Article

Reduced Lignin Decomposition and Enhanced Soil Organic Carbon Stability by Acid Rain: Evidence from ^{13}C Isotope and ^{13}C NMR Analyses

Jianping Wu ^{1,2}, Qi Deng ^{2,3,*}, Dafeng Hui ⁴ , Xin Xiong ³, Huiling Zhang ³, Mengdi Zhao ^{3,5}, Xuan Wang ^{3,5}, Minghui Hu ^{3,5}, Yongxian Su ^{1,2}, Hongou Zhang ¹, Guowei Chu ³ and Deqiang Zhang ^{2,3,*}

- ¹ Key Lab of Guangdong for Utilization of Remote Sensing and Geographical Information System, Guangdong Open Laboratory of Geospatial Information Technology and Application, Guangzhou Institute of Geography, Guangdong Academy of Sciences, Guangzhou 510070, China; wujianping@gdas.ac.cn (J.W.); suyongxian@gdas.ac.cn (Y.S.); hozhang@gdas.ac.cn (H.Z.)
- ² Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou 510070, China
- ³ Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China; xiongx@scbg.ac.cn (X.X.); zhanghuiling@scbg.ac.cn (H.Z.); zhaomengdi@scbg.ac.cn (M.Z.); wangxuan@scbg.ac.cn (X.W.); huminghui@scbg.ac.cn (M.H.); cgw@scbg.ac.cn (G.C.)
- ⁴ Department of Biological Sciences, Tennessee State University, Nashville, TN 37209, USA; dhui@tnstate.edu
- ⁵ College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100039, China
- * Correspondence: dengqi@scbg.ac.cn (Q.D.); zhangdeq@scib.ac.cn (D.Z.); Tel.: +86-020-37252919 (Q.D.)

Received: 8 October 2020; Accepted: 9 November 2020; Published: 12 November 2020

Abstract: Due to the emissions of air pollutants, acid rain in southern China poses a great threat to terrestrial ecosystems. However, its influences on ecological processes such as litter decomposition and soil organic carbon (SOC) accumulation are still not clear. The aim of this study was to understand the potential mechanisms of carbon sequestration change in response to long-term acid rain in a subtropical forest. A field experiment with simulated acid rain (SAR) treatment was conducted in a monsoon evergreen broadleaf forest in southern China. Four levels of SAR treatment were implemented by irrigating the plots with water of different pH values (4.5 as a control, 4.0, 3.5, and 3.0). The results showed that the concentration of SOC and recalcitrant index for the SAR pH = 3.0 treatment were significantly higher compared to the control. Lignin fractions in litter residue layers were significantly increased, while soil microbial biomass carbon and soil ligninolytic enzyme activities were reduced under the SAR treatment. The concentration of SOC and recalcitrant index had positive relationships with the litter residue lignin fraction, but negative relationships with soil ligninolytic enzyme activity. These findings indicate that soil carbon accumulation could be enhanced with more stable lignin input under prolonged acid rain in forest ecosystems in southern China.

Keywords: acid rain; ^{13}C NMR; $\delta^{13}\text{C}$; lignin fraction; soil ligninolytic enzyme activities; soil organic carbon

1. Introduction

With the increase in anthropogenic activity in recent decades, such as coal combustion, industrial air pollution, and automobile exhaust gases emissions, China has suffered from severe acid deposition, especially in the southern regions [1,2]. More than 50% of storms now contain acid rain and the annual average pH value of precipitation in southern China is generally below 4.5 [3,4]. Therefore, the prevalence of acid rain has increased public concern about the widespread impacts on terrestrial ecosystems in southern China [5].

Understanding the soil organic matter (SOM) dynamics in forest ecosystems is important because soils are the largest pool of carbon (C) on Earth [6,7]. As a key ecological process responsible for the transfer of organic matter from vegetation to soil, plant litter decomposition plays a critical role in global C cycling [8–10]. Plant organic matter fixed by photosynthesis is eventually deposited in soils, then decomposed and utilized by soil organisms, partially transformed into humic substances, and contributed to the SOM [11]. On the basis of previous studies, prolonged acid rain would change the quality and quantity of SOM into the soil, inhibits the decomposition of SOM, and alter the sequestration of soil C [12–15].

Our previous study at this experimental site also showed that acid rain affects the conservation of recalcitrant constituents [16]. However, the specific substances, relevant enzyme activities, and potential mechanisms are still not well understood. The lignin fraction, which acts as the fundamental materials for C sequestration, is often considered as a limiting factor of decomposition [17]. Since lignin is the second most abundant component of vascular plant tissues after cellulose, there is a large amount of lignin input into soil [18]. Lignin can react with other humic substances to form complexes that are highly resistant to microbial degradation [19]. Therefore, on the one hand, increasing the formation of the lignin fraction could increase soil organic carbon (SOC) stabilization [20]. On the other, since most organic inputs to soil are polymeric, the decomposition of soil C depends on the microbial production of extracellular enzymes that break down complex compounds into smaller products [18,21]. Soil oxidative enzymes (e.g., Ligninolytic enzymes, mainly phenol oxidase (PhOx) and peroxidase (Perox)), a group of highly versatile enzymes, are known for their role in the degradation of complex and recalcitrant compounds [22,23]. Hence, the activity of these enzymes greatly affects the changes in the preservation of more recalcitrant organic matter. Consequently, an improved understanding of the variation in the lignin fraction and soil ligninolytic enzymatic activities in response to prolonged acid rain in a forest in southern China may provide insights into soil C preservation and stabilization in tropical and subtropical forest ecosystems.

Over the past decades, discrimination of C isotopes during litter decomposition has been employed as a powerful tool for better understanding C flows into terrestrial ecosystems [17,24,25]. ^{13}C contents may differ among plant organs and among specific organic compounds [26,27]. Thus, selective consumption by the decomposing microbial community could change the stable C isotope ratios ($\delta^{13}\text{C}$) of organic matter, as compared with those of the original plant material [28]. McClaugherty and Berg (1987) [29] demonstrated that both cellulose and hemicelluloses are typically enriched in ^{13}C by 1–2% relative to whole-plant material, while lignin is depleted in ^{13}C by 2–6% relative to whole-plant material and by 4–7% relative to cellulose. It is suggested that lignin, because of its greater recalcitrance, should accumulate with litter decomposition, thus leading to depletion of $\delta^{13}\text{C}$ in SOM relative to the starting litter inputs [30–32]. Osono et al. (2008) [17] also found that the increase of lignin fraction is associated with a decrease in the $\delta^{13}\text{C}$ of *Swida controversa* residue. Consequently, the increase in lignin fractions and the relative increase of the residue in lignin-fraction-derived C during decomposition are reflected by $\delta^{13}\text{C}$ [33,34]. Therefore, understanding the changes in $\delta^{13}\text{C}$ of litter residues would be a critical step in deciphering the litter decomposition process and inferring the variation in the lignin fraction. Solid-state ^{13}C NMR spectroscopy is a powerful and rapidly developing technique that has been increasingly applied in the study of litterfall, SOM, and decomposition in recent years [35,36]. Several previous ^{13}C NMR studies identified the chemical changes in litter and other materials during decomposition and humification; they also provided useful information on the relative decomposability of different C components of organic matter [37,38]. Utilization of these two techniques could provide a better evaluation of litter residue chemical components such as lignin under prolonged acid rain.

In previous work, we confirmed that more recalcitrant organic matter was generated under prolonged simulated acid rain (SAR) treatment [16]. In this study, we hypothesized that: (1) SAR would facilitate SOC accumulation and stabilization due to the input of more recalcitrant constituents, such as the lignin fraction; and (2) it would reduce soil ligninolytic enzyme activities. The specific

objectives of this study were (1) to determine how the SAR treatment impacts SOC concentration; (2) to investigate the variations in litter residue lignin and soil ligninolytic enzyme activities under the SAR treatment; (3) to identify whether soil organic carbon accumulation and stabilization are associated with an increase in lignin fraction and a decrease in ligninolytic enzyme activities under the SAR treatment.

2. Materials and Methods

2.1. Site Description

This study was carried out in Dinghushan National Nature Reserve, Guangdong Province, China (112°30′–112°33′ E, 23°09′–23°11′ N). The reserve covers an area of 1155 ha and is located in subtropical humid climate zone [39]. Mean annual temperature is 21 °C with the maximum and minimum average monthly temperature of 28.0 °C in July and 12.6 °C in January, respectively. The mean annual precipitation of 1927 mm follows a distinct seasonal pattern, with >80% of the rain falls in the wet season from April to September and <20% in the dry season from October to March.

The experimental sites were set up in a monsoon evergreen broadleaf forest, the most mature forest in this region of forest succession. The forest is located at about 250–300 m above sea level and occupies approximately 600 ha. It has been protected from human impacts for more than 400 years [40]. Vegetation in the forest is typical of the south subtropical zone. The major species in this forest are *Castanopsis chinensis* Hance, *Schima superba* Chardn Champ, *Cryptocarya chinensis* (Hance) Hemsl., *Cryptocarya concinna* Hance, *Machilus chinensis* (Champ. EX Benth.) Hemsl., *Syzygium rehderianum* Merr. Perry in the overstory, and *Calamus rhabdocladus* Burret, *Ardisia quinqueгона* Bl., and *Hemigramma decurrens* (Hook.) Copel. in the understory. Tree heights range from 4 to 30 m and stem diameters range from 5 to 163 cm. The soil is classified as lateritic red earth, loamy in texture, and acidic [41]. Acid rain is a threat in this area with an annual average rainfall pH < 4.90 [3].

2.2. Experimental Treatments

The SAR experiment was initiated in June 2009. The SAR treatments were implemented by irrigating plots with water of different pH values: CK (the control, local lake water, pH $\approx$ 4.5), T1 (pH = 4.0), T2 (pH = 3.5), T3 (pH = 3.0). Twelve plots were established and randomly assigned into four SAR treatments, three replications for each treatment were used. Each plot was 10 $\times$ 10 m² and surrounded by a 3-m buffer strip. The SAR solutions were prepared by mixing the local lake water with H₂SO₄ and HNO₃ in a 1:1 mole ratio to reflect actual environmental conditions based on previous acid rain records. Additionally, the SAR solutions were applied to each plot below the canopy using a gasoline engine sprayer; the amount applied to each plot was 40 L per application. More details on the experimental design and methods can be found in Liang et al. (2013) [42] and Wu et al. (2016) [16].

2.3. Sample Collection and Analyses

Litter residue samples (15 cores; core diameter 20 cm), which were divided into litter (L), fermentation (F), and humified (H) layers, were taken randomly and combined to give one composite sample of each layer per plot in September 2016. The L layer was consisted as the initial stage, the F layer as the intermediate stage, and the H layer as the final stage of litter decomposition [43]. Thickness of the litter residue ranged from 3 to 5 cm. The L layer consisted of fresh or slightly decomposed litter from trees and the understory (0.5–2 cm). The F layer consisted of partly decomposed litter that was still identifiable (2–3 cm). The H layer consisted of decomposed organic matter that could not be identified (0.5 cm). A total of 36 (3 $\times$ 12) samples were collected in this study. These litter residue samples were cleaned and then oven-dried at 65 °C for 24 h for further analysis. The litter residue samples for total organic C, lignin concentration, solid-state ¹³C NMR, and stable C isotope analyses were further ground to pass through a 0.25-mm sieve.

Soil samples were collected from 0–10 cm, using a standard soil sampling tube (with an inside diameter of 2.5 cm). From each plot, we selected five cores randomly and mixed them into one sample, and a total of 12 samples were collected in this study. The fresh soil samples were passed through a 2-mm sieve to remove rocks and plant roots. Then, each sub-sample was divided into two parts. One part was stored at 4 °C for the soil microbial biomass carbon (SMBC) and soil enzyme activity analyses. The other part was air-dried and ground to pass through a 0.25-mm sieve for the SOC concentration and solid-state NMR analyses. The SOC concentration was determined using the Walkley-Black's wet combustion method [44].

The concentration of lignin fractions in the litter residue samples was estimated by gravimetry according to King and Heath (1967) [45], using hot sulfuric acid digestion. Briefly, each sample was treated with 72% sulfuric acid (*v/v*), diluted with distilled water to make a 2.5% sulfuric acid solution, and autoclaved at 120 °C for 60 min. After cooling, the residue was filtered, dried at 105 °C, and weighed as an acid-insoluble lignin residue. Although the acid-insoluble residue contains both true lignin and lignin-like materials produced during the decomposition processes, this acid-insoluble lignin fraction was considered to be a 'lignin' fraction for the sake of simplicity in this study.

The SOC concentration, total C contents of litter residues (Total C), and lignin fraction C content (Lignin C) of the subsamples from the three decomposition layers were measured by dry combustion on a vario ISOTOPE cube elemental analyzer (Elementar, Germany). The $\delta^{13}\text{C}$ of litter residues in different decomposition layers were analyzed on an IsoPrime 100 isotope ratio mass spectrometer (Elementar, Germany). The carbon isotope ratio is expressed relative to the Pee Dee Belemnite (PDB) standard. The results were calculated as delta carbon-13 values ($\delta^{13}\text{C}$) on a per mil basis (‰) according to the following formula:

$$\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C} \text{ sample}) - ({}^{13}\text{C}/{}^{12}\text{C} \text{ standard})]/({}^{13}\text{C}/{}^{12}\text{C} \text{ standard}) * 1000 \quad (1)$$

The carbon functional groups in the litter residue samples from the three decomposition layers and the 0–10 cm soil layer were analyzed using cross-polarization magic-angle-spinning (CPMAS) solid-state NMR spectroscopy (Bruker Ascend™ 300 WB, Rheinstetten, Germany). The soil samples were pretreated with hydrofluoric acid (HF, 10% *v/v*) to remove Fe^{3+} and Mn^{2+} from the soil and thus increase signal-to-noise ratio of instrument [46]. 7 mm diameter sample tubes were used with parameters set at a frequency of 75.5 MHz, MAS spinning frequency at 12 kHz; 2000 transients were collected for each sample, with a contact time of 35 ms, and a recycle time of 5 s [46]. The external standard used for chemical shift determination was adamantane.

The ^{13}C NMR spectra were divided into four regions representing the different chemical environments of a ^{13}C nucleus [18,47]: alkyl C (0–50 ppm), including side chains of amino acids, alicyclic-C in resin acids and structures derived from cutins and suberins; O-alkyl C (50–110 ppm), including oxygen-substituted C in alcohols, ethers, cellulose, hemicelluloses, and other polysaccharides; aromatic C (110–160 ppm), including condensed tannins, hydrolysable tannins and lignin; and carbonyl C (160–200 ppm), including secondary amide-C in proteins, carboxylic-C in hydrolysable tannins, carboxylic-C in resin acids, and carbonyl-C in lignin and carboxyl-C in hemicelluloses. The relative area of these chemical shift regions was calculated for each spectrum as the percentage of total area by integration using the MestReNova software package (Version 5.3.1, MestReNova, Mestrelab Research, Spain). The ratio of alkyl C to O-alkyl C (A/O-A ratio) is a useful index for the extent of litter decomposition [48]. The recalcitrance index ((alkyl C + aromatic C)/(O-alkyl C + carbonyl C)) is another indicator of organic matter stability [49].

The SMBC was determined by subjecting fresh soil samples to the chloroform fumigation-extraction method [50]. We quantified the potential activities of two soil oxidative enzymes PhOx and Perox, following the method described by Iyyemperumal and Shi (2008) [51], and using L-3, 4- dihydroxy phenylalanine (L-DOPA) as the substrate. For the 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 12,000× *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 Perox activity assay was the same as that for PhOx except for the addition of 1 mL 0.3% H₂O₂ to the mixture before incubation [51]. Enzymatic activity was expressed as micromoles per gram dry weight per hour.

2.4. Statistical Analysis

Regression analysis and Analysis of Variance (ANOVA) were performed using the general linear model procedure of the SPSS package for Windows (IBM Corporation, Armonk, NY, USA). Two-way ANOVA was used to test the interactive effect of SAR treatments and different decomposition layers on chemical shift region of NMR spectroscopy, $\delta^{13}\text{C}$ value, A/O-A ratio, lignin concentration (Lignin (%)), and the proportion of lignin fraction C to the residues total C (Lignin C/Total C ratio). One-way ANOVA with LSD test was used to further test the statistical significance of different decomposition layers on chemical shift region of NMR spectroscopy, A/O-A ratio, $\delta^{13}\text{C}$ value, Lignin (%), Lignin C/Total C ratio, respectively, and the SAR treatment on chemical shift region of NMR spectroscopy, A/O-A ratio, $\delta^{13}\text{C}$ value, Lignin (%), Lignin C/Total C ratio, ligninolytic enzyme activities, SMBC, SOC, recalcitrance index, respectively. Statistical significance was determined at $P < 0.05$.

3. Results

3.1. Soil Organic Carbon Concentration and Recalcitrance Index

The long-term SAR treatment had significant effects on SOC content and the soil recalcitrance index in the 0–10 cm soil layer ($P < 0.05$, Table 1). The SOC concentration in the T3 treatment was significantly higher (32.4%) than that of the CK treatment. The SAR treatment had no significant effect on the integrated intensities (%) of any chemical shift region (i.e., Alkyl C, O-alkyl C, Aromatic C, and Carbonyl C). However, the recalcitrance index in the T3 treatment was significantly higher than that of the CK treatment (Table 1).

Table 1. Soil organic carbon (SOC) concentration, the integrated intensities (%) of major ^{13}C cross-polarization magic-angle-spinning (CPMAS) NMR spectral region and recalcitrance index under the simulated acid rain (SAR) treatment in the 0–10 cm soil layer.

Treatment	SOC (g/kg)	Alkyl C	O-alkyl C	Aromatic C	Carbonyl C	Recalcitrance Index ¹
CK	27.79 ± 2.19b	46.70 ± 1.71	40.47 ± 1.05	2.13 ± 0.21	10.70 ± 0.5	0.96 ± 0.06b
T1	31.35 ± 2.79b	49.37 ± 1.71	39.03 ± 0.71	1.16 ± 0.70	10.43 ± 0.42	1.02 ± 0.04ab
T2	32.04 ± 2.53ab	48.37 ± 0.98	39.16 ± 1.86	2.10 ± 0.35	10.37 ± 0.68	1.02 ± 0.05ab
T3	36.80 ± 3.13a	50.16 ± 1.38	37.77 ± 3.87	2.27 ± 0.83	9.80 ± 3.20	1.10 ± 0.03a

¹ Recalcitrance index = (Alkyl C + Aromatic C)/(O-alkyl C + Carbonyl C). Different letters in the column indicate values are significantly in the SAR treatment at $P = 0.05$ level; values are means ± SD. CK is control treatment, pH ≈ 4.5; T1 is the pH = 4.0 treatment; T2 is the pH = 3.5 treatment and T3 is the pH = 3.0 treatment.

3.2. Lignin Fraction Concentration

The lignin fraction concentration was significantly affected by the decomposition layer, and showed an increasing trend from the L to H layer ($P < 0.001$, Table 2). The Lignin C/Total C ratios were also significantly affected by the decomposition layer ($P < 0.001$, Table 2). Compared to the L layer, the Lignin C/Total C ratios in the F and H layers were increased by 9.1 and 20.7%, respectively. Under the SAR treatment, the lignin fraction concentration was significantly higher (14.6%) in the T3 treatment than in the CK treatment ($P < 0.05$, Table 2), while there was no significant difference in the Lignin C/Total C ratio. Positive relationships of lignin concentration with SOC content and recalcitrance index were found to be significant in the H layer, but not in the L and F layers (Figure 1a,b). The SOC content and recalcitrance index increased linearly with increasing lignin concentration in the H layer ($P < 0.05$).

Table 2. Effects of simulated acid rain (SAR) treatment and litter residue decomposition layer on $\delta^{13}\text{C}$ value, lignin fraction concentration, and proportion of lignin fraction C to the total C (Lignin C/Total C).

		Lignin (%)	Lignin C/Total C (%)	$\delta^{13}\text{C}$ (‰)
Layer	L	43.44 ± 1.51C	37.05 ± 2.17C	−30.25 ± 0.07A
	F	45.21 ± 1.54B	40.42 ± 4.05B	−30.93 ± 0.11B
	H	52.10 ± 3.05A	44.73 ± 2.80A	−31.43 ± 0.11C
Treatment	CK	43.55 ± 2.20b	39.91 ± 3.50	−30.81 ± 0.47a
	T1	46.30 ± 3.55b	40.04 ± 4.35	−30.84 ± 0.53ab
	T2	47.23 ± 4.14ab	41.35 ± 4.93	−30.90 ± 0.53b
	T3	48.40 ± 5.64a	41.63 ± 5.11	−30.93 ± 0.55b
Analysis of variance (<i>P</i> value)				
Layer		<0.001	<0.001	<0.001
Treatment		<0.05	0.585	<0.05
Layer × Treatment		0.385	0.663	0.454

L, litter layer; F, fermentation layer; and H, humified layer. CK, control treatment; T1, pH = 4.0 treatment; T2, pH = 3.5 treatment; and T3, pH = 3.0 treatment. Values are means ± SD. Different superscript letters in the column indicate significant differences among litter residue decomposition layers (uppercase letters) and differences among the SAR treatments (lowercase letters) at $P < 0.05$.

Figure 1. Relationships between lignin concentration and soil organic carbon content (a, SOC), recalcitrance index (b, (Alkyl C + Aromatic C)/(O-alkyl C + Carbonyl C) ratio) in the L (litter), F (fermentation), and H (humified) layers. Relationships between ligninolytic enzyme activities and SOC content (c), and recalcitrance index (d). PhOx denotes phenol oxidase activity and Perox denotes peroxidase activity.

3.3. ^{13}C Isotope Dynamics and Association with the Lignin Fraction

The $\delta^{13}\text{C}$ of litter residue was significantly decreased ($P < 0.05$) as decomposition proceeded, ranging from −30.22‰ to −30.26‰ in the L layer, from −30.85‰ to −31.03‰ in the F layer, and from −31.32‰ to −31.50‰ in the H layer (Table 2). The SAR treatment significantly affected the $\delta^{13}\text{C}$ of the litter residue ($P < 0.05$, Table 2), and the $\delta^{13}\text{C}$ was significantly lower in the T2 and T3 treatments than in the CK treatment. There was a significant negative relationship between the Lignin C/Total C and

stable C isotope compositions of litter residues at different layers across all SAR treatments ($R^2 = 0.53$, $P < 0.001$, Figure 2).

Figure 2. Relationship between $\delta^{13}\text{C}$ values (‰) and proportion of lignin fraction C to the total C (Lignin C/Total C) (%) in litter residues of different decomposition layers under the SAR treatment (CK = control treatment; T1 = pH 4.0 treatment; T2 = pH 3.5 treatment; and T3 = pH 3.0 treatment). A linear regression model was fitted for all data points ($y = -27.49 - 0.08x$, $R^2 = 0.53$, $P < 0.0001$) as there were no significant differences in mode parameters among treatments.

3.4. ^{13}C NMR Spectroscopy of Litter Residue

Example spectra for the litter residues of different layers during litter decomposition are displayed in Figure 3. O-alkyl C was the most dominant part in the spectra followed by alkyl C, carbonyl C, and aromatic C (Table 3). The changes in the relative area of the chemical shift regions in different layers during litter decomposition were characterized by the relative increase in alkyl C, aromatic C, and carbonyl C, and the relative decrease in O-alkyl C. For example, litter residues in each SAR treatment showed a significant decrease during litter decomposition, as can be seen in the broad peak at 70–75 ppm, corresponding to the C2, C3, and C5 carbons in cellulose. However, the resonance region associated with aromatic C increased significantly in intensity as decomposition proceeded (Figure 3, Table 3). These peaks, at 148 and 153 ppm in the spectra, along with the peak at 133 ppm, were indicative of lignin compounds. The abundance of lignin-related C was also evident in the carbonyl C, which includes the carbonyl carbons of lignin, while also showing a significant increase in integrated intensity (Figure 3, Table 3). The A/O-A ratio generally increased significantly during the litter decomposition process under different SAR treatments (Table 3).

The SAR treatment had a significant influence on the most integrated intensity value of ^{13}C NMR spectral regions (except Carbonyl C) and A/O-A ratio ($P < 0.05$, Table 3). The Alkyl C and Aromatic C in the T3 treatment were significantly higher than in the CK treatment, while the O-alkyl C was significantly lower in the T2 and T3 treatments as compared to the CK treatment. The A/O-A ratios in the T1, T2, and T3 treatments were increased by 8.8, 17.6, and 29.4%, respectively, compared to the CK treatment (Table 3).

Figure 3. The ^{13}C cross-polarization magic-angle-spinning (CPMAS) NMR spectra of litter residues at different layers (L, litter layer; F, fermentation layer; and H, humified layer) as decomposition proceeded. Examples are shown for CK = control treatment, T1 = pH 4.0 treatment, T2 = pH 3.5 treatment, and T3 = pH 3.0 treatments. Superscripts indicate the peaks' chemical shift.

Table 3. Effects of simulated acid rain (SAR) treatment and litter residue decomposition layer on the integrated intensity of major ^{13}C cross-polarization magic-angle-spinning (CPMAS) NMR spectral regions and the ratio of alkyl C to O-alkyl C (A/O-A ratio).

E		Alkyl C	O-alkyl C	Aromatic C	Carbonyl C	A/O-A Ratio
Layer	L	19.90 ± 2.48C	72.06 ± 2.97A	2.58 ± 0.48B	5.47 ± 0.87B	0.28 ± 0.05C
	F	23.93 ± 2.60B	65.80 ± 3.54B	4.76 ± 1.39A	5.52 ± 1.06B	0.37 ± 0.06B
	H	29.64 ± 2.79A	57.13 ± 3.52C	5.37 ± 1.10A	7.86 ± 1.20A	0.52 ± 0.08A
Treatment	CK	22.63 ± 3.35b	67.77 ± 5.60a	3.28 ± 1.07b	6.32 ± 1.63	0.34 ± 0.08b
	T1	23.61 ± 4.50b	65.73 ± 6.42ab	4.19 ± 1.40a	6.47 ± 1.44	0.37 ± 0.11b
	T2	25.22 ± 5.03ab	64.09 ± 7.28b	4.67 ± 1.98a	6.02 ± 1.64	0.40 ± 0.12ab
	T3	26.48 ± 5.81a	62.40 ± 8.45b	4.80 ± 1.59a	6.31 ± 1.61	0.44 ± 0.15a
Analysis of variance (<i>P</i> value)						
Layer		<0.001	<0.001	<0.001	<0.001	<0.001
Treatment		<0.01	<0.01	<0.01	0.86	<0.001
Layer × Treatment		0.134	0.179	0.111	0.385	<0.05

L, litter layer; F, fermentation layer; and H, humified layer. CK, control treatment; T1, pH = 4.0 treatment; T2, pH = 3.5 treatment; and T3, pH = 3.0 treatment. Values are means ± SD. Different superscript letters in the column indicate significant differences among litter residue decomposition layers (uppercase letters) and differences among SAR treatments (lowercase letters) at $P < 0.05$.

3.5. Soil Microbial Biomass Carbon and Ligninolytic Enzyme Activities

The SMBC concentration was significantly affected by the SAR treatment in the topsoil ($P < 0.05$, Figure 4A). The SMBC in the T2 and T3 treatments were significantly lower (24.8 and 33.2%, respectively) than those in the control. The SAR treatment significantly influenced PhOx and Perox activities ($P < 0.05$, Figure 4). The mean soil enzyme activities of the CK treatment were 0.74 ± 0.18 and $0.52 \pm 0.04 \mu\text{mol g}^{-1}$ dry soil h^{-1} for the PhOx and Perox, respectively. For the PhOx, the activity was significantly reduced as pH value decreased ($P < 0.05$). The activity of Perox in the T3 treatment was decreased by 31.4% compared to the CK treatment. Using regression analysis, we found that the SOC content and recalcitrance index had a negative relationship with ligninolytic enzyme activities ($P < 0.05$). The SOC content and recalcitrance index increased linearly with decreasing ligninolytic enzyme activities (Figure 1c,d).

Figure 4. Soil microbial biomass carbon (SMBC, A) and ligninolytic enzyme activities (B,C) under the effect of the acid rain treatments. CK is control treatment, pH $\approx$ 4.5; T1 is the pH = 4.0 treatment; T2 is the pH = 3.5 treatment; and T3 is the pH = 3.0 treatment. PhOx denotes phenol oxidase and Perox denotes peroxidase. Different superscript letters indicate significant differences among acid treatments at $P < 0.05$. Values are means $\pm$ SD.

4. Discussion

4.1. Effect of SAR on the Lignin Fraction

Two lines of evidence showed that the lignin fraction increased significantly as decomposition proceeded (i.e., from the L to F and H layers) (Table 1). (1) The decrease in $\delta^{13}\text{C}$ of the litter residue was greater in the lower layer (H) where the concentration of the lignin fraction was higher than in the upper layers (L and F). The significant negative correlations between $\delta^{13}\text{C}$ and the ratio of Lignin C/Total C of the litter residue within the decomposition layers (Figure 2) suggested that the variation of lignin in the litter residue can be reflected by ^{13}C isotope dynamics in this ecosystem [52]. (2) ^{13}C NMR analysis showed that alkyl C and aromatic C increased significantly as the decomposition proceeded, resulting in the increase of lignin compounds in the late stages of decay. Similar results were reported in previous studies [17,32,34,53]. The significant differences in the lignin fractions in the different decomposition layers might be related to fast mass loss of leaf litter through leaching during the early stage of decomposition. Nearly 50% of leaf litter is composed of labile, water-soluble constituents such as carbohydrates [54]. Loss of carbohydrates from the leaf litter structure explains the large loss in mass in the upper layers of litter decomposition. Since the most easily leached constituents (e.g., soluble carbohydrates) are more enriched in ^{13}C than recalcitrant constituents (e.g., lignin) [30,53], the remaining litter would be lower in $\delta^{13}\text{C}$. After the initial leaching stage moving from the L to the F and H layers, the mass loss rate is reduced as easily degradable constituents are exhausted and decomposition is limited by an increase of phenolic compounds in the H layer [55].

One interesting finding of this study was that, under the SAR treatment, the $\delta^{13}\text{C}$ showed a trend of decline as the pH value decreased (Table 2). Since a more recalcitrant lignin fraction is ^{13}C -depleted compared to the less refractory C fractions and bulk leaf litter [30], the relative increase of lignin might

be reflected by more negative $\delta^{13}\text{C}$ in the litter residue [52]. More specific chemical and spectroscopic analyses using ^{13}C NMR analysis suggested that the O-alkyl C resonance region, which is dominated by peaks related to carbohydrate structures, decreased significantly under the SAR treatment (Table 2). On the contrary, the alkyl C and aromatic C fractions, which represent more recalcitrant compounds such as resins and lignin, increased under the SAR treatment. One possible reason for the increase of lignin under the SAR treatment is that prolonged acid rain aggravates the soil acidification, and lower soil pH can change the population and biological activity of soil decomposers due to the toxicity of high H^+ loads [56]. In this study, we did find that the microbial biomass decreased as the low pH treatment had a significant lower SMBC concentration (Figure 4A). Our previous studies [16,42] and other SAR experiments [14,57] showed that increasing acid rain inputs proportionally restrain the activities of microorganism, and lead to a marked loss of microbial biomass.

4.2. Effect of SAR on Soil Ligninolytic Enzymatic Activity

Ligninolytic enzymes play an important role in the degradation of recalcitrant compounds, so the change in enzymatic activity would greatly influence C cycling [22,58], and directly affect the changes in the preservation of more stable organic C in forest soil. In this study, the SAR treatment reduced soil microbial biomass and decreased the associated enzyme activities by restraining the rates of synthesis and the release of exoenzymes by soil microorganisms (Figure 4) [59,60]. The enzymatic potential for oxidizing the recalcitrant fractions of soil organic material is strongly related to soil pH, and as a result, the oxidative enzyme activities are restrained by low soil pH [61]. We also found that the SOC concentration and recalcitrant index were negatively correlated with ligninolytic enzyme activities under the SAR treatment (Figure 1c,d), and the relatively stable compounds might be preserved and resistant to further microbial decomposition [62].

4.3. Effect of SAR on Soil Organic Carbon

We hypothesized that soil organic C would be higher under prolonged acid rain in the forest soil. The results in our study, indeed, showed an increasing trend as regards SOC concentration in response to long-term acid rain. Under the SAR treatment, SOC also became more stable as the lower pH treatment had a significantly increased soil recalcitrant index (Table 1). These results are in accordance with our and other previous studies [12,16,57,63], which reported that soil organic carbon accumulates as a long-term consequence of continued acid rain. Different mechanisms may help explain soil C accumulation and stabilization in response to prolonged acid rain such as more C input from litterfall and roots, more stable C preserved in the soil, and less was utilized by micro-organisms under the SAR treatment. As acid rain might not enhance the C inputs, we focused more on the changes in the lignin fraction and soil ligninolytic enzymatic activities. On the basis of our results, we believe that prolonged acid rain decreased the ligninolytic enzymatic activities, retained more lignin fraction carbon, and resulted in a higher SOC in the soil.

5. Conclusions

After more than seven years of the SAR treatment in a subtropical forest in southern China, we found a significant increase in the soil organic carbon concentration and recalcitrant index under heavy SAR treatment. Using the ^{13}C isotope and the solid-state ^{13}C NMR techniques, we showed that the lignin fraction in the later decomposition layer significantly increased as the pH value decreased. The reduced activities of soil ligninolytic enzymes under the SAR treatment contributed to the increases in the lignin fraction, the recalcitrant index, and the SOC concentration. The information generated in our study indicates that prolonged acid rain facilitates SOC stabilization and accumulation in subtropical forest. However, acid rain is widely considered to be a serious environmental problem, and the increasing soil acidification poses a serious threat to terrestrial ecosystems. While prolonged acid rain might mitigate a fraction of potential carbon emissions, the results from this study should not be viewed as an endorsement for soil acidification in terrestrial ecosystems.

Author Contributions: Conceptualization, D.Z. and Q.D.; methodology, X.X. and H.Z. (Huiling Zhang); investigation, J.W., X.W. and M.H.; data curation, G.C. and M.Z.; formal analysis, Y.S.; writing—original draft preparation, J.W.; writing—review and editing, D.H., D.Z. and Q.D.; supervision, D.Z. and H.Z. (Hongou Zhang). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 41773088, 41907289; the Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), grant number GML2019ZD0301; the GDAS' project of Science and Technology Development, grant number 2019GDASYL-0103002, GDASYL-20200302001 and 2020GDASYL-20200102002; and the Science and Technology Program of Guangzhou, China, grant number 202002030335.

Acknowledgments: The authors are grateful to Ze Meng, Jianqi Zhao, Dingsheng Mo and Chuanyin Xiang for their extraordinary help and support on both field samplings and laboratory analyses.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wang, W.X.; Xu, P.J. Research progress in precipitation chemistry in China. *Prog. Chem.* **2009**, *21*, 266–281.
2. Du, E.; de Vries, W.; Liu, X.; Fang, J.; Galloway, J.N.; Jiang, Y. Spatial boundary of urban 'acid islands' in southern China. *Sci. Rep.-UK* **2015**, *5*, 12625. [[CrossRef](#)] [[PubMed](#)]
3. Liu, J.X.; Zhou, G.Y.; Zhang, D.Q. Effects of acidic solutions on element dynamics in the monsoon evergreen broad-leaved forest at Dinghushan, China. *Environ. Sci. Pollut. Res.* **2007**, *3*, 215–218. [[CrossRef](#)] [[PubMed](#)]
4. Hu, B.Q.; Yi, P.; Duan, N.; Zhao, D.G.; Zhao, J.M.; Cheng, K. Study on the influence factors of accurate definition of the spatial distribution of acid rain area in China. *China Environ. Sci.* **2015**, *3*, 917–924. (In Chinese)
5. Liu, K.H.; Fang, Y.T.; Yu, F.M.; Liu, Q.; Li, E.R.; Peng, S.L. Soil acidification in response to acid deposition in three subtropical forests of subtropical China. *Pedosphere* **2010**, *20*, 399–408. [[CrossRef](#)]
6. Ono, K.; Hirai, K.; Morita, S.; Ohse, K.; Hiradate, S. Organic carbon accumulation processes on a forest floor during an early humification stage in a temperate deciduous forest in Japan: Evaluations of chemical compositional changes by ^{13}C NMR and their decomposition rates from litterbag experiment. *Geoderma* **2009**, *151*, 351–356. [[CrossRef](#)]
7. Wang, H.; Liu, S.R.; Wang, J.X.; Shi, Z.M.; Lu, L.H.; Guo, W.F.; Jia, H.Y. Dynamics and speciation of organic carbon during decomposition of leaf litter and fine roots in four subtropical plantations of China. *For. Ecol. Manag.* **2013**, *300*, 43–52. [[CrossRef](#)]
8. Pandey, R.R.; Sharma, G.; Tripathi, S.K.; Singh, A.K. Litterfall, litter decomposition and nutrient dynamics in a subtropical natural oak forest and managed plantation in northeastern India. *For. Ecol. Manag.* **2007**, *240*, 96–104. [[CrossRef](#)]
9. Xu, S.G.; Liu, Y.F.; Cui, Y.G.; Pei, Z.Y. Litter decomposition in a subtropical plantation in Qianyanzhou, China. *J. For. Res.* **2011**, *16*, 8–15. [[CrossRef](#)]
10. Innangi, M.; Danise, T.; D'Alessandro, F.; Curcio, E.; Fioretto, A. Dynamics of Organic Matter in Leaf Litter and Topsoil within an Italian Alder (*Alnus cordata* (Loisel.) Desf.) Ecosystem. *Forests* **2017**, *8*, 240. [[CrossRef](#)]
11. Ono, K.; Hiradate, S.; Ohse, K.; Hirai, K. Humification processes of needle litter on forest floors in Japanese cedar (*Cryptomeria japonica*) and Hinoki cypress (*Chamaecyparis obtusa*) plantations in Japan. *Plant Soil* **2011**, *338*, 171–181. [[CrossRef](#)]
12. Motavalli, P.P.; Palm, C.A.; Parton, C.A.; Elliott, E.T.; Frey, S.D. Soil pH and organic C dynamics in tropical forest soil: Evidence from laboratory and simulation studies. *Soil Biol. Biochem.* **1995**, *27*, 1587–1599. [[CrossRef](#)]
13. Wang, R.L.; Christian, S.; Dayan, F.E.; Song, Y.Y.; Su, Y.J.; Zeng, R.S. Simulated acid accelerates litter decomposition and enhances the allelopathic potential of the invasive plant *Wedelia trilobata* (creeping daisy). *Weed Sci.* **2012**, *60*, 462–467. [[CrossRef](#)]
14. Lv, Y.N.; Wang, C.Y.; Jia, Y.Y.; Wang, W.W.; Ma, X.; Du, J.J.; Pu, G.Z.; Tian, X.J. Effects of sulfuric, nitric, and mixed acid rain on litter decomposition, soil microbial biomass, and enzyme activities in subtropical forests of China. *Appl. Soil Ecol.* **2014**, *79*, 1–9. [[CrossRef](#)]
15. Li, Y.; Wang, Y.; Wang, Y.; Wang, B. Effects of simulated acid rain on soil respiration and its component in a mixed coniferous-broadleaved forest of the three gorges reservoir area in Southwest China. *For. Ecosyst.* **2019**, *6*, 32. [[CrossRef](#)]

16. Wu, J.P.; Liang, G.H.; Hui, D.F.; Deng, Q.; Xiong, X.; Qiu, Q.; Liu, J.; Chu, G.; Zhou, G.; Zhang, D. Prolonged acid rain facilitates soil organic carbon accumulation in a mature forest in Southern China. *Sci. Total Environ.* **2016**, *544*, 94–102. [[CrossRef](#)]
17. Osono, T.; Takeda, H.; Azuma, J. Carbon isotope dynamics during leaf litter decomposition with reference to lignin fractions. *Ecol. Res.* **2008**, *23*, 51–55. [[CrossRef](#)]
18. Kögel-Knabner, I. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* **2002**, *34*, 139–162. [[CrossRef](#)]
19. Kemmitt, S.J.; Wright, D.; Goulding, K.W.T.; Jones, D.L. pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biol. Biochem.* **2006**, *38*, 898–911. [[CrossRef](#)]
20. Dijkstra, F.A.; Hobbie, S.E.; Knops, J.M.H.; Reich, P.B. Nitrogen deposition and plant species interact to influence soil carbon stabilization. *Ecol. Lett.* **2004**, *7*, 192–198. [[CrossRef](#)]
21. Allison, S.D.; Jastrow, J.D. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. *Soil Biol. Biochem.* **2006**, *38*, 3245–3256. [[CrossRef](#)]
22. Dhakar, K.; Kooliyottil, R.; Joshi, A.; Pandey, A. Simultaneous production of ligninolytic enzymes by a temperature and pH tolerant strain of *Aspergillus niger* under different cultural conditions. *Indian J. Biotechnol.* **2015**, *14*, 81–86.
23. Schimel, J.; Becerra, C.A.; Blankinship, J. Estimating decay dynamics for enzyme activities in soils from different ecosystem. *Soil Biol. Biochem.* **2017**, *114*, 5–11. [[CrossRef](#)]
24. Bernoux, M.; Cerri, C.C.; Neill, C.; de Moraes, J.F.L. The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma* **1998**, *82*, 43–58. [[CrossRef](#)]
25. Gioacchini, P.; Masia, A.; Canaccini, F.; Boldreghini, P.; Tonon, G. Isotopic discrimination during litter decomposition and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ soil profiles in a young artificial stand and in an old floodplain forest. *Isot. Environ. Health Stud.* **2006**, *42*, 135–149. [[CrossRef](#)] [[PubMed](#)]
26. Collister, J.W.; Rieley, G.; Stern, B.; Eglinton, G.; Fry, B. Compound-specific $\delta^{13}\text{C}$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms. *Org. Geochem.* **1994**, *21*, 619–627. [[CrossRef](#)]
27. Hobbie, E.A.; Werner, R.A. Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: A review and synthesis. *New Phytol.* **2004**, *161*, 371–385. [[CrossRef](#)]
28. Dümig, A.; Rumpel, C.; Digna, M.F.; Kögel-Knabner, I. The role of lignin for the $\delta^{13}\text{C}$ signature in C4 grassland and C3 forest soils. *Soil Biol. Biochem.* **2013**, *57*, 1–13. [[CrossRef](#)]
29. McClaugherty, C.A.; Berg, B. Cellulose, lignin and nitrogen concentrations as rate regulating factors in late stages of forest litter decomposition. *Pedobiologia* **1987**, *30*, 101–112.
30. Benner, R.; Fogel, M.L.; Sprague, E.K.; Hodson, R.E. Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. *Nature* **1987**, *329*, 708–710. [[CrossRef](#)]
31. Feng, X. A theoretical analysis of carbon isotope evolution of decomposing plant litters and soil organic matter. *Glob. Biogeochem. Cycle* **2002**, *16*, 66–1–66–11. [[CrossRef](#)]
32. Preston, C.M.; Trofymow, J.A.; Flanagan, L.B. Decomposition, $\delta^{13}\text{C}$, and the “lignin paradox”. *Can. J. Soil Sci.* **2006**, *86*, 235–245. [[CrossRef](#)]
33. Osono, T.; Takeda, H. Limit values for decomposition and convergence process of lignocelluloses fraction in decomposing leaf litter of 14 tree species in a cool temperate forest. *Ecol. Res.* **2005**, *20*, 51–58. [[CrossRef](#)]
34. Osono, T.; Hobara, S.; Koba, K.; Kameda, K.; Takeda, H. Immobilization of avian excreta-derived nutrients and reduced lignin decomposition in needle and twig litter in a temperate coniferous forest. *Soil Biol. Biochem.* **2006**, *38*, 517–525. [[CrossRef](#)]
35. Mathers, N.J.; Mao, X.A.; Xu, Z.H.; Saffigna, P.G.; Berners-Price, S.J.; Perera, M.C.S. Recent advances in the application of ^{13}C and ^{15}N NMR spectroscopy to soil organic matter studies. *Aust. J. Soil Res.* **2000**, *38*, 69–787. [[CrossRef](#)]
36. Johnson, C.E.; Blumfield, T.J.; Boyd, S.; Xu, Z.H. A ^{13}C NMR study of decomposing logging residues in an Australian hoop pine plantation. *J. Soil. Sediment.* **2013**, *13*, 854–862. [[CrossRef](#)]
37. Preston, C.M.; Nault, J.R.; Trofymow, J.A. Chemical changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 2. ^{13}C abundance, solid-state ^{13}C NMR spectroscopy and the meaning of lignin. *Ecosystems* **2010**, *12*, 1078–1102. [[CrossRef](#)]
38. Ono, K.; Hiradate, S.; Morita, S.; Hirai, K. Fate of organic carbon during decomposition of different litter types in Japan. *Biogeochemistry* **2013**, *112*, 7–21. [[CrossRef](#)]

39. Mo, J.M.; Sandra, B.; Xue, J.H.; Fang, Y.T.; Li, Z.A. Response of litter decomposition to simulated N deposition in disturbed, rehabilitated and mature forests in subtropical China. *Plant Soil* **2006**, *282*, 135–151. [\[CrossRef\]](#)
40. Zhou, H.; Li, M.; Zhou, Y.; He, D.; Huang, Y. The Vegetation Map of Dinghushan Biosphere Reserve with Reference to Its Illustration. In *Tropical and Subtropical Forest Ecosystem 4*; Science Press: Guangzhou, China, 1986; pp. 43–49. (In Chinese)
41. Ding, M.; Brown, S.; Lugo, A.E. *A Continental Subtropical Forest in China Compared with an Insular Subtropical Forest in the Caribbean*; Gen. Tech. Rep. IIF-17, USDA, Forest Service; Institute of Tropical Forestry: Rio Piedras, PR, USA, 2001; p. 46.
42. Liang, G.H.; Liu, X.Z.; Chen, X.M.; Qiu, Q.Y.; Zhang, D.Q.; Chu, G.W.; Liu, J.X.; Liu, S.Z.; Zhou, G.Y. Response of soil respiration to acid rain in forests of different maturity in southern China. *PLoS ONE* **2013**, *8*, e62207. [\[CrossRef\]](#)
43. Chertov, O.G.; Komarov, A.S. SOMM—A model of soil organic matter dynamics. *Ecol. Model.* **1997**, *94*, 177–189. [\[CrossRef\]](#)
44. Fang, X.; Zhao, L.; Zhou, G.; Huang, W.; Liu, J. Increased litter input increases litter decomposition and soil respiration but has minor effects on soil organic carbon in subtropical forests. *Plant Soil* **2015**, *392*, 139–153. [\[CrossRef\]](#)
45. King, H.G.C.; Heath, G.W. The chemical analysis of small samples of leaf material and the relationship between the disappearance and composition of leaves. *Pedobiologia* **1967**, *7*, 192–197.
46. Chen, X.; Zhang, D.; Liang, G.; Qiu, Q.; Liu, J.; Zhou, G.; Liu, S.; Chu, G.; Yan, J. Effects of precipitation on soil organic carbon fractions in three subtropical forests in southern China. *J. Plant Ecol.* **2016**, *9*, 10–19. [\[CrossRef\]](#)
47. Hyodo, F.; Inoue, T.; Azuma, J.; Tayasu, I.; Abe, T. Role of the mutualistic fungus in lignin degradation in the fungus-growing termite *Macrotermes gilvus* (Isoptera; Macrotermitinae). *Soil Biol. Biochem.* **2000**, *32*, 653–658. [\[CrossRef\]](#)
48. Baldock, J.A.; Oades, J.M.; Nelson, P.N.; Skene, T.M.; Golchin, A.; Clarke, P. Assessing the extent of decomposition of natural organic materials using solid-state ¹³C NMR spectroscopy. *Aust. J. Soil Res.* **1997**, *35*, 1061–1083. [\[CrossRef\]](#)
49. Ostertag, R.; Marin-Spiotta, E.; Silver, W.L.; Schulten, J. Litterfall and decomposition in relation to soil carbon pools along a secondary forest chronosequence in Puerto Rico. *Ecosystems* **2008**, *5*, 701–714. [\[CrossRef\]](#)
50. Martens, R. Current methods for measuring microbial biomass C in soil: Potentials and limitations. *Biol. Fert. Soils* **1995**, *19*, 87–99. [\[CrossRef\]](#)
51. Iyyemperumal, K.; Shi, W. Soil enzyme activities in two forage systems following application of different rates of swine lagoon effluent or ammonium nitrate. *Appl. Soil Ecol.* **2008**, *38*, 128–136. [\[CrossRef\]](#)
52. Connin, S.L.; Feng, X.; Virginia, R.A. Isotopic discrimination during long-term decomposition in an arid land ecosystem. *Soil Biol Biochem.* **2001**, *33*, 41–51. [\[CrossRef\]](#)
53. Adams, M.A.; Grierson, P.F. Stable isotopes at natural abundance in terrestrial plant ecology and ecophysiology: An update. *Plant Biol.* **2001**, *3*, 299–310. [\[CrossRef\]](#)
54. Maass, W.S.G.; Craigie, J.S. Examinations of some soluble constituents of Sphagnum gametophytes. *Can. J. Bot.* **1964**, *42*, 805–813. [\[CrossRef\]](#)
55. Rudolph, H.; Samland, J. Occurrence and metabolism of Sphagnum acid in the cell walls of bryophytes. *Phytochemistry* **1985**, *24*, 745–749. [\[CrossRef\]](#)
56. Falappi, D.; Farini, A.; Ranalli, G.; Sorlini, C. Effects of simulated acid rain on some microbiological parameters of subacid soil. *Chemosphere* **1994**, *28*, 1087–1095. [\[CrossRef\]](#)
57. Wang, C.Y.; Guo, P.; Han, G.M.; Feng, X.G.; Zhang, P.; Tian, X.J. Effect of simulated acid rain on the litter decomposition of *Quercus acutissima* and *Pinus massoniana* in forest soil microcosms and the relationship with soil enzyme activities. *Sci. Total Environ.* **2010**, *408*, 2706–2713. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Waldrop, M.P.; Zak, D.R.; Sinsabaugh, R.L.; Gallo, M.; Lauber, C. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecol. Appl.* **2004**, *14*, 1172–1177. [\[CrossRef\]](#)
59. Enowashu, E.; Poll, C.; Lamersdorf, N.; Kandeler, E. Microbial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. *Appl. Soil Ecol.* **2009**, *43*, 11–21. [\[CrossRef\]](#)
60. Xiao, S.; Wang, G.G.; Tang, C.; Fang, H.; Duan, J.; Yu, X. Effects of One-Year Simulated Nitrogen and Acid Deposition on Soil Respiration in a Subtropical Plantation in China. *Forests* **2020**, *11*, 235. [\[CrossRef\]](#)

61. Sinsabaugh, R.; Lauber, C.L.; Weintraub, M.N.; Ahmed, B.; Allison, S.D.; Crenshaw, C.; Contosta, A.R.; Cusack, D.; Frey, S.; Gallo, M.E.; et al. Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **2008**, *11*, 1252–1264. [[CrossRef](#)]
62. Zech, W.; Ziegler, F.; Kögel-Knabner, I.; Haumaier, L. Humic substances distribution and transformation in forest soils. *Sci. Total Environ.* **1992**, *117–118*, 155–174. [[CrossRef](#)]
63. Curtin, D.; Campbell, C.A.; Jalil, A. Effects of acidity on mineralization: pH-dependence of organic matter mineralization in weakly acidic soils. *Soil Biol. Biochem.* **1998**, *30*, 57–64. [[CrossRef](#)]

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).