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RESEARCH ARTICLE

Effects of prolonged acid rain on labile and stable soil organic carbon fractions in a subtropical forest

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Abstract Due to the air pollutant emissions, acid rain in southern China may strongly influence soil organic carbon (SOC) decomposition and stabilization by disrupting microbial communities and associated enzymatic processes. In this study, we conducted a field experiment in a subtropical forest in southern China, by implementing simulated acid rain (SAR) treatments with varying pH levels (4.5 as a control, 4.0, 3.5, and 3.0) to investigate the effects of acid rain on soil microbial community composition, carbon (C)-degrading enzyme activities, an both labile and stable SOC fractions. Results showed that SAR treatments significantly altered the soil microbial community and reduced several key C-degrading enzyme activities (e.g., β-glucosidase by −22% − −55%, phenol oxidase by −32% − −71%, and peroxidase by −4% − −71%). Accordingly, SAR treatments increased liable SOC content by 3%−150% and stable SOC content by 1%−52%, leading to increases in total SOC content by 5%−29%. These findings demonstrate that acid rain can suppress soil microbial productions of C-degrading enzymes, thereby promoting the accumulation of both labile and stable SOC fractions. The differential responses of labile and stable SOC fractions to prolonged acid rain exposure may have important implications for the long-term sequestration and stability of SOC in subtropical forests soils in southern China.

Keywords prolonged acid rain, soil organic carbon fractions, carbon sequestration, microbial community, soil enzyme activity

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1 Introduction

Forest ecosystems are critical reservoirs of carbon (C), with soils storing 3−4 times more C than aboveground vegetation (Scharlemann et al., 2014). Tropical and subtropical forests, which comprise nearly half of the global forest area, contribute approximately 30% of the global soil C stock (Crowther et al., 2019). Given the substantial quantities of soil C stored and cycled in these forests, even minor changes in the SOC pool can significantly affect the global C balance (Zhou et al., 2013; Cavaleri et al., 2015; Zhang et al., 2024). Therefore, understanding the complex processes of soil C sequestration and turnover in tropical and subtropical forests is crucial for managing global C fluxes.

Acid rain remains a widespread environmental concern that strongly alter the wide biogeochemical cycle of ecosystems, including ecosystem C balance (Yu et al., 2017). Especially in recent decades, due to the rapid development of industry and agriculture across emerging economies, acid rain has already spread from the temperate Northern Hemisphere to tropical and subtropical regions (Duan et al., 2016). It is reported that the rates of N and S deposition in southern China and India have reached the highest levels in Europe and North America (Decina et al., 2020). Such high rates of acid deposition in these regions have lowered the soil pH of some forests to < 4 (Lu et al., 2014). Decreased soil pH may strongly influence SOC decomposition by disrupting microbial communities and enzymatic processes. However, while many studies of the acid rain effects on SOC have been conducted, the consequences remain controversial, showing increases (Wang et al., 2010; Qiu

et al., 2024), decreases (Wang et al., 2012; Liu et al., 2024) or negligible effects (Kemmitt et al., 2006; Kroeger et al., 2024). These inconsistent results points to the necessity of further examination of how SOC respond to acid rain.

Soil organic carbon is conventionally classified into labile and stable fractions based on its persistence and susceptibility to decomposition (Schmidt et al., 2011; Lehmann and Kleber, 2015). The labile fraction, including acid-hydrolysable organic carbon (AOC), readily oxidizable carbon (ROC), and particulate organic carbon with sizes greater than 53 μm (POC), comprises organic compounds that are highly active and sensitive to external environmental changes (von Lützow et al., 2007; Wagai et al., 2013). These compounds, derived from decomposed plant residues, microbial biomass, and their by-products, are prone to oxidation, mineralization, and rapid turnover (Rovira et al., 2010; Feng and Simpson, 2011). In contrast, the stable fraction, including non-acid-hydrolysable organic carbon (NAOC), non-readily oxidizable organic carbon (NROC), and particulate organic carbon smaller than 53 μm (NPOC), consists of chemically recalcitrant compounds that persist in the soil over extended periods (Gentsch et al., 2018; Karhu et al., 2019). These stable SOC components are typically associated with mineral surfaces, which can be protected from microbial decomposition especially in the future warming climate (Jagadamma and Lal, 2010; Villarino et al., 2021). Therefore, improving our understanding of how SOC fractions respond to prolonged acid rain exposure is essential for evaluating the long-term sequestration potential of subtropical forest soils in southern China.

The decomposition of SOC predominantly mediated by soil microorganisms and the secreted extracellular enzymes (Allison et al., 2010; Nannipieri et al., 2012; Crowther et al., 2019). For instance, fungi primarily produce oxidases, which facilitates the breakdown of phenolic compounds that are characteristic of chemically resistant organic matter (Tian and Shi, 2014; Fang et al., 2020; Püspök et al., 2023). However, bacteria prefer to decompose the liable fractions of organic matter by secreting hydrolases (Cheng et al., 2017). Additionally, Gram-positive (G^+) bacteria and actinomycetes have been identified as efficient decomposers of recalcitrant carbon compounds, including lignin and chitin (Zaitlin et al., 2004; Santos et al., 2012). Acid rain-induced soil acidification can lead to significant shifts in microbial community structure, potentially altering the activity of specialized enzymes (Liu et al., 2010; Tian et al., 2019; Cheng et al., 2020), and thereby driving changes in SOC and its fractions (Ge et al., 2021; Bhattacharyya et al., 2022). However, most existing studies have been conducted in soils with alkaline to near-neutral pH levels (Aliasgharzad et al., 2010; Lim et al., 2011), and relatively little is known about microbial responses to acid rain and their implications for SOC fractions in the subtropical forests of southern China, where soil pH is already very low.

To address these knowledge gaps, we conducted a long-term field experiment to assess the effects of simulated acid rain (SAR) on SOC content and its various fractions in a subtropical forest in southern China. In addition, we analyzed soil microbial community composition and the activity of C-degrading enzymes to explore potential mechanisms driving SOC fraction changes under SAR treatment. Our previous studies have shown that SAR treatment significantly enhances the input of stable substances from litter to the soil layer (Wu et al., 2016, 2020). In this study, we aimed to explore how different SOC fractions respond to the prolonged SAR exposure and to elucidate the microbial processes associated with these changes. We hypothesized that SAR-induced soil acidification would alter the compositions of dominant microbial communities and inhibit all of the key C-degrading enzymes, thereby enhancing SOC in both labile and stable fractions.

2 Material and methods

2.1 Site description

This study was conducted in Dinghushan National Nature Reserve, spanning 1133 ha in Guangdong, southern China (112°30′−112°33′E, 23°09′−23°11′N). The reserve is characterized by a typical monsoon and subtropical humid climate, with an average annual temperature of 21.5°C and a relative humidity of 80%. The mean annual rainfall is 1956 mm with a distinct seasonality, and nearly 80% of the precipitation occurring during the warm-wet season from April to September (Lu et al., 2014). The experimental site was set up in a monsoon evergreen broadleaf forest, representing the most mature stage of forest succession in this region (Chen et al., 2022). The forest is located 250–300 m above sea level and occupies approximately 600 ha. The dominant species were *Castanopsis chinensis*, *Cryptocarya concinna*, *Cryptocarya chinensis*, *Machilus chinensis*, and *Schima superba* (Yan et al., 2006). The soil type at the study site is classified as lateritic red earth, featuring a loamy in texture and an acid pH. Acid rain poses a significant environmental threat in this area, evidenced by an annual average rainfall pH consistently below 4.50 over the past decades (Chen et al., 2022; Hu et al., 2022).

2.2 Experimental design and treatments

The SAR experiment was established in June 2009 as a randomized complete block design with three replicates. Each block consisted of four plots (10×10 m^2), separated by 3-m buffer zone to minimize edge effects. Four SAR

treatments were randomly assigned within the four plots within each block by irrigating the soil with water solutions of varying pH levels: CK (control, utilizing local lake water, pH ≈ 4.5), T1 (pH = 4.0), T2 (pH = 3.5), T3 (pH = 3.0). The SAR solutions were prepared by mixing local lake water with sulfuric acid (H_2SO_4) and nitric acid (HNO_3) at a 1:1 molar ratio, reflecting the ionic composition of acid rain based on historical precipitation data. Treatments were applied beneath the forest canopy using a gasoline-powered sprayer, with each plot receiving 40 L of solutions per application. Applications were conducted twice per month throughout the experimental period. For more detailed information on the experimental setup and methodology, please refer to Liang et al. (2013) and Wu et al., (2020).

2.3 Sample collection and analyses

In June 2018, soil samples were collected at a depth of 0−10 cm soil layer in each plots, using a standard soil sampling tube (2.5 cm inside diameter). In each plot, we randomly collected five soil cores and mixed them into one sample. A total of 12 mixed samples were collected. Fresh soil samples were passed through a 2-mm sieve to remove rocks and plant roots, and stored at 4°C for analyzing soil microbial biomass carbon (SMBC), phospholipid fatty acid (PLFA) and soil enzyme activities. The sub-samples were air-dried and analyzed for SOC fractions and chemical properties.

The soil pH value was determined by mixing 10 g soil with 25 mL of deionized CO_2-free water for 5 min, leaving it to equilibrium for approximately 30 min (Dick et al., 2000). SOC concentration was determined by the Walkley-Black's wet digestion method (Nelson and Sommers, 1982). The SMBC was determined by subjecting the fresh soil samples to the chloroform fumigation-extraction technique (Martens, 1995; Chen et al., 2018).

Non-acid-hydrolysable organic carbon (NAOC) was obtained by acid hydrolysis to isolate resistant pools of SOC using the method described in Rovira and Vallejo (2002) and Tan et al. (2004). Briefly, approximately 1000 mg of ground soil samples were refluxed at 105°C for 18 h in 25 mL of 6 M HCL, with occasional shaking by using a digestion block. After refluxing, the suspension was washed with de-ionized water and filtered through a glass-fiber filter. Finally, the residue was oven-dried at 60°C to a constant weight, and subsequently analyzed for NAOC content. For each sample, the difference between SOC and NAOC concentrations is regarded as the AOC content.

Particulate organic carbon (POC, 53−2000 μm) was measured by modifying the procedure described in Chen et al. (2012) and Fang et al. (2020). 15 g of air-dried soil were dispersed in 100 mL of 5 $g \cdot L^{-1}$ sodium hexametaphosphate solution by handshaking the mixture for 5 min. The dispersed soil sample was placed on a reciprocal shaker (90 $r \cdot min^{-1}$) for 18 h, and subsequently, was passed subsequently through 53 μm stainless steel sieve and rinsed thoroughly with distilled water. The material remaining on the sieve was defined as the POC fraction. The dispersed soil sample that passed through the 53 μm sieve was considered as the NPOC fraction. All of the samples were dried at 60°C for 12 h, weighed, then finely ground to determine the C content. The organic C content in each fraction was converted to bulk soil POC or N-POC content according to the fraction mass ratios.

Readily oxidizable organic carbon (ROC) was determined using the method described in Blair et al. (1995) and Wang et al. (2024). 1 g air-dried soil samples containing about 15−30 mg carbon were weighed into centrifuge tubes containing 25 mL of 333 mmol $KMnO_4$ solution, which were shaken for 1 h, and then centrifuged for 5 min. The supernatants were diluted 1: 250 with deionized water. The absorbance of the diluted samples and standards were read on a UV spectrophotometer at 565 nm. The change in the concentration of $KMnO_4$ was used to estimate the amount of C oxidized, assuming that 1 mmol MnO4 is consumed in the oxidation of 9 mg carbon. For each sample, the difference between SOC and ROC concentrations is regarded as the NROC content.

2.4 PLFA analyses

Soil microbial community structure was evaluated by measuring the profile of phospholipid fatty acids (PLFAs) following the method as described by Fang et al. (2020). The PLFAs were extracted from 8 g of dry soil, fractionated and analyzed. Peak areas were converted to nanomole per gram of dry soil using internal standards (19:0 nonadecanoic methylester). The microbial community structure was investigated on the basis of specific PLFAs for different microbial groups (He et al., 2023). The PLFAs used to indicate gram-negative (G^-) bacterial abundance included cyclic 17:0cy, 19:0cy, 16:1ω7c, and 18:1ω7c, while gram-positive (G^+) bacterial abundance was identified using 15:0a, 15:0i, 16:0i, 17:0a, and 17:0i. Total bacterial abundance was determined by summing the PLFA biomarkers for G^- and G^+ bacteria, along with 14: 0, 15:0, 16: 0, and 18:0. The biomarker for arbuscular mycorrhizal fungi (AMF) was 16:1ω5c. Fungal abundance was calculated as the sum of the PLFA biomarkers 18: 1ω9c, 18: 2ω6, 9c and 18: 3ω6c along with AMF abundance. Actinomycetes were identified using the PLFAs 10Me16:0, 10Me17:0 and 10Me18:0. Total microbial biomass was calculated as the sum of all detected PLFAs. The fungal-to-bacterial biomass ratio (F:B ratio) was determined by dividing total fungal PLFA markers by total bacterial PLFA markers (Liu et al., 2021).

2.5 Activities of soil enzyme analyses

Activities of soil enzyme involved in C cycling were measured, including *β*-glucosidase (BG), cellobiohydrolase (CBH), phenol oxidase (PhOx) and peroxidase (Perox). For BG activity (Eivazi and Tabatabai, 1988), 1.0 g of soil was mixed with 4 mL of modified universal buffer at pH 6.0, and 1 mL 0.025 M *p*-nitrophenyl-*β*-*D*-glucopyranoside. The mixture was then incubated for 1 h at 37°C. Reactions were stopped by adding 0.5 M $CaCl_2$ and 0.1 M trihydroxymethyl aminomethane which was buffered to pH 12. Controls were performed with the substrate being added after the reactions were stopped. The mixtures were filtered through Whatman filter paper and measured colorimetrically at 400 nm (Tabatabai, 1994; Fang et al., 2016) with a UV-VIS spectrophotometer (UV-1700, Shimadzu, Columbia, MD, USA). The procedures for the assay of CBH activities was the same as for BG activity except using *p*-nitrophenyl-b-D-Cellobioside as the substrate and buffering pH of reaction systems to 5.0 (Parham and Deng, 2000; Wang et al., 2015). PhOx and Perox were quantified as described by Iyyemperumal and Shi (2008), and using *L*-3, 4- dihydroxy phenylalanine (*L*-DOPA) as the substrate. For PhOx activity, 1 g of soil was mixed with 4.5 mL of modified universal buffer at pH 5.0 and 4.5 mL 0.01 M *L*-DOPA. The mixture was then rapidly mixed and incubated for 1 h at 25°C. After that, it was immediately centrifuged at 12000 g at 5°C for 5 min to terminate the reaction. The products were filtered through Whatman filter paper and quantified using a fluorescence spectrometer by measuring the absorbance at 450 nm. The assay of Perox activity was the same as PhOx except adding 1 mL 0.3% H_2O_2 to the mixture before incubation. Enzyme activity was expressed as micromoles per gram dry weight and per hour ($\mu mol \cdot g^{-1}$ dry soil$\cdot h^{-1}$).

2.6 Statistical analysis

One-way ANOVA with LSD test was used to determine the effects of SAR treatment on all response variables. This experimental setup followed a completely randomized design, which allowed for independent replication of treatment effects without the need for block stratification. While a block design can be beneficial in accounting for spatial heterogeneity, the selected completely randomized design approach was deemed appropriate given the relatively uniform topography and soil conditions at the study site, as previously characterized by Liang et al. (2013). Correlation among microbial parameters, soil enzyme activities, SOC and its liable/stable fractions were tested by the Pearson correlation coefficient. All statistical analyses were performed by IBM SPSS Statistics 22 software (IBM Corp., New York, USA), and statistical significance was set at $P < 0.05$ unless otherwise stated.

3 Results

3.1 Soil pH value and SOC fractions

During the experimental period, soil pH levels significantly decreased under the SAR treatments, with notable differences observed between the control and the more acidic T2 and T3 treatments (Table 1, $P < 0.05$). SOC concentration increased by 5%−29% under the SAR treatment, with the greatest increase observed in the T3 treatment (Table 1). All liable SOC fractions showed significant increases with decreasing soil pH (Fig. 1), including a 3%−22% increase in AOC, a 19%−40% increase in ROC, and a 34%−150% increase in POC. The T3 treatment exhibited the highest concentrations of labile SOC fractions compared to the control, suggesting that prolonged exposure to high acidity inhibits microbial processes responsible for decomposing these labile carbon sources, thereby reducing their mineralization rates and allowing for their accumulation in the soil. Similarly, all stable SOC fractions also increased significantly under SAR treatments, with NAOC increasing by 11%−52%, NROC by 1%−27% and NPOC by 1%−14%. NAOC concentrations were significantly higher in the T2 and T3 treatments, while NROC and NPOC increases were most pronounced in the T3 treatments compared to the control (Fig. 1). The increased accumulation of stable SOC fractions under prolonged SAR exposure indicates a shift toward more chemically recalcitrant C forms that are less susceptible to microbial utilization and enzymatic degradation.

Table 1 Effects of simulated acid rain (SAR) treatment on soil pH, soil organic carbon content (SOC, $g \cdot kg^{-1}$), soil microbial biomass carbon (SMBC, $g \cdot kg^{-1}$), and recalcitrance index of SOC (*RI*)

Treatment	pH	SOC	SMBC	*RI*[#]
CK	3.85 ± 0.02a	28.36 ± 1.07b	442.06 ± 14.27a	0.96 ± 0.06b
T1	3.81 ± 0.03ab	29.71 ± 1.91b	411.75 ± 17.66ab	1.02 ± 0.04ab
T2	3.77 ± 0.04b	33.38 ± 1.78ab	341.03 ± 23.31b	1.02 ± 0.05ab
T3	3.70 ± 0.03c	36.65 ± 1.36a	288.57 ± 25.37b	1.10 ± 0.03a

Notes: Values are means ± SE. Different superscript letters indicate differences at a significance level of $P < 0.05$. [#]Data from Wu et al. (2020). The recalcitrance index (*RI*) is a reliable indicator of organic carbon (C) stability. *RI* = (Alkyl C + Aromatic C) / (O-alkyl C + Carbonyl C).

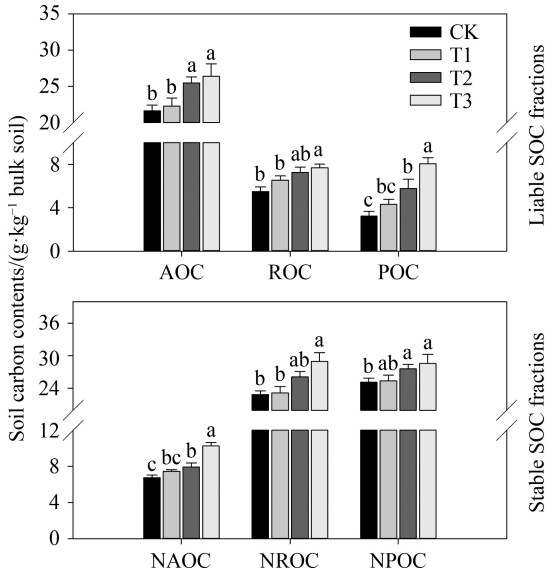

Fig. 1 Effect of simulated acid rain treatment on liable and stable soil organic carbon (SOC) fractions. The simulated acid rain treatments are CK: Control, pH ≈ 4.5; T1: pH = 4.0; T2: pH = 3.5; T3: pH = 3.0. Error bars are standard errors of the mean. Different lowercase letters above bars for some variable denote significant difference ($P < 0.05$) among simulated acid treatments. AOC, acid-hydrolysable organic carbon; ROC, readily oxidation organic carbon; POC, particulate organic carbon; NAOC, non-acid-hydrolysable organic carbon; NROC, non-readily oxidation organic carbon; NPOC, non-particulate organic carbon.

Pearson correlation analyses revealed strong positive correlations between total SOC and both labile and stable SOC fractions (Table 2, $P < 0.05$), indicating that variation in these fractions is a primary driver of total SOC dynamics. Overall, these findings suggest that prolonged acid rain exposure not only facilitates the accumulation of labile SOC but also enhances SOC stability by promoting the preservation of stable carbon compounds, thereby contributing to increased soil carbon sequestration in subtropical forest ecosystems.

3.2 Soil microbial community composition

SMBC concentrations were significantly affected by the SAR treatments (Table 1, $P < 0.05$). Specifically, SMBC decreased by 22.8% and 48.4% in the T2 and T3 treatments, respectively, compared to the control. We measured total microbial phospholipid fatty acids (PLFAs) along with biomarkers specific to bacteria, fungi, arbuscular mycorrhizal fungi, Gram-positive bacteria, Gram-negative bacteria, and actinomycetes. As illustrated in Fig. 2, most microbial PLFA groups were significantly impacted by SAR treatments ($P < 0.05$), except for actinomycetes and Gram-negative bacteria. Total microbial PLFAs and bacterial PLFAs were significantly lower in the T2 and T3 treatments compared to control. Fungal and Gram-positive bacterial PLFAs, along with the fungal-to-bacterial ratio, were significantly reduced under T1, T2, and T3 treatments relative to CK. AMF PLFAs also showed a pronounced decline in the T3 treatment when compared to both T1 and CK. In contrast, the biomass of actinomycetes and Gram-negative bacteria remained statistically unchanged across treatments (Fig. 2).

Pearson correlation analysis revealed strong positive relationships between microbial group abundances and total SOC, as well as both labile and stable SOC fractions (Table 2). These correlations indicate that microbial communities and SOC fractions respond in a coupled manner under prolonged SAR exposure. The observed reductions in microbial biomass and key functional groups, together with the accumulation of SOC fractions, highlight the intricate interactions between soil microbial communities and carbon dynamics under prolonged acid rain exposure.

3.3 Activities of carbon-degrading enzymes

We measured the activities of hydrolytic enzymes involved in soil carbon decomposition, as presented in Fig. 3. The SAR treatments had no significant effect on cellobiohydrolase (CBH) activity ($P > 0.05$). However, β-glucosidase (BG) activity was markedly reduced under SAR treatments, with both T2 and T3 treatments exhibiting significantly lower BG activities compared to the CK ($P < 0.05$).

Furthermore, we also evaluated the activities of oxidative enzymes related to soil carbon decomposition. The results indicated that SAR treatments significantly decreased the activities of phenol oxidase (PhOx) and peroxidase (Perox, $P < 0.05$). Specifically, PhOx activity was significantly lower in all SAR treatments compared to CK, while Perox activity was significantly reduced in the T2 and T3 treatments relative to CK (Fig. 3).

Pearson correlation analysis revealed that SOC content and its stable fractions were negatively correlated with oxidase enzyme activities ($P < 0.05$). Consequently, SOC and its stable fractions increased significantly as oxidase enzyme activities decreased (Table 2).

4 Discussion

4.1 Effect of SAR on soil microbial communities and enzyme activities

Consistent with our hypotheses, the simulated acid rain (SAR) treatment resulted in a decrease in overall microbial abundance and induced shifts in the composition of key microbial groups (Fig. 2). These outcomes align with findings from numerous previous studies (Fierer and Jackson, 2006; Cha et al., 2013). The most plausible explanation for these changes is the increased extractable aluminum (Al) toxicity associated

Table 2 Pearson correlation between soil organic carbon (SOC) and its fractions, microbial communities, enzyme activities under the effect of simulated acid rain treatment

	SOC	AOC	ROC	POC	NAOC	NROC	NPOC	SMBC	tPLFAs	Bacteria	Fungi	G^+	G^-	Act	AMF	BG	CBH	PhOx	Perox
SOC	1																		
AOC	0.927**	1																	
ROC	0.813**	0.672*	1																
POC	0.729**	0.486	0.817**	1															
NAOC	0.600*	0.255	0.661*	0.841**	1														
NROC	0.985**	0.937**	0.699*	0.650*	0.538	1													
NPOC	0.852**	0.935**	0.521	0.263	0.202	0.891**	1												
SMBC	−0.659*	−0.380	−0.638*	−0.754**	−0.887**	−0.618*	−0.352	1											
tPLFAs	−0.839**	−0.735**	−0.844**	−0.648*	−0.595*	−0.778**	−0.688*	0.660*	1										
Bacteria	−0.693*	−0.496	−0.781**	−0.693*	−0.728**	−0.617*	−0.447	0.619*	0.889**	1									
Fungi	−0.595*	−0.419	−0.733**	−0.542	−0.641*	−0.511	−0.525*	0.631*	0.807**	0.852**	1								
G^+	−0.771**	−0.617*	−0.843**	−0.677*	−0.670*	−0.694*	−0.569	0.643*	0.917**	0.883**	0.923**	1							
G^-	−0.466	−0.362	−0.465	−0.365	−0.427	−0.433	−0.378	0.401	0.656*	0.756**	0.539	0.523	1						
Act	−0.166	−0.063	−0.211	−0.225	−0.294	−0.140	−0.062	0.169	0.328	0.562	0.203	0.158	0.822**	1					
AMF	−0.541*	−0.427	−0.606*	−0.468	−0.486	−0.484	−0.406	0.428	0.621*	0.732**	0.647*	0.610*	0.786**	0.693*	1				
BG	−0.781**	−0.569	−0.852**	−0.848**	−0.796**	−0.703*	−0.451	0.869**	0.765**	0.696*	0.758**	0.781**	0.374	0.062	0.483	1			
CBH	0.2435	0.078	0.465	0.401	0.459	0.159	0.036	−0.386	−0.405	−0.424	−0.503	−0.460	−0.116	0.161	0.073	−0.586*	1		
PhOx	−0.746**	−0.551	−0.866**	−0.765**	−0.747**	−0.656*	−0.566*	0.776**	0.915**	0.856**	0.845**	0.901**	0.532	0.229	0.595*	0.898**	−0.583*	1	
Perox	−0.829**	−0.637*	−0.807**	−0.802**	−0.777**	−0.776**	−0.555*	0.869**	0.801**	0.747**	0.704*	0.745**	0.619*	0.289	0.555	0.905**	−0.440	0.805**	1

Notes: AOC, acid-hydrolysable organic carbon; ROC, readily oxidation organic carbon; POC, particulate organic carbon; NAOC, non-acid-hydrolysable organic carbon; NROC, non-readily oxidation organic carbon; NPOC, non-particulate organic carbon; SMBC, soil microbial biomass carbon; tPLFAs, total PLFA concentration; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria; Act, actinomycetes; AMF, arbuscular mycorrhizal fungi; BG, β-glucosidase, CBH, Cellobiohydrolase; PhOx, Phenol oxidase; Perox, peroxidase.

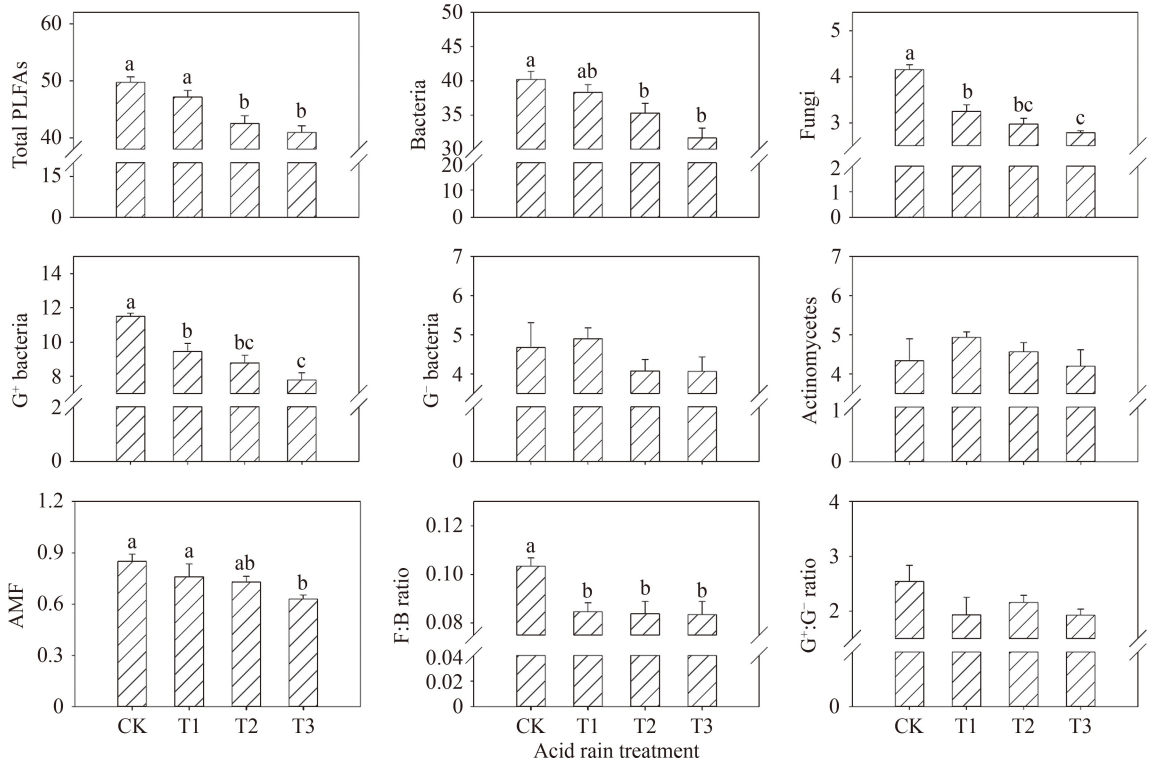

Fig. 2 Effect of simulated acid rain treatment on soil microbial phospholipids fatty acid (PLFAs) biomarkers content (nmol·g^{-1} dry soil) and biomarker ratios. The simulated acid rain treatments are CK: Control, pH≈4.5; T1: pH = 4.0; T2: pH = 3.5; T3: pH = 3.0. Error bars are standard errors of the mean. Different lowercase letters above bars for some variable denote significant difference (P < 0.05) among simulated acid treatments. Total PLFAs, total PLFA concentration; G$^+$ bacteria, Gram-positive bacteria; G$^-$ bacteria, Gram-negative bacteria; AMF, arbuscular mycorrhizal fungi; F: B ratio, the ratio of fungal biomass to bacterial biomass; G$^+$: G$^-$ ratio, the ratio of Gram-positive bacterial biomass to Gram-negative bacterial biomass.

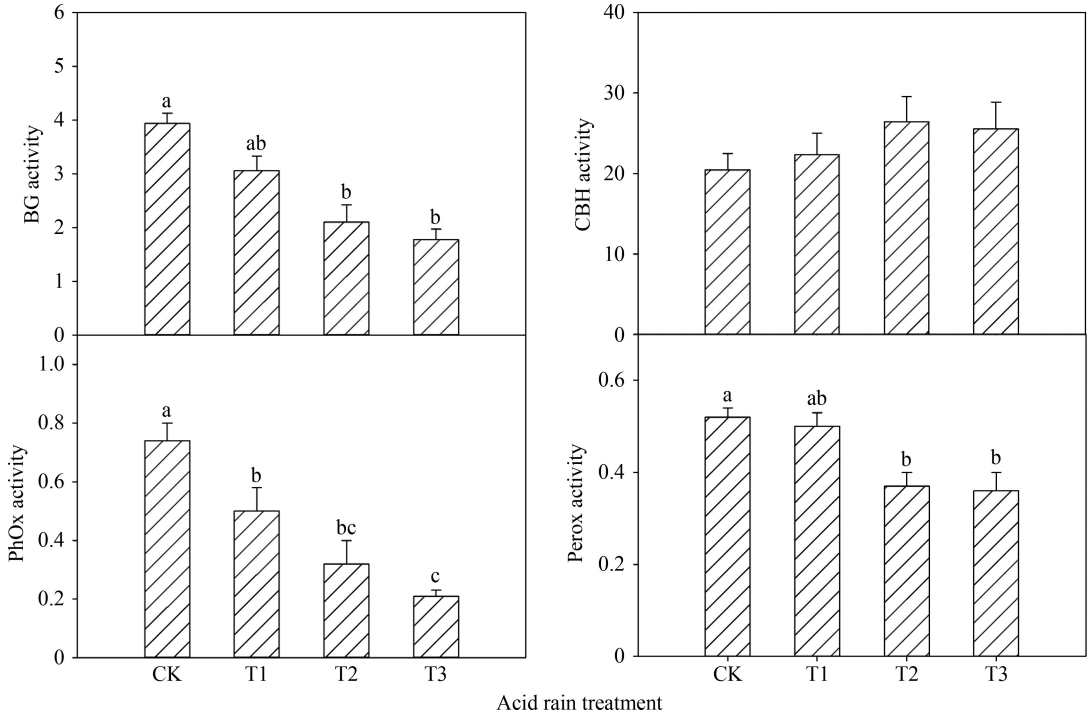

Fig. 3 Effect of simulated acid rain treatment on the activities of carbon-degrading enzymes (μmol·g^{-1} dry soil·h^{-1}).The simulated acid rain treatments are CK: Control, pH≈4.5; T1: pH = 4.0; T2: pH = 3.5; T3: pH = 3.0. Error bars are standard errors of the mean. Different lowercase letters above bars for some variable denote significant difference (P < 0.05) among simulated acid treatments. BG, β-glucosidase activity; CBH, cellobiohydrolase activity; PhOx, phenol oxidase activity; Perox, peroxidase activity.

with declining soil pH (Chen et al., 2022; Hu et al., 2022). Aciego Pietri and Brookes (2009) reported a significant rise in available Al concentrations when soil pH dropped below 5, escalating from nearly negligible levels above pH 5 to approximately 600 mg Al kg^{-1} soil at pH 4. In contrast to our observation of a reduced F: B ratio (Table 1), some studies reported that bacterial populations decrease more sharply than fungal populations with increasing soil acidity (Aliasgharzad et al., 2010; Lim et al., 2011). However, these studies were predominantly conducted in grassland ecosystems with lower acidity levels (pH > 4). Our study site, a subtropical forest soil with a pH below 4, likely experiences acidity levels that exceed the tolerance threshold of soil fungi to Al toxicity. At such low pH levels, fungal abundance may become increasingly sensitive to further decreases in soil acidity. Another contributing factor to the observed microbial community shifts may be the impact of SAR treatment on plant growth. The increased soil acidity could have significantly restricted plant development, leading to a reduction in the allocation of C sources to roots. This limitation diminishes the availability of C substrates for fungal utilization, thereby further influencing the balance between fungal and bacterial populations (Li et al., 2021; Hu et al., 2022).

Soil microorganisms produce specific enzymes, which plays a crucial roles in biogeochemical cycling within the soil ecosystem. In line with the observed changes in microbial abundance, our study also found that the SAR treatment significantly diminished the activities of BG, PhOx, and Perox. This reduction can be attributed to the increased deprotonation of phenolic compounds under lower pH conditions, which elevates their redox potential and decreases their solubility. These chemical changes may inhibit the catalytic efficiency of enzymes responsible for SOC degradation (Sinsabaugh, 2010). Results showed positive correlations between soil pH and the activities of PhOx and Perox, corroborating the global enzymatic trends reported by Sinsabaugh et al. (2008). Conversely, while Sinsabaugh et al. (2008) identified a slight negative relationship between soil pH and CBH activity, our study demonstrated a more complex, CBH activity rose at the beginning and then declined with acidity level enhancement across the SAR treatments (Fig. 3). This suggests that CBH maintains relatively high activity in moderately acidic soils but becomes inhibited under more extreme acidity levels. Additionally, the SAR treatment may indirectly reduce enzyme activities by suppressing plant growth, which in turn decreased the supply of root-derived C that is typically utilized by fungi (Liang et al., 2013; Wu et al., 2024). This reduction in root-derived C could further suppress fungal-mediated enzyme production (e.g., arbuscular mycorrhizal fungi), contributing to the overall decrease in C-degrading enzyme activities observed in our study.

4.2 Effect of SAR on liable and stable SOC fractions

Labile SOC fractions possess a degree of solubility and instability, making them susceptible to oxidation, decomposition, and mineralization. These fractions are particularly responsive to external environmental changes, with their forms and spatial distributions serving as sensitive indicators of ecosystem health (Datta et al., 2010; Karhu et al., 2019; Fang et al., 2020). Acid rain can influence labile SOC fractions through multiple interrelated mechanisms, primarily by altering soil pH, affecting microbial activity, and modifying C inputs. The reduction in soil pH due to SAR exposure enhances the solubility of certain organic carbon compounds, particularly the water-extractable fraction, leading to increased leaching losses of AOC (Wang et al., 2022). However, we found that AOC content increased significantly across the SAR treatments (Fig. 1), suggesting that acidification-induced constraints on microbial decomposition may have surpassed the effects of leaching, thereby leading to the accumulation of AOC in the soil. Similarly, ROC, representing the SOC fraction most susceptible to microbial oxidation, exhibited a notable increase under SAR treatments ($P < 0.05$). This accumulation may be attributed to a suppression of microbial oxidative enzyme activity (e.g., PhOx, Perox) under lower pH conditions (Sinsabaugh, 2010), leading to a slower decomposition rate of these easily oxidizable carbon substrates. Additionally, the decline in fungal biomass, particularly arbuscular mycorrhizal fungi, may have limited root-associated microbial decomposition processes, further contributing to the increase of ROC. For POC, which is largely derived from plant litter and root turnover, also showed significantly higher concentrations in the high-acidity treatments. This suggests that acid rain-induced shifts in microbial community structure and enzyme activity reduced the efficiency of particulate organic matter breakdown, allowing for its accumulation. The observed changes in labile SOC fractions imply that SAR-induced acidification slows microbial decomposition, leading to the short-term preservation of easily decomposable carbon sources. However, given the inherent instability of labile SOC, future environmental fluctuations could trigger its rapid decomposition, posing potential risks to long-term SOC sequestration.

Stable SOC fractions (NAOC, NROC, and NPOC) also showed significant increases under the SAR treatment, particularly in the high-acidity T3 treatment. This accumulation can be explained by several interrelated mechanisms. One primary mechanism is the “substrate priority utilization principle”, which suggests that soil microorganisms preferentially utilize labile SOC sources due to their higher bioavailability, reducing their reliance on stable fractions (Hagedorn et al., 2003; DeForest et al., 2004). As prolonged SAR exposure enhances the

accumulation of labile SOC, microbial consumption of more recalcitrant carbon pools declines, promoting their preservation and buildup in the soil. Secondly, the inhibition of microbial oxidative enzyme activity under low pH conditions likely contributes to the retention of stable SOC fractions. The decreased activities of PhOx and Perox, key enzymes involved in the breakdown of complex organic matter, limit the microbial degradation of chemically resistant carbon fractions (Sinsabaugh, 2010). Additionally, the suppression of fungal biomass, particularly saprotrophic and mycorrhizal fungi, further constrains decomposition processes, enhancing the stabilization of SOC (Six et al., 2004; Totsche et al., 2018). Finally, acid rain-induced soil aggregation and organo-mineral interactions provide additional stabilization pathways for SOC. Lower pH conditions promote the formation of humic substances and enhance the association of organic carbon with soil minerals, effectively shielding SOC from microbial attack (Wu et al., 2024). The formation of these aggregates effectively shields stable SOC from microbial assess and enzymatic degradation, facilitating its long-term stabilization in the soil ecosystem.

4.3 Implications and limitations

Our results indicate that prolonged exposure to SAR, particularly under the high-acidity T3 treatment, significantly increased total SOC content by promoting the accumulation of both labile and stable SOC fractions. Pearson correlation analyses further confirm that changes in these fractions were closely associated with variations in total SOC, underscoring their critical role in shaping SOC dynamics. The simultaneous accumulation of both labile and stable SOC fractions highlights the complex interplay between acid rain, microbial activity, and soil carbon stabilization mechanisms. Although suppression of microbial decomposition under acidified conditions may enhance short-term C storage, the substantial increases in labile SOC fractions raises concerns about the long-term stability of SOC, as these components are inherently more susceptible to mineralization and leaching. Future shifts in environmental conditions, such as fluctuations in soil moisture or increased temperature, may trigger a rapid decomposition of these labile SOC, potentially offsetting the observed C gains. In contrast, the stable SOC is primarily associated with mineral surfaces, which can be potentially protected from simulated microbial decomposition by warming. Thus, the increase of stable SOC fractions observed in this study may facilitate long-term soil carbon sequestration, but further research is needed.

In addition, acid rain remains a critical environmental concern, with long-term implications for soil C dynamics and ecosystem resilience. While our study offers important insights into the short-term effects of SAR on SOC fractions, it is important to acknowledge that our findings are based on a single soil sampling event, which may not fully capture seasonal and interannual variations in SOC decomposition. The long-term accumulation or depletion of SOC is influenced by the dynamics of microbial activity, enzymatic processes, and plant–soil interactions, which require extended monitoring to comprehensively assess their temporal patterns. Therefore, long-term field monitoring and multi-seasonal sampling are essential to fully understand the enduring impacts of acid rain on soil carbon sequestration processes. Future research should aim to unravel the underlying microbial and biochemical mechanisms and develop targeted strategies to mitigate the adverse effects of acidification on soil C storage in subtropical forest ecosystems.

5 Conclusions

In this study, we investigated the effects of long-term simulated acid rain (SAR) on total soil organic carbon (SOC) and its fractions in a monsoon evergreen broadleaf forest. Our results showed that total SOC increased significantly with decreasing SAR pH, driven by the accumulation of both labile (AOC, ROC, and POC) and stable (NAOC, NROC, and NPOC) fractions under high-acidity treatments. This accumulation was strongly associated with reduced microbial activity and suppressed carbon-degrading enzymes functions, including β-glucosidase, phenol oxidase, and peroxidase. Although the increased in stable SOC fractions suggests enhanced potential for long-term carbon sequestration, the more substantial increase in labile SOC fractions indicates possible vulnerability to future environmental disturbances. Future research should focus on long-term field observations to assess the dynamics of SOC under continuous acid deposition combined with other environmental changes.

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