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Biochar Application and Mowing Independently and Interactively Influence Soil Enzyme Activity and Carbon Sequestration in Karst and Red Soils in Southern China

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Abstract: Soil organic carbon (SOC), a critical component of the global carbon cycle, represents the largest terrestrial carbon reservoir, and is thus a major component of influencing climate regulation and ecosystem health. Grasslands store substantial carbon in their soils, but this carbon reservoir is easily degraded by both grazing and mowing, particularly in vulnerable karst landscapes. This study investigates the potential of biochar, a carbon-rich soil amendment, as a management tool to maintain SOC or mitigate the degradation of SOC during mowing in karst grasslands in Southern China, using both red acidic and calcareous soils as experimental variables. T SOC fractions, soil enzyme activities, and soil pH were measured to determine the effect of mowing and biochar application on carbon stability and microbial activity. Consistent with expectations, mowing increases belowground biomass and promotes carbon loss through increased microbial activity, particularly in calcareous soils where mowing also decreases soil pH, increasing acidity and reducing the stability of Ca–carbon complexes. Biochar, however, counteracted these effects, increasing both particulate organic carbon (POC) and mineral-associated organic carbon (MAOC), especially in red soils where the addition of biochar greatly increased soil pH (from 5.4 to 6.33) (an effect not observed in the already-alkaline karst soils). Enzyme activities related to carbon degradation, such as β-D-Glucosidase and peroxidase, increased in biochar-amended soils (β-D-Glucosidase increased from 12.77 to 24.53 nmol/g/h and peroxidase increased from 1.1 to 2.36 mg/g/2h), each of which contribute to the degradation of carbon containing organic matter so that it may be ultimately stored in more recalcitrant forms. Mowing led to reduced polyphenol oxidase activity, but the presence of biochar mitigated these losses, protecting SOC pools (increased from 0.03 to 0.79 mg/g/2h). This study highlights biochar as an effective tool for enhancing SOC stability in karst grasslands, particularly in acidic soils, and suggests that integrating biochar into mowing regimes may optimize carbon sequestration while reducing fire risk. These findings offer valuable theoretical guidance for developing sustainable land management in sensitive ecosystems.

Keywords: grasslands; mitigation; pH; soil enzyme activities; soil organic carbon (SOC)

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1. Introduction

Soils hold more carbon than the atmosphere and vegetation combined [1,2]. Understanding the mechanisms that stabilize this soil carbon reservoir is crucial for mitigating climate change and maintaining ecosystem health [3,4]. Grasslands, which cover approximately 40% of the Earth's surface, represent a significant portion of the global soil carbon pool, with much of the carbon stored in the soil rather than in aboveground biomass [5]. Conserving and managing soil carbon in grasslands are critical for reducing CO_2 emissions and enhancing carbon sequestration.

Karst landscapes, which are characterized by porous carbonate rock and thin soils, are particularly sensitive to land-use changes [6–8]. In these regions, grazing is commonly practiced but can increase CO_2 emissions by promoting soil decomposition and accelerating the loss of soil organic carbon (SOC) [9]. Grazing disturbs the soil and increases root turnover, enhancing microbial activity and the breakdown of organic matter [10,11]. Thus, grazed karst regions represent significant contributors to SOC loss, necessitating improved land management practices to prevent further degradation [12,13].

One proposed alternative to grazing is allowing grasslands to grow unmanaged, but this strategy can lead to an accumulation of litter, increasing wildfire risks. In response, land managers often turn to mowing to reduce fuel loads and mitigate fire hazards [14]. However, mowing also carries risks, as it can reduce the stability of SOC by increasing decomposition rates. Mowing stimulates plant allocation of resources underground, potentially providing increased substrate to microbial associates and generally promoting decomposition, potentially to offset nutrient limitations associated with compensatory growth [15,16]. Thus, while mowing can reduce fire risk, mowing may also exacerbate SOC losses, presenting a dilemma for sustainable land management.

Biochar, a carbon-rich product produced by pyrolyzing organic material, offers a potential solution to stabilize SOC and mitigate carbon losses from mowing. Biochar's high surface area, porosity, and nutrient retention capacity make it a promising amendment for improving soil properties and promoting carbon sequestration [17]. Biochar can alter microbial activity, increase carbon stability, and buffer soil pH, particularly in degraded or acidic soils [18]. However, the effects of biochar on SOC fractions and soil enzyme activities in less disturbed ecosystems, such as karst grasslands, remain understudied.

This study investigates the potential of biochar to stabilize SOC in mowed karst grasslands. To better understand the mechanisms involved, we included a contrasting soil type: an acidic red soil (oxisol), depleted in base cations, which represents an older and more weathered soil than our karst soil of interest. By including both soil types, the study can determine whether biochar's pH-raising properties contribute to its ability to protect soil carbon. Additionally, the study measured key soil enzyme activities to further explore how biochar and mowing interact to influence SOC dynamics. Enzymes such as β-D-Glucosidase, peroxidase, and leucine aminopeptidase play pivotal roles in carbon and nitrogen cycling, and their activity offers insight into how biochar and mowing affect SOC turnover.

Thus, we used a mesocosm experiment to address the knowledge gaps described above. The objectives of this study were to evaluate how biochar amendment influences soil organic carbon (SOC) content and stability in mowed karst grasslands by altering extracellular enzyme activities and to determine whether these effects depend on parent soil type. We hypothesized that mowing increases soil carbon losses via enhanced belowground biomass, microbial activity, and soil acidity, whereas biochar application mitigates these negative impacts and improves SOC stability, particularly in acidic soils. This study provides valuable theoretical guidance for optimizing land management in vulnerable grassland ecosystems, contributing to climate change mitigation and soil health protection.

2. Materials and Methods

2.1. Study Site

The experiment took place in a greenhouse in Guilin City, Guangxi Zhuang Autonomous Region, southwest China's karst region (coordinates: 24°15′23″–26°23′30″ N, 109°36′50″–111°29′30″ E). The region has a subtropical monsoon climate with mean annual temperatures approximately 19.1 °C, with lowest monthly mean temperature in January at an average of 8 °C and the highest in July at an average of 28 °C. The mean precipitation ranges from 1160 to 1378 mm with a distinct seasonal pattern. The study region is mountainous, interwoven with karst areas and non-karst areas. We used two soil types: Fe-rich red soil (pH 5.2–6.6) and calcareous soil (pH 7.8–8.2), both of which were topsoil from nearby non-karst and karst grasslands, respectively. Soils were air-dried and homogenized for potting experiments.

2.2. Experimental Design

On 1 March 2022, we designed a mesocosm experiment using a randomized block design to test the effects of biochar and mowing on soil organic carbon and enzyme activities of two soil types in karst grassland. Within each block, we assigned four treatments: control (CK), biochar addition (B), mowing (M), and biochar + mowing (BM), with four replicates in each treatment. Each pot was 30 cm in diameter at the top, 23 cm at the bottom, and 23 cm high, and each pot was filled with approximately 8 kg of soil.

Separately, biochar was produced by pyrolyzing corn stalks at 500 °C (we should add a citation here for the biochar method). This biochar was mixed into the soil at a rate of 100 g biochar/kg of soil for a total of 0.8 kg of biochar per pot on average. Following this, each pot was planted with 20 tall fescue plants (*Festuca arundinacea* Schreb.), a C_4 grass species adapted to Guilin's climate. Plants were watered daily to ensure the soil moisture content remained at 70–80% of its water holding capacity. Weeds were controlled through regular manual removal to prevent competition for resources and maintain experimental conditions. The pots were not fertilized or limed, to isolate the effect of biochar as a management tool.

On 15 March 2023, one year after planting, we measured leaf height and, using allometric equations to estimate height–biomass relationships, cut each plant at the height necessary to remove 50% of aboveground biomass. The plant shoot materials were ultimately mowed to an average canopy height of ~10 cm and ~20 cm above the ground for red soil without and with biochar, respectively, and ~18 cm and ~25 cm above the ground for calcareous soil without and with biochar, respectively. The harvested plant parts were removed from the plots. On 30 March 2023, the remaining plants were harvested in their entirety, dried at 65 °C for 48 h, and aboveground and belowground biomasses were recorded. Soil samples were collected from each pot at a 0–15 cm depth and sieved to remove rock and root residues. Soil samples were split into two fractions: one fraction of air-dried soil was sieved through a 0.15 mm mesh to obtain a more homogeneous sample, while the other fraction of field-moist soil was transported to the lab and stored at 4 °C. The field-moist soil was later passed through a 2 mm sieve and thoroughly mixed. The fresh soil was used for determination of soil-available N, microbial biomass C, and soil enzyme activities. Air-dried samples were used for determination of soil pH, total N, total P, total organic C, and carbon fractions.

2.3. Chemical Analysis

Soil pH was measured in a 1:2.5 soil–water suspension with a pH meter. SOC and total N contents were determined using an elemental analyzer (Vario EL III; Elementar, Langenselbold, Germany). Soil TP was determined by acid digestion with a H_2SO_4 + $HClO_4$ solution and analyzed using the molybdenum blue method. The available N (NH_4-N and NO_3-N) was extracted from moist soil using 2 M KCl solution (with a soil–solution ratio of 1:5) and was analyzed on a continuous-flow autoanalyzer [19]. Microbial biomass carbon (MBC) was determined by chloroform fumigation, measured with a total organic carbon (TOC) analyzer [20].

The bulk soil sample was separated into two operationally defined soil fractions, a free light fraction and a heavy fraction (>1.8 g cm^3), following a density fractionation technique. Briefly, approximately 10 g air-dried soil (<2 mm) was weighed into a 100 mL polycarbonate centrifuge tube, and 60 mL of 1.8 g cm^{-3} NaI solution was added. Samples were shaken at 300 rpm for 2 h and then centrifuged at 3000× *g* for 15 min. After centrifuging, tubes were allowed to stand for 24 h, and the supernatant was removed by pipetting. After repeating the procedure three times for each sample to ensure complete recovery of the light fraction, both the light fraction and the heavy fraction were washed by deionized water to remove residual NaI. Then, the separated soil fractions were dried in an oven at 80 °C and then ground to a homogenized fine powder for organic C analysis using the CN analyzer [21].

2.4. Enzyme Activity Assays

Each soil sample was assayed for hydrolytic enzymes β-D-Glucosidase (BG) and leucine aminopeptidase (LAP) and oxidative enzymes polyphenol oxidase (PPO) and peroxidase (PER), using standard fluorometric and colorimetric techniques [22,23]. In brief, 96-well microplates were used to conduct enzyme assays. For each sample, 1 g of fresh soil was suspended in 50 mL of 50 mm sodium acetate buffer (pH 6.0), using either methylumbelliferyl enzyme substrates (for β-D-Glucosidase and leucine aminopeptidase) or L-3,4-dihydroxyphenylalanine (L-DOPA, 25 µM) as the substrate (for polyphenol oxidase and peroxidase) [24]. Eight replicates of each plate were incubated in the dark at 25 °C for 2.5 h for hydrolytic enzymes and for 4 h for oxidative enzymes. Fluorescence was quantified at 360 and 460 nm excitation and emission wavelengths (hydrolytic enzymes), respectively, and absorbance was measured at 450 nm (oxidative enzymes) in a microplate reader (Synergy 2, Biotek, Winooski, VT, USA).

2.5. Data Analyses

We tested the data for normality before conducting a three-way split-plot ANOVA to evaluate the effects of biochar addition, mowing, and soil type on soil enzyme activity. Biochar, mowing, and their interaction were treated as fixed factors, while blocks ($n = 4$) were treated as random factors. The results showed a significant interactive effect between biochar addition and mowing, influenced by soil types. To further investigate these effects, a two-way split-plot ANOVA was conducted separately for each soil type, with biochar, mowing, and their interaction as fixed factors and blocks ($n = 4$) as random factors. Significant interactions between biochar and mowing were explored using independent *t*-tests. This analysis allowed us to examine the effects of mowing on SOC fractions, enzyme activities, belowground biomass, pH, MBC, and available nitrogen within each biochar treatment for different soil types, as well as the effects of biochar within mowing treatments across soil types. All statistical analyses were performed using SPSS 29.0 with statistical significance set at $p < 0.05$.

3. Results

Results from Table 1 show that both red soil and calcareous soil have different initial soil properties. The soil MBC, SOC, TN, TP, and enzyme activities were significantly higher in both alkaline calcareous soils than in red soils (Table 1).

Table 1. The soil physicochemical properties of the initial experimental soil including pH, microbial biomass carbon (MBC), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), polyphenol oxidase (PPO), peroxidase (PER), β-D-Glucosidase (βG), and leucine aminopeptidase (LAP) of red soils and calcareous soils, respectively. Data are means ± SD. Different letters indicate significant differences between red and calcareous soils ($p \leq 0.05$).

Soil Type	pH	MBC (mg/kg)	SOC (g/kg)	TN (g/kg)	TP (g/kg)	PPO (mg/g/2h)	PER (mg/g/2h)	βG (nmol/g/h)	LAP (nmol/g/h)
Red soil	5.28 ± 0.03 [b]	37.55 ± 1.94 [b]	3.16 ± 0.22 [b]	0.43 ± 0.02 [b]	0.33 ± 0.01 [b]	0.34 ± 0.02 [b]	0.91 ± 0.02 [b]	10.98 ± 1.29 [b]	14.99 ± 2.19 [b]
Calcareous soil	7.78 ± 0.02 [a]	336.52 ± 18.3 [a]	23.61 ± 0.76 [a]	2.16 ± 0.02 [a]	0.72 ± 0.03 [a]	0.79 ± 0.10 [a]	1.92 ± 0.10 [a]	29.08 ± 3.06 [a]	208.62 ± 9.58 [a]

3.1. Effect of Biochar on Soil Organic Carbon (SOC) Stocks

Biochar significantly increased particulate organic carbon (POC) in both red and calcareous soils ($p < 0.001$). Red soils exhibited a greater increase in POC compared to calcareous soils. Similarly, mineral-associated organic carbon (MAOC) increased in both soils following biochar addition, with the increase being more pronounced in red soils (Figure 1).

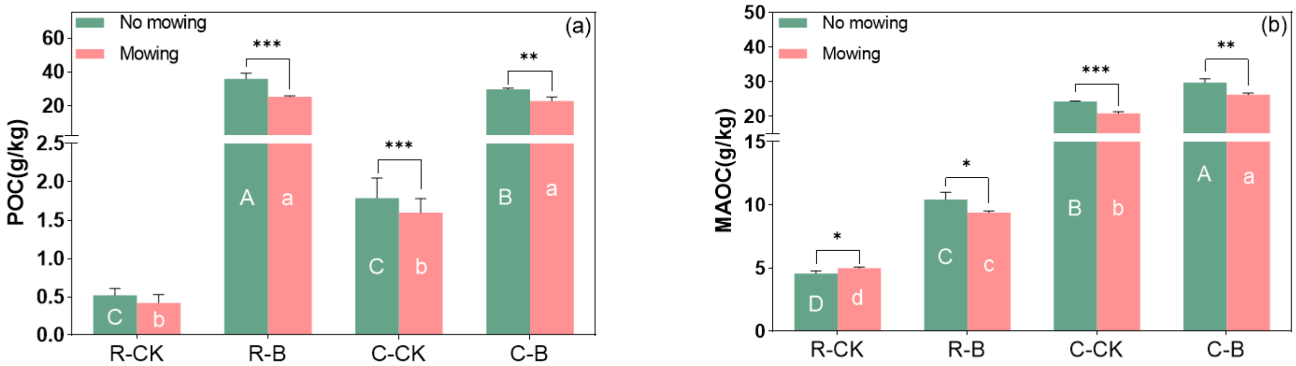

Figure 1. Effect of mowing and biochar on soil particulate organic carbon (POC) (**a**) and mineral-associated organic carbon (MAOC) (**b**) in different soil types (no biochar, CK; with biochar, B; red soil, R; and calcareous soil, C). Values represent mean ± SE ($n = 4$). Different uppercase letters indicate significant differences among treatments under no mowing, different lowercase letters indicate significant differences among treatments under mowing ($p \leq 0.05$). Asterisk indicates significant differences between mowing and no mowing treatments (* $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$).

3.2. Patterns Across Enzymes and Carbon Sequestration

Biochar addition influenced enzyme activities. There were significant interaction effects between the soil type and biochar addition (Table 2). Two-way ANOVA indicated that the effects of biochar and mowing are different in different soil types (Table 3). Polyphenol oxidase (PPO) activity remained higher in control soils compared to mowed soils across both soil types. In red soils, biochar increased PPO activity ($p < 0.001$), but in calcareous soils, biochar did not affect PPO levels. Peroxidase (PER) activity followed a similar trend, with a significant increase in red soils after biochar addition ($p < 0.001$) but no influence of biochar addition on PER activity in calcareous soils (Figure 2).

Table 2. The results of the three-way ANOVA on soil type (ST), biochar amendment (B), mowing (M), and their interactions on soil enzyme activities, using ST, B, M, and their interaction as fixed terms and block as random-effect. PPO: polyphenol oxidase; PER: peroxidase; βG: β-D-Glucosidase; and LAP: leucine aminopeptidase. Asterisk indicates significant differences (* $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$).

Treatment	PPO		PER		βG		LAP	
	f	*p*	*f*	*p*	*f*	*p*	*f*	*p*
ST	1255.79	<0.001 ***	754.59	<0.001 ***	55.26	0.005 **	795.28	<0.001 ***
M	523.35	<0.001 ***	313.62	<0.001 ***	0.93	0.355	62.30	<0.001 ***
B	242.71	<0.001 ***	290.99	<0.001 ***	17.32	0.006 **	64.94	<0.001 ***
ST × M	0.35	0.566	654.00	<0.001 ***	0.05	0.831	82.74	<0.001 ***
ST × B	101.82	<0.001 ***	305.77	<0.001 ***	21.78	0.003 **	99.11	<0.001 ***
B × M	13.41	0.003 **	17.44	0.001 **	0.07	0.797	10.43	0.007 **
ST × B × M	0.82	0.383	5.80	0.033 *	0.02	0.887	20.90	0.001 **

Table 3. The results of two-way ANOVA testing the effect of biochar amendment (B), mowing (M), and their interactions on soil enzyme activities in different soil types, using B, M, and their interaction as fixed terms and block as random-effect. R: red soil; C: calcareous soil; PPO: polyphenol oxidase; PER: peroxidase; βG: β-D-Glucosidase; and LAP: leucine aminopeptidase. Asterisk indicates significant differences (** $p < 0.01$; and *** $p < 0.001$).

Treatment	PPO		PER		βG		LAP	
	f	*p*	*f*	*p*	*f*	*p*	*f*	*p*
R-M	2148.48	<0.001 ***	285.27	<0.001 ***	0.95	0.367	33.198	0.001 **
R-B	666.46	<0.001 ***	397.30	<0.001 ***	79.73	0.003 **	52.359	0.005 **
R-B × M	81.39	<0.001 ***	14.42	0.009 **	0.01	0.926	41.318	0.001 **
C-M	132.67	<0.001 ***	495.19	<0.001 ***	0.22	0.657	72.957	<0.001 ***
C-B	10.00	0.051	0.18	0.697	0.09	0.79	82.542	0.003 **
C-B × M	2.03	0.204	11.46	0.015 **	0.07	0.806	15.379	0.008 **

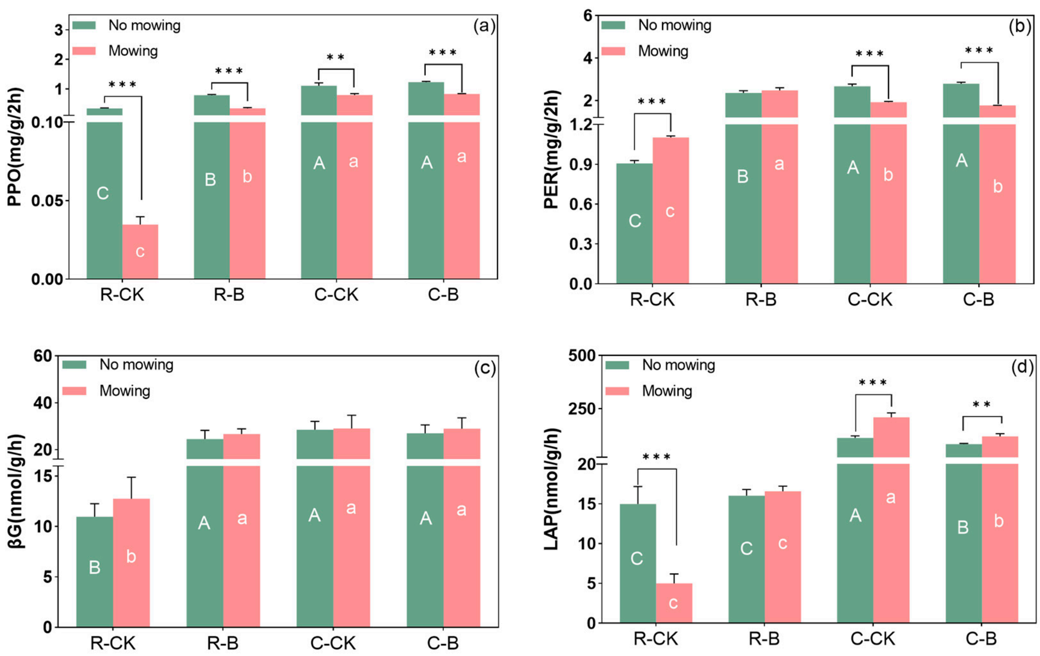

Figure 2. Effect of mowing and biochar on polyphenol oxidase (PPO) (**a**), peroxidase (PER) (**b**), β-D-Glucosidase (βG) (**c**), and leucine aminopeptidase (LAP) (**d**) in different soil types (no biochar, CK; with biochar, B; red soil, R; and calcareous soil, C). Values represent mean ± SE ($n = 4$). Different uppercase letters indicate significant differences among treatments under no mowing, different lowercase letters indicate significant differences among treatments under mowing ($p \leq 0.05$). Asterisk indicates significant differences between mowing and no mowing treatments (** $p < 0.01$; and *** $p < 0.001$).

Biochar raised β-D-Glucosidase (BG) activity in red soils to levels comparable with those in calcareous soils, and biochar-amended red soil β-D-Glucosidase (BG) activity was protected from any influence by mowing. In contrast, biochar did not significantly alter BG activity in calcareous soils. Leucine aminopeptidase (LAP) activity showed no change in response to biochar in either soil type, though LAP activity remained higher in calcareous soils across all treatments, regardless of biochar or mowing (Figure 2).

3.3. Effects of Mowing on SOC and Enzyme Activity

Mowing consistently reduced particulate organic carbon (POC) in both soil types, with a larger decrease observed in calcareous soils compared to red soils. Biochar helped mitigate these reductions, particularly in calcareous soils, where the gap between biochar-amended and non-amended soils was smaller, indicating that biochar provided greater protection against POC loss in calcareous soils (Figure 1b).

Mowing also reduced MAOC in calcareous soils, regardless of biochar addition, with a more significant decrease in non-amended soils. In red soils, MAOC increased slightly with mowing, but biochar reversed this trend, leading to a slight decrease in MAOC. Mowing reduced PPO and PER activities in both soils, with more pronounced reductions in calcareous soils, particularly in biochar-amended treatments. LAP activity increased significantly in mowed calcareous soils ($p < 0.001$), but biochar mitigated this increase. In red soils, mowing without biochar reduced LAP activity, while biochar kept LAP levels consistent across treatments (Figure 2).

3.4. General Soil Properties

Belowground biomass, soil pH, and soil microbial biomass C responded significantly to biochar and mowing treatments. Mowing significantly increased belowground biomass ($p < 0.001$), as did biochar, with the highest levels observed when both treatments were combined. Biochar increased soil pH significantly in red soils but did not affect pH in calcareous soils. Mowing reduced soil pH in calcareous soils, particularly in biochar-amended treatments ($p < 0.05$), while in red soils, mowing actually increased pH. Biochar significantly increased soil MBC in calcareous soils but did not affect MBC in red soils. Mowing significantly increased MBC in both soil types (Figure 3a–c).

Ammonium nitrogen (NH_4-N) levels were lowest in biochar-amended soils across all treatments, regardless of mowing. Control soils in both red and calcareous treatments had significantly higher NH_4-N concentrations than their biochar-amended counterparts ($p < 0.001$). Mowing increased NH_4-N in red soils but had minimal impact on NH_4-N in calcareous soils. Nitrate nitrogen (NO_3-N) levels increased with mowing in red soils, both in control and biochar-amended treatments, though NO_3-N levels were consistently lower in biochar-amended red soils. In calcareous soils, NO_3-N levels were higher than in red soils, and mowing reduced NO_3-N concentrations. Biochar reduced NO_3-N levels in both soils. Total available nitrogen (AN) increased with mowing in red soils and decreased with mowing in calcareous soils ($p < 0.05$). Biochar significantly reduced AN in both soil types, particularly in red soils (Figure 3d–f).

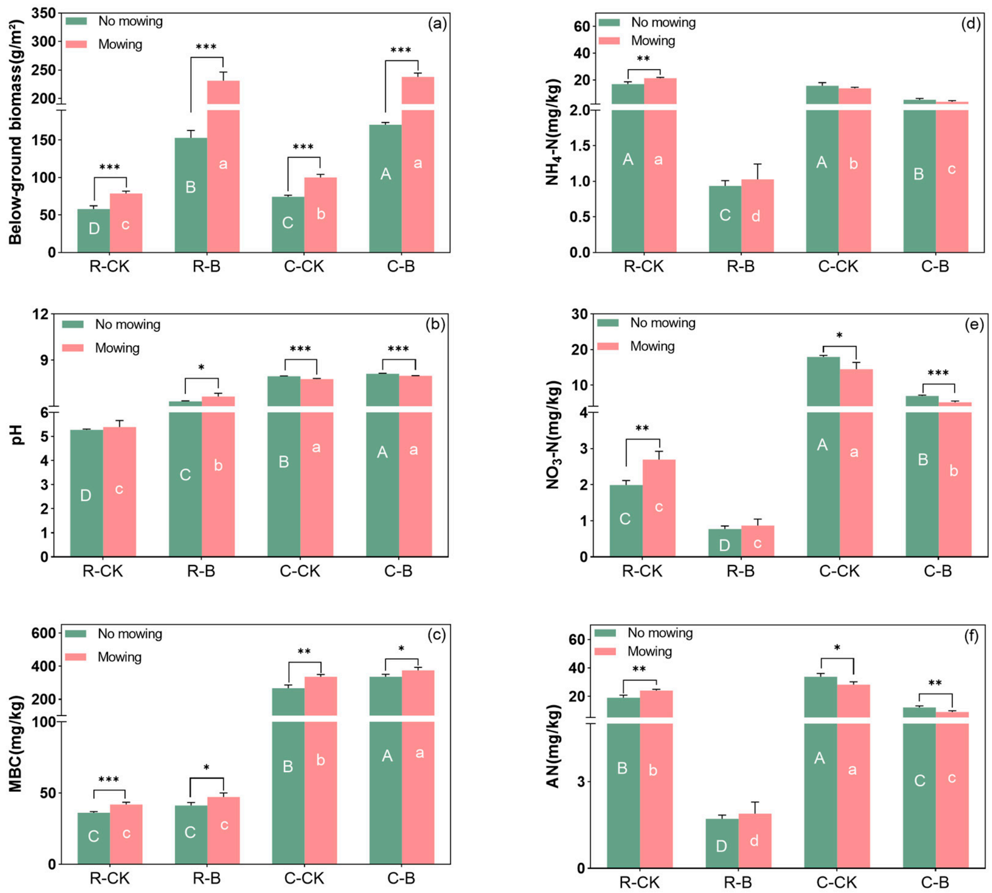

Figure 3. Effect of mowing and biochar on belowground biomass (**a**), pH (**b**), microbial biomass carbon (MBC) (**c**), ammonium nitrogen (NH_4-N) (**d**), nitrate nitrogen (NO_3-N) (**e**), and total available nitrogen (AN) (**f**) in different soil types (no biochar, CK; with biochar, B; red soil, R; and calcareous soil, C). Values represent mean ± SE ($n = 4$). Different uppercase letters indicate significant differences among treatments under no mowing, different lowercase letters indicate significant differences among treatments under mowing ($p \leq 0.05$). Asterisk indicates significant differences between mowing and no mowing treatments (* $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$).

4. Discussion

4.1. Impact of Biochar and Mowing on Soil Organic Carbon Dynamics

Our pot experiment demonstrates that biochar amendment, mowing, and parent soil type each influence total soil organic carbon (SOC) content and its distribution into particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) fractions. Mowing consistently reduced POC and MAOC in both soil types (Figure 1). This reduction likely results from enhanced microbial decomposition following increased belowground biomass and root exudation (Figure 3). Increased belowground biomass was observed in both soil types following mowing, indicating that plants invested more carbon belowground when aboveground biomass was removed [25]. This shift likely stimulated root exudation, contributing to SOC decomposition. These effects were most pronounced in calcareous soils, where mowing had a substantial influence on pH and dramatically influenced the stability of Ca–carbon complexes [21], as opposed to in the red soil, which was less affected (because the red soil represents a soil that has already lost easily weathered calcium complexes).

However, in the calcareous karst soil, biochar could mitigate the reduction in carbon pools caused by mowing, resulting in a net positive effect on SOC when both treatments were combined (Figure 1), which is consistent with our hypothesis. This effect was attributed to biochar's ability to raise soil pH, alter extracellular enzyme activity, and enhance carbon retention. This aligns with previous studies suggesting that biochar can improve soil structure, nutrient retention, and microbial activity through its porous structure and high adsorption capacity, facilitating root growth and microbial colonization [18]. In comparison, the most significant impact of biochar was observed in red soils, where the pH-raising effect played a critical role in protecting both POC and MAOC. The significant pH increase in acidic red soils enhanced conditions for microbial activity and the function of extracellular enzymes such as β-D-Glucosidase (βG), polyphenol oxidase (PPO), and peroxidase (PER). These enzymes are critical for decomposing plant-derived cellulose and stabilizing microbial necromass, likely facilitating the stabilization of organic matter in more resistant carbon pools [26,27]. The pronounced effect of biochar in red soils, where its alkalinity enhances SOC stability in more acidic environments, suggests that biochar can significantly promote carbon stabilization even under conditions of increased decomposition. This makes biochar a valuable tool for grassland management, particularly in karst landscapes currently undergoing rapid rocky desertification. In both soil types, biochar increased belowground biomass, further supporting the hypothesis that biochar improves soil conditions for plant growth and carbon retention.

4.2. Enzyme Activity and Nutrient Cycling

Our results show that enzyme activities respond to biochar and mowing with clear implications for carbon and nitrogen cycling. Biochar addition increased PPO, PER, andβG activities in red soils, enhancing the breakdown of lignin and cellulose [28], key components of plant-derived carbon. Meanwhile, we found that microbial biomass carbon (MBC) increased (Figure 3c). This enzyme activity, in turn, may have facilitated the incorporation of carbon into more recalcitrant soil fractions, such as MAOC, through microbial decomposition pathways [27].

PPO activity was reduced by mowing across both soil types. Importantly, in the act of mowing, we removed litter, and thus the decreased PPO activity may simply reflect the reduced input of lignin in plant biomass, which PPO is normally used to degrade [29]. This reduction in PPO, combined with increased LAP activity (Figure 2), suggests a shift toward nitrogen-limited decomposition under mowing conditions, as microbes appear to prioritize the production of LAP to acquire nitrogen. LAP, which is involved in breaking down proteins to release nitrogen, was particularly elevated in mowed calcareous soils, where belowground biomass increased. This increase could represent a stress response, potentially linked to the octadecanoid pathway [30], a defense response mechanism similar to that seen in solanaceous plants. LAP may also serve a dual function, aiding in nitrogen acquisition and regulating plant responses to biotic and abiotic stresses [31].

Interestingly, mowing decreased LAP in red soils, although mowing did increase in belowground biomass. This decrease, coupled with increased MBC and MAOC, suggests that nitrogen availability may have satisfied microbial nitrogen demands, reducing the need for LAP activity. This finding supports the idea that soil inorganic nitrogen content can regulate LAP activity, with higher nitrogen availability inhibiting the enzyme's activity and promoting the accumulation of microbial necromass (Figure 3d–f) [32,33]. Whereas, in calcareous soils, where available N is constant or decreases after mowing, it is possible that microorganisms produce more LAP to generate available N for plant uptake.

4.3. Red Soil as a Control for Parent Soil Effects

Adding biochar to red soils caused a significantly larger increase in both POC and MAOC than the same biochar addition to calcareous soils ($p < 0.001$). Enzyme activities also responded more strongly to biochar in red soils, particularly for PPO, PER, and BG. In calcareous soils, biochar had no significant impact on PPO or PER activities, suggesting that red soils were more sensitive to biochar's effects on enzyme-driven carbon cycling. The increase in β-D-Glucosidase activity in biochar-amended red soils, bringing it to levels similar to those in calcareous soils, indicates that biochar altered carbon-degrading enzyme activity in red soils to match the baseline activity in calcareous soils.

4.4. Implications for Carbon Sequestration in Karst Grasslands

The interplay between biochar, mowing, and enzyme dynamics has important implications for managing karst grasslands to improve carbon sequestration. Mowing reduced POC and MAOC, likely due to increased microbial activity and nitrogen limitation. The increase in LAP activity in calcareous soils under mowing suggests a shift toward nitrogen cycling, possibly driven by stress responses and belowground biomass increases. While mowing can stimulate root exudation and belowground biomass, leading to enhanced carbon inputs, it also accelerates decomposition. However, biochar's ability to enhance carbon retention, even under conditions of increased microbial activity induced by mowing, highlights its potential as a soil amendment for maintaining or increasing SOC pools. The increased enzyme activity observed in biochar-amended red soils, particularly PER and βG, points to enhanced decomposition and an increased rate of carbon cycling, which can lead to greater incorporation of carbon into stable fractions like MAOC. Thus, integrating biochar into mowing regimes could mitigate these negative effects, preserving carbon stores while promoting root growth and nutrient acquisition.

For karst grassland management, a strategy that incorporates biochar alongside mowing could offer a sustainable approach to improving carbon sequestration. By promoting belowground biomass growth and maintaining SOC pools, biochar amendments could help offset the decomposition losses associated with mowing or grazing, ultimately contributing to long-term carbon storage in grassland ecosystems.

5. Conclusions

This study explored the combined effects of biochar addition and mowing on SOC dynamics in karst grasslands, using red and calcareous soils to assess parent soil effects. The results demonstrate that mowing increases belowground biomass but promotes carbon loss through increased microbial activity and soil acidification, particularly in calcareous soils. Biochar addition, however, significantly counteracted these effects, increasing both POC and MAOC fractions, especially in red soils, where biochar's pH-raising properties also enhanced enzyme activities such as β-D-Glucosidase, polyphenol oxidase, and peroxidase, which may contribute to SOC stabilization by promoting the decomposition of plant residues and movement of readily degraded carbon into more recalcitrant soil carbon pools. However, biochar mitigated these negative effects, demonstrating its potential to buffer against the destabilizing impact of mowing on SOC pools.

In conclusion, the findings suggest that biochar is a valuable tool for managing carbon sequestration in karst grasslands, particularly when considering both the carbon loss associated with grazing or the potential carbon loss associated with mowing to prevent fire. The dramatic increase in SOC of red soils associated with changing pH brought on by biochar addition points to how a major role in the effectiveness of biochar may be the alkalizing effect of raising soil pH. By improving SOC retention and promoting belowground biomass, biochar offers a sustainable strategy for reducing CO_2 emissions while managing fire risks in grasslands. Overall, integrating biochar into mowing regimes in karst landscapes can optimize carbon sequestration, mitigate soil carbon loss, and maintain soil health. The differential effects observed between red and calcareous soils highlight the importance of tailoring management practices to specific soil types and environmental conditions. This targeted approach is crucial for maximizing the benefits of biochar in vulnerable grassland ecosystems.

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