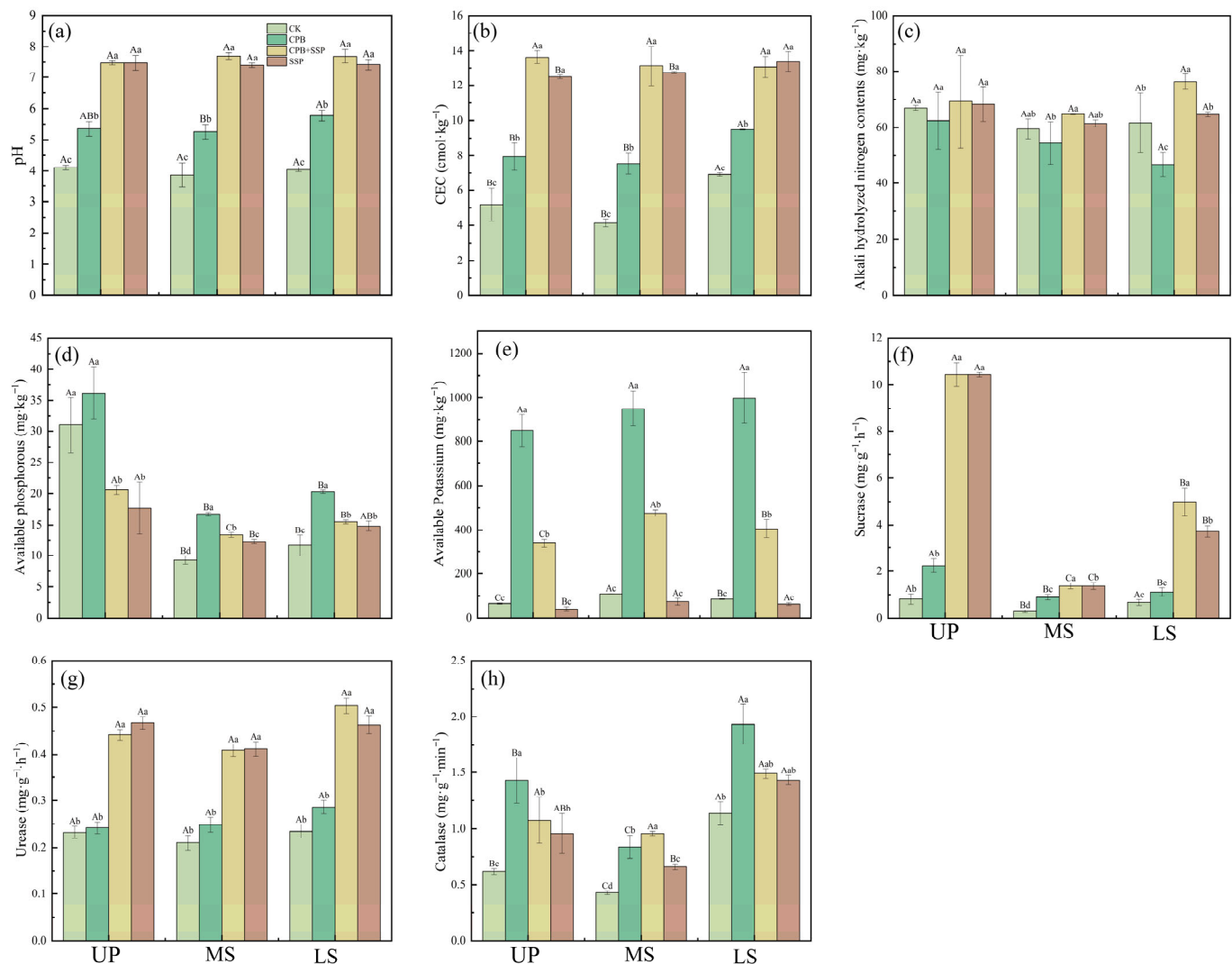


Figure 2. Effects of different amendments on soil physical and chemical properties and enzyme activities. (a) soil pH; (b) soil Cation Exchange Capacity (CEC); (c) soil alkali-hydrolyzable nitrogen (AN); (d) soil available phosphorus (AP); (e) soil available potassium (AK); (f) soil sucrase; (g) soil urease; (h) soil catalase. The figures containing different lowercase letters indicate significant differences between data points for different levels of the amendment factor under the same slope factor ($p < 0.05$). The different capital letters in the figure indicate significant differences between data at different slope factor levels under the same modifier factor ($p < 0.05$). citrus peel biochar (CPB), field snail shell powder (SSP), and their composite amendment (CPB + SSP).

3.3. Effects of Different Amendments on Soil Organic Carbon Mineralization

The mineralization of SOC exhibited distinct temporal and spatial patterns influenced by slope position and amendment type. Across all slope positions, mineralization rates peaked during early incubation (days 0–40) and gradually declined thereafter (Figure 3a–c). Notably, SSP consistently stimulated mineralization, particularly at the lower slope ($9.61 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) where rates surged by 58.95% versus the CK ($6.048 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$). In contrast, CPB ($1405.54 \text{ mg} \cdot \text{kg}^{-1}$) suppressed mineralization at the lower slope, reducing cumulative CO_2 emissions by 17.9% at the lower slope after day 60. The CPB-SSP ($2352.56 \text{ mg} \cdot \text{kg}^{-1}$) displayed intermediate effects, enhancing mineralization by 37.3% at the lower slope (CK $1712.88 \text{ mg} \cdot \text{kg}^{-1}$).

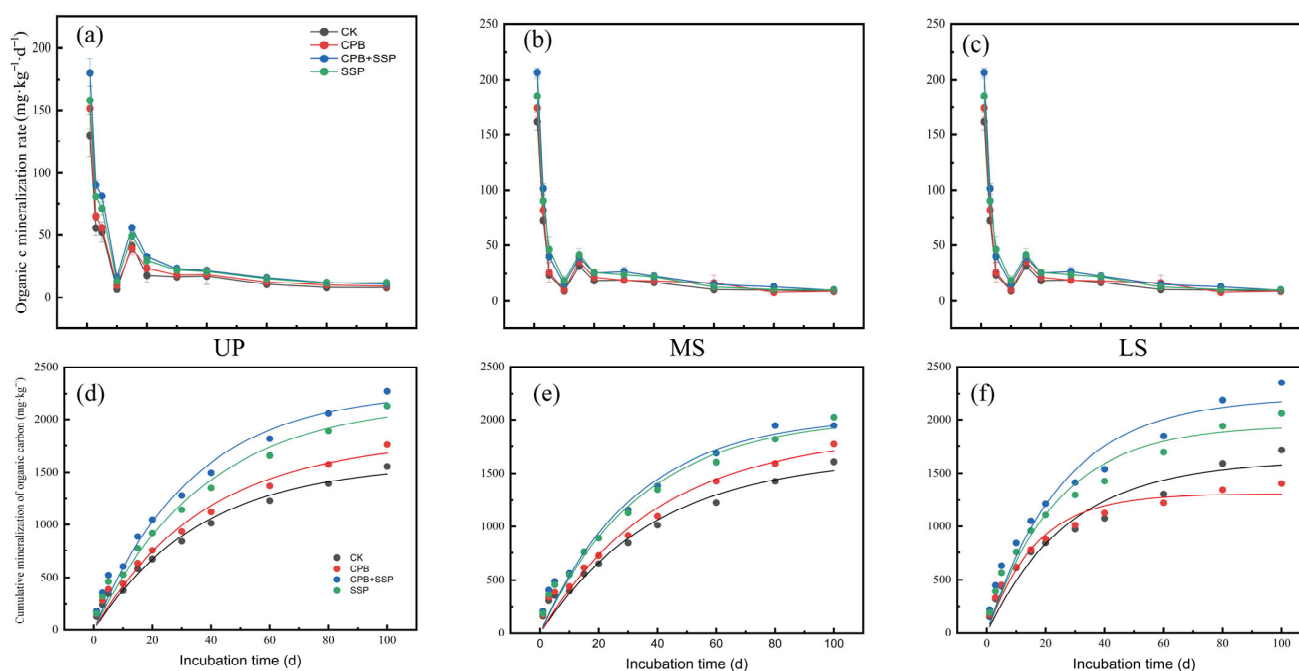


Figure 3. Effects of different amendments on soil mineralization. (a) Organic c formation rate of modifier at upper position, (b) Organic c formation rate of modifier at middle position, (c) Organic c formation rate of modifier at lower position; (d) Cumulative mineralization of organic carbon of modifier at upper position, (e) Cumulative mineralization of organic carbon of modifier at middle position, (f) Cumulative mineralization of organic carbon of modifier at lower position. citrus peel biochar (CPB), field snail shell powder (SSP), and their composite amendment (CPB + SSP).

Kinetic parameters further elucidated these trends (Table 4): The potential mineralizable carbon pool (C_0) under SSP and CPB-SSP treatments exceeded CK by 25–36% at the upper slope, reflecting heightened microbial activity. At the lower slope, CPB reduced C_0 by 19.4% but increased the mineralization rate constant (k) by 68.4%, indicating accelerated turnover of labile carbon fractions despite overall carbon conservation. Mineralization activity was strongly slope-dependent, with efficacy ranking lower slope > middle slope > upper slope for both SSP and CPB-SSP treatments ($p < 0.05$).

Table 4. Kinetic parameters of soil organic carbon mineralization in different slope positions affected by citrus peel biochar and field snail shell powder.

Different Treatments		Fitting Parameters			
		$C_0/\text{mg}\cdot\text{kg}^{-1}$	k/d^{-1}	R^2	C_0/SOC
UP	CK	1587.89 ± 94.33	0.0301 ± 0.003	0.973	0.3835
	CPB	1803.42 ± 117.09	0.0266 ± 0.003	0.970	0.2443
	CPB + SSP	2272.68 ± 123.57	0.0301 ± 0.003	0.973	0.4591
	SSP	2171.39 ± 130.47	0.0267 ± 0.003	0.974	0.5619
MS	CK	1655.06 ± 132.19	0.0255 ± 0.004	0.956	0.6961
	CPB	1859.68 ± 146.01	0.0250 ± 0.004	0.960	0.2946
	CPB + SSP	2037.87 ± 125.76	0.0308 ± 0.004	0.962	0.3721
	SSP	2029.00 ± 122.56	0.0294 ± 0.004	0.967	0.6093
LS	CK	1622.62 ± 117.11	0.0361 ± 0.006	0.930	0.4359
	CPB	1308.39 ± 50.34	0.0608 ± 0.007	0.959	0.1420
	CPB + SSP	2212.59 ± 137.55	0.0392 ± 0.006	0.940	0.3701
	SSP	1950.59 ± 100.64	0.0422 ± 0.005	0.954	0.5551

Note: C_0 : the potential mineralization amount of soil organic carbon; k : the soil mineralization rate constant; citrus peel biochar (CPB), field snail shell powder (SSP), and their composite amendment (CPB + SSP).

3.4. Effects of Different Amendments on Soil Organic Carbon and Its Active Carbon Fractions

As shown in Figure 4a, the CPB treatment increased the soil SOC content by $3.2 \text{ g}\cdot\text{kg}^{-1}$, $4.5 \text{ g}\cdot\text{kg}^{-1}$, and $5.5 \text{ g}\cdot\text{kg}^{-1}$ at the upper, middle, and lower slope positions, respectively.

The CPB + SSP treatment increased the soil SOC content by $0.8 \text{ g}\cdot\text{kg}^{-1}$, $3.6 \text{ g}\cdot\text{kg}^{-1}$, and $2.2 \text{ g}\cdot\text{kg}^{-1}$ at the upper, middle, and lower slope positions, respectively. The SSP treatment decreased the soil SOC content by $0.3 \text{ g}\cdot\text{kg}^{-1}$ and $0.2 \text{ g}\cdot\text{kg}^{-1}$ at the upper and lower slope positions, respectively, while it increased the soil SOC content by $0.7 \text{ g}\cdot\text{kg}^{-1}$ at the middle slope position. As shown in Figure 4b, among different soil amendments for dissolved organic carbon (DOC), the CPB + SSP treatment showed the best improvement effect at the three slope positions. The CPB treatment increased the soil DOC content by $10.5 \text{ mg}\cdot\text{kg}^{-1}$, $11.4 \text{ mg}\cdot\text{kg}^{-1}$, and $5.5 \text{ mg}\cdot\text{kg}^{-1}$ at the upper, middle, and lower slope positions, respectively. The SSP treatment increased the soil DOC content by $2 \text{ mg}\cdot\text{kg}^{-1}$, $7.1 \text{ mg}\cdot\text{kg}^{-1}$, and $4.5 \text{ mg}\cdot\text{kg}^{-1}$ at the upper, middle, and lower slope positions, respectively. As shown in Figure 4c, both CPB and CPB + SSP treatments significantly enhanced readily oxidizable organic carbon (ROC) at the middle slope position. The CPB treatment increased soil ROC by $0.19 \text{ g}\cdot\text{kg}^{-1}$ (upper), $0.29 \text{ g}\cdot\text{kg}^{-1}$ (middle), and $0.38 \text{ g}\cdot\text{kg}^{-1}$ (lower). The CPB + SSP treatment increased ROC by $0.18 \text{ g}\cdot\text{kg}^{-1}$ (upper), $0.22 \text{ g}\cdot\text{kg}^{-1}$ (middle), and $0.5 \text{ g}\cdot\text{kg}^{-1}$ (lower). The SSP treatment increased the soil ROC content by $0.07 \text{ g}\cdot\text{kg}^{-1}$, $0.2 \text{ g}\cdot\text{kg}^{-1}$, and $0.27 \text{ g}\cdot\text{kg}^{-1}$ at the upper, middle, and lower slope positions, respectively. As shown in Figure 4d, each treatment had a significant effect on MBC at different slope positions, and CPB + SSP treatment had the best effect on improving microbial biomass carbon (MBC) at different slope positions.

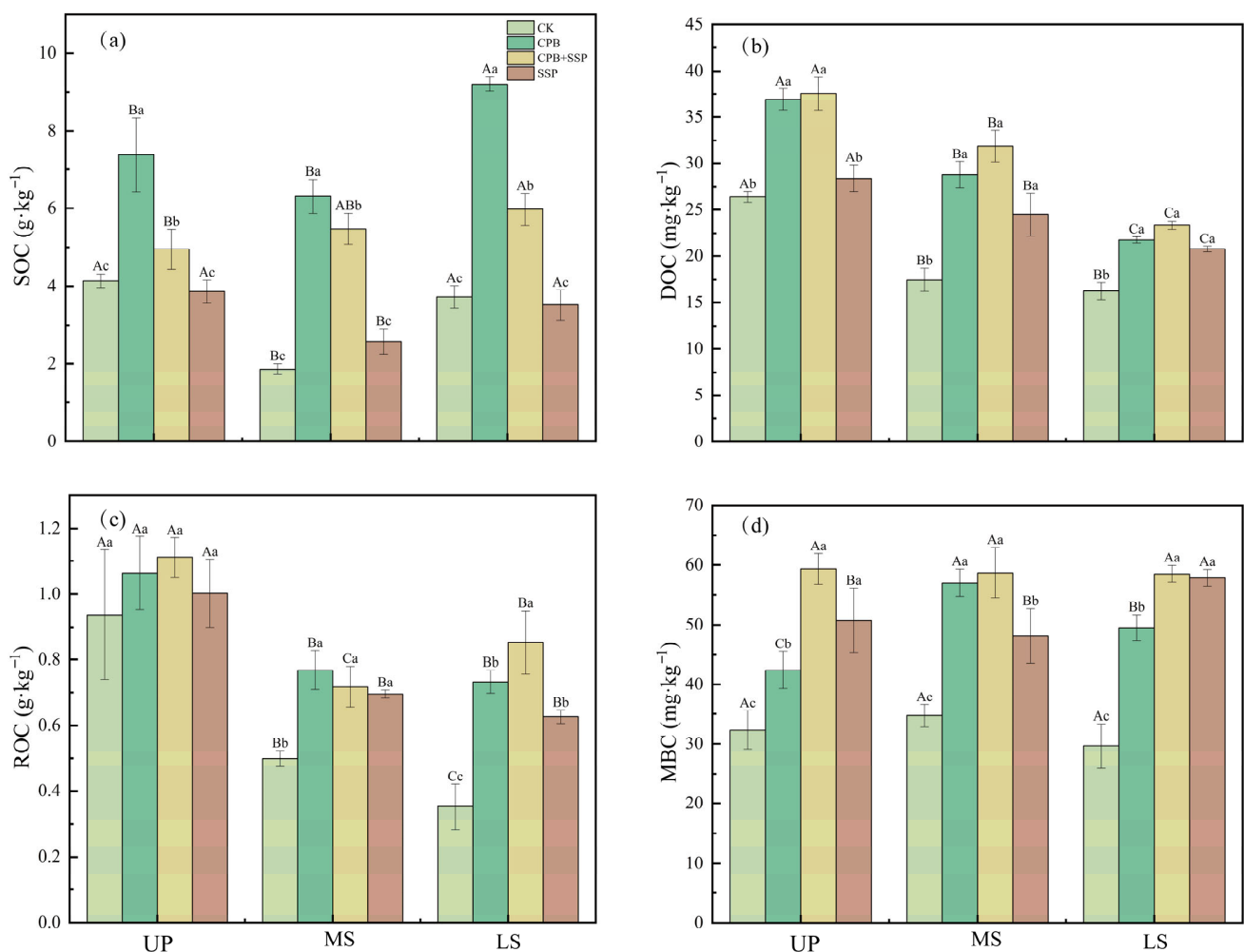


Figure 4. Effects of different amendments on soil organic carbon and its active carbon components. (a) soil organic carbon (SOC); (b) dissolved organic carbon (DOC); (c) readily oxidizable organic carbon

(ROC); (d) microbial biomass carbon (MBC). The figures containing different lowercase letters indicate significant differences between data points for different levels of the amendment factor under the same slope factor ($p < 0.05$). The different capital letters in the figure indicate significant differences between data at different slope factor levels under the same modifier factor ($p < 0.05$) citrus peel biochar (CPB), field snail shell powder (SSP), and their composite amendment (CPB + SSP).

3.5. Analyses of Correlation, Random Forest Model, and Structural Equation Model

From the heatmap in Figure 5, it can be observed that the significant effects on CO₂ emissions in the CPB and CPB + SSP treatments are similar. CEC and available nitrogen significantly affect CO₂ emissions, while pH, SOC, and DOC all have significant negative effects. In the CPB + SSP and SSP treatments, sucrase has a significant effect on CO₂ emissions. In the CPB and SSP treatments, available potassium has a significant negative effect on SOC. However, this effect is weakened in the treatment with the combined application of CPB + SSP. The random forest model was used to evaluate the important influencing factors of the soil CO₂ emission index. MBC, ROC, urease, and available nitrogen are important predictors of soil CO₂ emissions. The partial least squares structural equation model (PLS-SEM) explains 72% of soil CO₂ emissions. The soil nutrient components and SOC have a direct impact on CO₂ emissions.

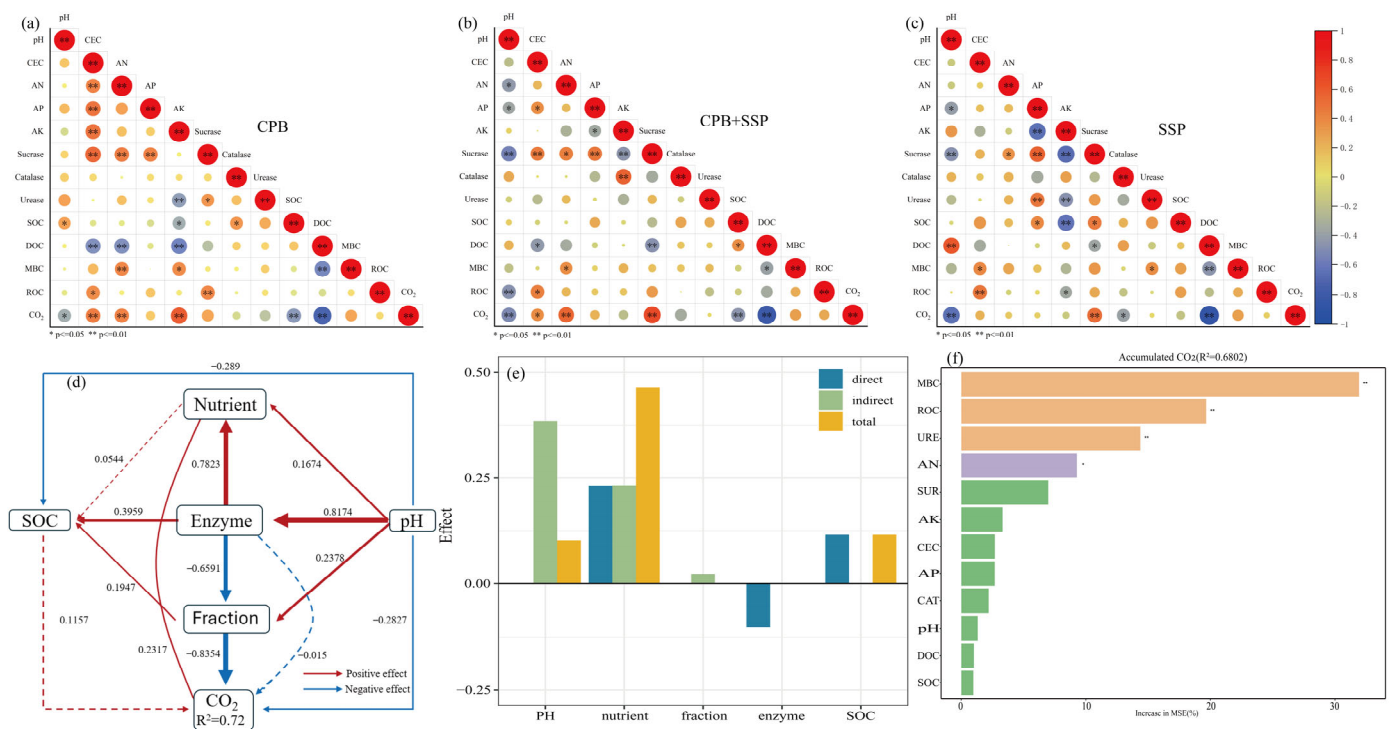


Figure 5. Heatmap of the correlation of amendments, partial least squares structural equation model, and random forest model. (a) CPB—related heat map; (b) CPB + SSP—related heat map; (c) SSP—related heat map; (d) The main pathways illustrating how environmental factors influence CO₂ emissions, the dotted line indicates that the coefficient is too small; (e) Normalized effects for PLS-SEM. (f) random forest model. citrus peel biochar (CPB), field snail shell powder (SSP), and their composite amendment (CPB + SSP). (* $p < 0.05$, ** $p < 0.01$).

4. Discussion

4.1. Regulation of Soil SOC and CO₂ Emissions by Amendments

Our results demonstrate distinct mechanisms through which citrus peel biochar (CPB), field snail shell powder (SSP), and their composite amendment (CPB + SSP) regulate SOC dynamics and CO₂ emissions in sloping citrus orchards.

CPB significantly increased SOC content across all slopes (up to $5.5 \text{ g} \cdot \text{kg}^{-1}$ at lower slope; Figure 4a), primarily due to the direct input of its highly stable carbon (77.81%; Table 3). Its porous structure and alkaline nature (pH 9.6) facilitated physical adsorption and chemical bonding of organic matter, reducing microbial accessibility and loss. Crucially, CPB suppressed mineralization rates, particularly at middle and lower slopes (e.g., 17.9% reduction at lower slope; Figure 3a, Table 4). This suppression is likely attributed to its high C/N ratio (38.59; Table 3), inducing microbial nitrogen limitation, thereby restricting metabolic activity and carbon mineralization [39,40]. Although CPB accelerated the turnover rate (k) of some unstable carbon at the lower slope (Table 4), the substantial input of stable biochar-C far exceeded potential mineralization increases (which were suppressed), resulting in unequivocal net carbon sequestration. The formation of micro-aggregates involving biochar's inert carbon further enhanced carbon pool stability by reducing the exposure of active fractions like ROC (Figure 4c) [41].

SSP effectively neutralized soil acidity (pH increase to 7.5; Figure 2a) via CaCO_3 dissolution (Figure 1), stimulating microbial activity. This led to significantly increased mineralization rates across all slopes (e.g., +58.95% at the lower slope; Figure 3a) and higher cumulative CO_2 emissions [42,43]. The pH increase promoted bacteria-dominated decomposition [44,45], accelerating mineralization of unstable carbon (e.g., DOC; Figure 4b) [46]. While Ca^{2+} from SSP increased CEC (Figure 2b) and potentially formed complexes with organic acids (short-term DOC increase; Figure 4b), its net contribution to SOC accumulation was minimal or even negative at upper and lower slopes (Figure 4a) [40,47,48]. This suggests SSP primarily accelerated the mineralization of existing SOC without providing a major new stable carbon source to offset losses [49]. The high Ca^{2+} input also induced nutrient imbalances ($\text{K}^+/\text{Ca}^{2+}$ ratio, P fixation; Figure 2e) [50,51].

CPB + SSP synergistically combined the benefits of CPB and SSP. It achieved effective pH neutralization (Figure 2a) and the highest CEC increase ($8.9 \text{ cmol} \cdot \text{kg}^{-1}$; Figure 2b), enhancing organic matter adsorption. CPB + SSP significantly increased active carbon fractions (DOC, ROC, MBC; Figure 4b–d), indicating selective retention through biochar pore-trapping and Ca^{2+} complexation, providing sustainable microbial substrates. Although CPB + SSP stimulated mineralization compared to CK (e.g., +37.3% at lower slope; Figure 3a), it simultaneously increased SOC content (e.g., $+2.2 \text{ g} \cdot \text{kg}^{-1}$ at lower slope; Figure 4a). This net gain highlights CPB + SSP's ability to balance active carbon release (supporting microbial activity and fertility) with carbon stabilization. The substantial input of stable biochar-C, combined with the protection of existing and newly formed labile carbon, outweighed the increased mineralization losses driven by SSP-induced microbial activation [52].

4.2. Slope Position Modulates Amendment Efficacy

Slope position effects profoundly influenced amendment outcomes, necessitating position-specific strategies: Lower Slopes: Exhibited the strongest SOC response to CPB (Figure 4a), attributable to higher initial SOC ($4.00 \text{ g} \cdot \text{kg}^{-1}$; Table 1) and nutrient deposition. Biochar's erosion mitigation via aggregate formation [41] and N-limitation effect counteracted inherent mineralization risks [53], making it ideal for carbon sequestration. Middle Slopes: Characterized by low initial SOC ($3.46 \text{ g} \cdot \text{kg}^{-1}$) and available phosphorus ($10.98 \text{ mg} \cdot \text{kg}^{-1}$; Table 1), this position benefited most from CPB + SSP. The composite mitigated phosphorus fixation by Ca^{2+} (Figure 2d) through biochar's ability to reduce Fe/Al-P sorption [54], while enhancing ROC and DOC (Figure 4b,c)—key substrates for fertility. COB+SSP's urease stimulation (Figure 2g) further alleviated N constraints, contrasting with nutrient limitation theories [55]. Upper Slopes: Limited amendment efficacy, likely due to erosion-driven physical carbon loss [56,57]. Though CPB raised pH (Figure 2a) [15,58],

reducing Al^{3+} toxicity, its high-carbon input could not offset runoff-induced SOC displacement. These position-dependent responses highlight that slope context dictates optimal amendment selection—a nuance overlooked in flat-land studies [25].

4.3. Model Integration and Practical Implications

Integration of machine learning (Random Forest—RF) and structural equation modeling (PLS-SEM) provided deeper insights into the factors controlling CO_2 emissions and SOC dynamics under amendments (Figure 5).

Key Predictors: The RF model identified MBC, ROC, urease activity, and AN as the most important predictors of CO_2 emissions (Figure 5f). This directly links emissions to MBC, the pool of ROC, microbial activity (urease), and AN, corroborating the mechanistic roles discussed above.

Pathway Analysis: The PLS-SEM explained 72% of the variation in CO_2 emissions (Figure 5d,e). It confirmed significant direct effects of soil nutrient components (e.g., AN, AK) and SOC fractions (including negative effects of SOC and DOC) on emissions. It also revealed indirect pathways; notably, amendments primarily influenced emissions indirectly by altering soil pH and CEC [59–61], which subsequently affected microbial activity (MBC, enzymes) and carbon pool characteristics (SOC, DOC, ROC) [62–64]. This underscores that the primary mechanism of amendment action on mineralization is through modifying the soil physicochemical environment (pH, CEC) and its cascading effects on microbes and carbon availability, rather than direct effects.

This model-aided insight informs scalable strategies:

1. Lower Slopes: Apply CPB (4%) for maximal carbon sequestration.
2. Middle Slopes: Use CPB + SSP (2% CPB + 2% SSP) to balance SOC storage and fertility (via DOC/ROC).
3. Upper Slopes: Prioritize erosion control (e.g., terracing) before amendment use.

By converting citrus waste (CPB) and aquaculture byproducts (SSP) into soil amendments, this approach supports circular agriculture while addressing soil degradation and climate goals (e.g., carbon neutrality). However, long-term field trials are essential to validate persistence under real-world erosion and cropping cycles.

The controlled laboratory incubation conditions, while ideal for isolating specific biogeochemical processes, cannot fully replicate the complex interplay of variables present in a field environment, such as fluctuating rainfall patterns, temperature extremes, root exudation, and soil erosion. Consequently, the magnitude of the reported effects on SOC mineralization and stabilization might differ under real-world conditions. More importantly, our study focused exclusively on soil biogeochemical responses and did not evaluate the ultimate agronomic efficacy of the amendments—namely, the response of the citrus plants themselves. The effectiveness of any soil amendment must be ultimately validated through improved plant physiology, nutrient uptake, and fruit yield and quality.

5. Conclusions

This study demonstrates that slope position critically regulates SOC responses to amendments in citrus orchards. CPB maximized SOC sequestration at lower slopes ($+5.5 \text{ g} \cdot \text{kg}^{-1}$) by combining high carbon input with suppressed mineralization (17.9%). Conversely, CPB + SSP optimized carbon stability and active fractions (DOC, ROC) at middle slopes, mitigating carbon loss despite low initial SOC. Machine learning (RF/PLS-SEM) confirmed MBC and ROC as key drivers of CO_2 emissions, mediated by pH and enzyme activities. Lower slopes benefit from CPB for carbon sequestration, while middle slopes require CPB + SSP to balance fertility and carbon retention. Future field studies must validate these laboratory findings under real-world erosion and crop growth conditions.

Conclusions derive from controlled incubations; long-term field trials are needed to assess amendment persistence and scalability. Future studies should focus on: long-term field trials to validate the persistence of amendment effects under natural erosion and cropping cycles, and economic and environmental life-cycle assessments of CPB and SSP production and application to evaluate scalability and sustainability.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15092209/s1>, Figure S1: FTIR of the citrus peel biochar and field snail shell powder. References [65–70] are cited in the Supplementary Materials file.

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