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Response of Soil Respiration to Simulated Acid Rain with Different Ratios of SO_4^{2-} to NO_3^- in *Cunninghamia lanceolata* (Lamb.) Hook. and *Michelia macclurei* Dandy Plantations

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Abstract: Acid rain is one of the most serious environmental issues in Southern China. The composition of acid rain has gradually changed from sulfuric acid rain (SAR) to nitric acid rain (NAR) due to the rapid development of industry, and controls on SO_2 emissions. However, a comprehensive understanding of how changes in the type of acid rain affect soil respiration (Rs) in forest ecosystems is still lacking. In this study, we investigated the influence of simulated acid rain with different SO_4^{2-}/NO_3^- ratios, namely, SAR (4:1), MAR (mixed acid rain, 1:1), and NAR (1:4), on Rs in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations from 2019 to 2020. A trenching method was used to partition Rs into heterotrophic respiration (Rh) and autotrophic respiration (Ra). The results showed that acid rain did not significantly influence Rs in the two plantations, which could be mainly attributed to the unchanged soil pH. Neither SAR, MAR, nor NAR affected Ra in the two plantations, possibly due to the unchanged root biomass. The SAR treatment only significantly increased Rh in the MM plantation, not in the CL plantation. The temperature sensitivity (Q_{10}) of Rs and its components was not significantly different among different acid rain types in either of the plantations. Our results suggest that the impact of acid rain on Rs and its components depends on the forest ecosystem and the type of acid rain. Different biological processes complicate the response of soil CO_2 emissions to acid rain pollution.

Keywords: simulated acid rain; SO_4^{2-}/NO_3^- ratio; heterotrophic respiration; temperature sensitivity; plantation

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1. Introduction

Atmospheric emissions of sulfur dioxide (SO_2) and nitrogen oxide (NO_x) are increasing due to anthropogenic activities such as industry and fertilization, which cause severe acid rain [1,2]. As one of the most serious global environmental issues, acid rain has affected approximately 40% of China, and it is particularly serious in southern China [3]. Sulfuric acid rain (SAR) was considered to primarily impact the ecosystem in the past [4]. However, after the implementation of several policies to control SO_2 emissions, the amount of sulfate ions (SO_4^{2-}) in precipitation decreased [5]. Meanwhile, the level of NO_x emissions increased sharply with the increasing number of motor vehicles, thereby contributing to a rapid increase in the relative content of nitrate ions (NO_3^-) in precipitation [6]. Consequently, the ratio of SO_4^{2-}/NO_3^- in precipitation in southern China may continue to gradually

decrease in the future [7,8]. In such a situation, forest ecosystems would face a more complex challenge.

Soil respiration (Rs), as one of the two largest terrestrial carbon (C) fluxes [9], accounts for 70%–90% of the global ecosystem C emissions [10]. Therefore, small alterations in Rs may influence the global C budget [11]. The effects of acid rain on Rs have received considerable attention in research regarding forest ecosystems [12–14]. Acid rain can cause soil acidification, which inhibits Rs by interfering with root growth and soil organic matter decomposition [15]. However, some studies have shown that simulated acid rain does not always lead to a decrease in Rs. For example, neutral and positive effects of acid rain on Rs have been reported [16–18]. These contradictory results may be partly due to differences in types of acid rain. On one hand, nitric acid rain (NAR) is more likely to lead to soil acidification compared to SAR [19,20]. On the other hand, the fertilizer effect of NO_3^- from acid rain leads to a more complicated response of Rs [13].

The potential effects of acid rain on Rs might vary with different biological processes. Autotrophic respiration (Ra) and heterotrophic respiration (Rh) are different components of Rs and occur in different ways; the former originates from the respiration of the roots and rhizosphere, and the latter is mostly derived from the mineralization of organic matter [21]. Acid rain can affect Rh and Ra to different degrees by altering the microbial community structure and/or soil enzyme activities as well as root growth through excessive H^+ inputs. Chen et al. [22] reported that simulated acid rain inhibited Ra, but did not affect Rh, due to the toxicity of H^+ to the roots in a subtropical secondary forest. In addition, NO_3^- from acid rain was shown to have different effects on Ra and Rh. Studies reported that Ra was more sensitive to N addition than Rh [23], although N (as a limiting nutrient) may have positive effects on microbial activity and plant growth. Therefore, acid rain with different ratios of SO_4^{2-} to NO_3^- is expected to affect Ra, Rh, and thus Rs, in forest ecosystems differently.

The temperature sensitivity (Q_{10}) of soil respiration is an important parameter used to predict its potential feedback to rising air temperatures [24,25]. Changes in the Q_{10} of soil respirations largely determine the net C flux from the soil to the atmosphere [26]. The results of previous studies showed that Ra is more temperature-sensitive than Rh [27]. Acid rain may change the proportion or the Q_{10} of respiration components, and thus affect the carbon dynamics [28]. In addition, acid rain may influence Q_{10} by altering environmental parameters, including soil temperature and moisture [29]. However, few studies have explored how the transition from SAR to NAR affects the Q_{10} of Rs and its components.

Subtropical forests, especially plantations, make up a large proportion of vegetation-covered terrestrial ecosystems and play a considerable role in maintaining the stability of the global C balance in the global ecosystem [30–32]. In this study, we carried out an-experiment to investigate the response of Rs and its components to simulated acid rain with different ratios of SO_4^{2-} to NO_3^- in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations. We hypothesized that (1) the negative effect of acid rain on Rs would increase with a decreasing SO_4^{2-}/NO_3^- ratio due to the stronger effect of NO_3^- on acidification; (2) Ra and Rh would have different responses to the transition of acid rain types due to the fertilizer effect of NAR on Ra; and (3) the Q_{10} value of Rs would decrease with an increase in Ra under NAR treatment because Ra is more sensitive to temperature than Rh.

2. Materials and Methods

2.1. Site Description

This study was performed at the Huitong National Research Station of Forest Ecosystem (26°40′–27°09′ N, 109°26′–110°08′ E) located in Hunan Province, Southern China. This region is characterized as a mid-subtropical monsoon climate with typical humid and warm conditions. The mean annual temperature is 16.5 °C with maximum and minimum monthly average temperatures of 29.0 °C in July and 1.9 °C in January [27]. The average annual precipitation is approximately 1200 mm, the annual mean pH of rainfall is approximately

4.7, the acidity of rainfall reaches 3.6 in the worst circumstance, and the frequency of acidic precipitation is approximately 85% [33]. The soil was classified as reddish oxisol soil [34].

2.2. Experiment Design

The field experiment was conducted in adjacent 34-year-old CL and MM plantations in April 2016. The mean values of diameter at breast height (DBH) were 24.6 cm and 21.1 cm in the CL and MM plantations, respectively. A completely randomized design was applied in our study. Sixteen plots were established in each plantation, with each plot measuring 4 m × 4 m. The distance between the two plots was more than 3 m to prevent interference caused by mutual influence. All plots were sprayed with corresponding acidic solutions, which were control (local spring water, pH = 5.6), SAR (the mole ratio of H_2SO_4 to HNO_3 is 4:1, pH = 3), mixed acid rain (MAR, the mole ratio of H_2SO_4 to HNO_3 is 1:1, pH = 3), and NAR (the mole ratio of H_2SO_4 to HNO_3 is 1:4, pH = 3) solutions. The cumulative amount of spring water or acidic solution was 60 $mm \cdot m^{-2} \cdot yr^{-1}$, and the spraying frequency was twice a month.

2.3. Measurements of Rs, Rh, Soil Temperature and Soil Moisture

A trenching method was adopted to distinguish Rs into heterotrophic and autotrophic components [35]. Trenches were cut (>60 cm) into the soil to sever roots entering the plots in April 2018. Each plot was split into untrenched and trenched subplots. Trenches with an area of 1 m × 1m were excavated to a depth of 60 cm or to bedrock. The soil was backfilled after the trench was lined with polyvinyl chloride (PVC) panels, and the understory vegetation in the trenched subplot was carefully removed. Two PVC collars (10.8 cm in diameter and 5 cm in height) were inserted into the soil at the center of each subplot, and a length of 2 cm was exposed above the ground, and 3 cm was set below the ground. From January 2019 to December 2020, the soil CO_2 efflux of each collar was measured between 8:30 am and 12:00 am once per month by using the Li-8100 Infrared Gas Analyzer (Li-Cor Inc., Lincoln, NE, USA). The soil temperature and moisture (v/v) at 5 cm were measured simultaneously with soil CO_2 efflux measurements using a portable temperature and moisture probe provided in the Li-8100 system.

2.4. Soil Properties and Root Biomass

Soil samples from 0 to 10 cm depths were collected from each subplot using an auger (5 cm in diameter) to measure the root biomass (only untrenched subplots) and soil chemical properties in July 2020. Soil samples were brought back to the laboratory, passed through a 2 mm sieve, and fine roots with diameters less than 2 mm were collected. All the fine roots were dried at 60 °C to a constant mass. All of the soil samples were divided into two portions for refrigeration at 4 °C and for air drying. The pH of the soil was determined in 1:2.5 (w/v) soil: CO_2-free water solutions with a pH meter (FE28, Five Easy Plus, Shanghai, China). Ammonium and nitrate were extracted using a 2 $mol \cdot L^{-1}$ KCl solution and determined on a Flow-Injection Autoanalyzer (AA3, SEAL, Hamburg, Germany) using the indophenol blue colorimetric method and the Cu-plated cadmium reduction–diazotization coupled colorimetric method [36]. The available phosphorus (P) in the soil was extracted with the mixture solution of 0.03 $mol \cdot L^{-1}$ NH_4F and 0.025 $mol \cdot L^{-1}$ HCl and determined colorimetrically using the molybdenum antimony anti-colorimetric method [37] on a Flow-Injection Autoanalyzer. The available sulfur (S) in the soil was extracted using monocalcium phosphate-acetic acid (2.04 g of calcium dihydrogen phosphate was dissolved in 2 $mol \cdot L^{-1}$ of acetic acid) and determined using the barium sulfate turbidimetric method [38] on a microplate spectrophotometer (Epochta, Biotek Instruments Inc., Winooski, VT, USA).

The microbial community of the fresh soil sample was determined through the analysis of phospholipid fatty acids (PLFAs) within one week. According to White et al. [39] and Bardgett et al. [40], PLFAs were extracted from 3 g of the lyophilized soil samples by using a 20 mL solvent mixture of chloroform, methanol, and citric (1:2:0.8) for 2 h, eluted selectively through activated silica columns, and subjected to mild methanolysis. A toluene methanol

mixture (toluene:methanol = 1:1) and KOH methanol solution (prepared with methanol) were added for methylation. After being purified and methylated, PLFAs were analyzed using Agilent 6890N gas chromatography and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA). The fatty acids 16:1 w9c, 16:1 w7c, 18:1 w7c, 18:1 w5c, cy19:0 w7c, and cy17:0 w7c represent Gram-negative bacteria (GN) [41,42], while i14:0, i15:0, a-15:0, i16:0, i17:0, and a17:0 represent Gram-positive bacteria (GP) [43]. The unsaturated PLFAs, 18: 1w9c and 18:2 w6c represent fungi [44,45].

Soil β-glucosidase (BG), soil leucine aminopeptidase (LAP), soil N-acetylglucosaminidase (NAG), and soil acid phosphatase activity (AP) were evaluated using the fluorescence colorimetric method [46]. Soil suspensions were prepared by homogenizing 1 g of fresh soil in 125 mL of buffer (50 mol·L^{-1} sodium acetate). The substrates were 4-MUB-β-D-glucoside, L-Leucine-7-amino-4-methylcoumarin, 4-MUB-*N*-acetyl-β-D-glucosaminide, and 4-MUB-phosphate, respectively. The microplates were incubated at 25 °C in the dark for 4 h. Fluorescence was determined using a microplate spectrophotometer at 365 nm excitation and 450 nm emission. The phenol oxidase (PHO) and urease (URE) activities were measured spectrophotometrically using L-3,4-dihydroxyphenylala-nine (DOPA) and urea as the substrate [46]. The microplates were incubated at 20 °C in the dark for 18 h. The activity was quantified by measuring the absorbance at 450 nm and 610 nm using a microplate spectrophotometer.

2.5. Statistical Analysis

The mean of two collar measurements in each subplot was calculated as Rs or Rh. The difference between Rs measured in the untrenched subplot and Rh in the corresponding trenched subplot was calculated as Ra:

$$Ra = Rs - Rh$$

Temperature sensitivity (Q_{10}) was defined as the exponential relationship between Rs, Rh, or Ra and soil temperature, and it was computed as follows:

$$R = ae^{bT}$$

$$Q_{10} = e^{10b}$$

where R (μmol·m^{-2}·s^{-1}) is Rs, Rh, or Ra; a and b are the model parameters; and T (°C) is the soil temperature corresponding to Rs, Rh, or Ra.

The effects of acid rain on Rs, Rh, Ra, soil temperature, and moisture were examined with a repeated-measure analysis of ANOVA ($p < 0.05$). A two-way ANOVA was performed to determine the main and interactive effect of acid rain and year on mean annual Rs, Rh, and Ra. The LSD method was adapted to confirm the difference among SAR, MAR, NAR, and CK. One-way ANOVA with LSD was used to determine differences in the root biomass, microbial community composition, enzymes, and soil chemical properties among the four treatments. All the data analyses were performed using SPSS 26.0 (IBM SPSS Statistics, Armonk, NY, USA) and graphic drawing was carried out using Origin 2021 (Origin Lab, Northampton, MA, USA) software.

3. Results

3.1. Soil Temperature and Moisture

In the CL and MM plantations, the soil temperature after acid rain treatments exhibited similar seasonal patterns, with the highest temperature occurring around August and the lowest occurring around January. Soil temperature was not significantly different among all acid rain treatments in the untrenched and trenched subplots in both plantations (Figure 1). The soil moisture also showed obvious seasonal dynamics and was not significantly different between the different treatments in both plantations (Figure 2).

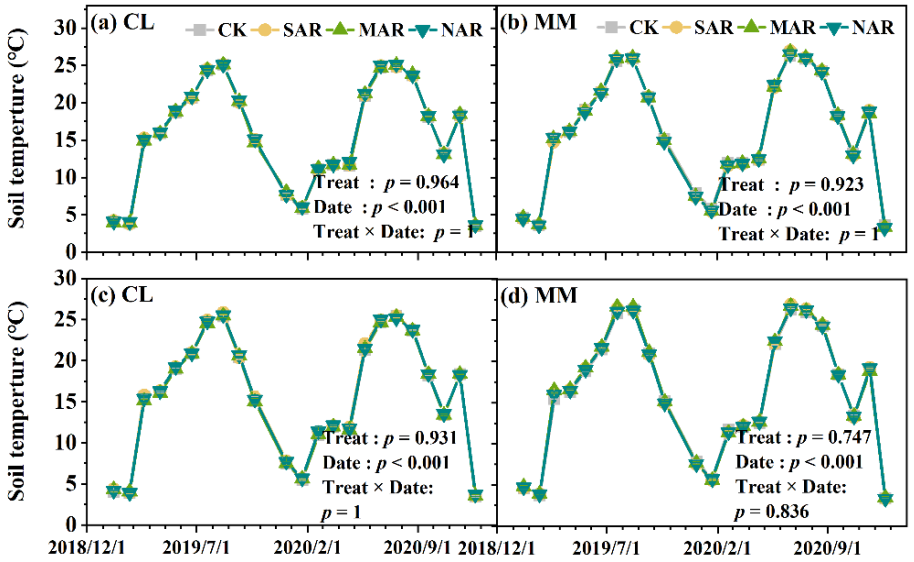

Figure 1. Seasonal variations in soil temperature in untrenched plots (**a**,**b**) and trenched plots (**c**,**d**) under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL, (**a**,**c**)) and *Michelia macclurei* Dandy (MM, (**b**,**d**)) plantations from January 2019 to December 2020. Vertical bars represent standard errors (n = 4).

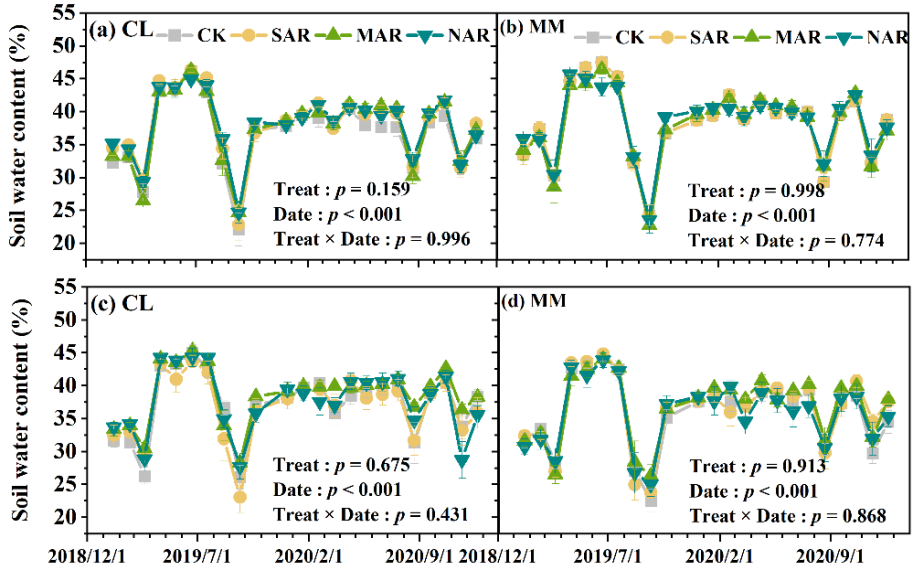

Figure 2. Seasonal variations in soil water content in untrenched plots (**a**,**b**) and trenched plots (**c**,**d**) under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL, (**a**,**c**)) and *Michelia macclurei* Dandy (MM, (**b**,**d**)) plantations from January 2019 to December 2020. Vertical bars represent standard errors (n = 4).

3.2. Rs and Its Components

The seasonal dynamics of Rs, Rh, and Ra in different acid rain treatments following the change in soil temperature at 5 cm depth had a bell-shaped curve in the CL and MM plantations from 2019 to 2020 (Figure 3). The repeated-measure ANOVA showed that acid rain did not alter Rs, Rh, and Ra in the two plantations, but it had a significant effect on Rh in the MM plantation ($p < 0.05$, Figure 3d).

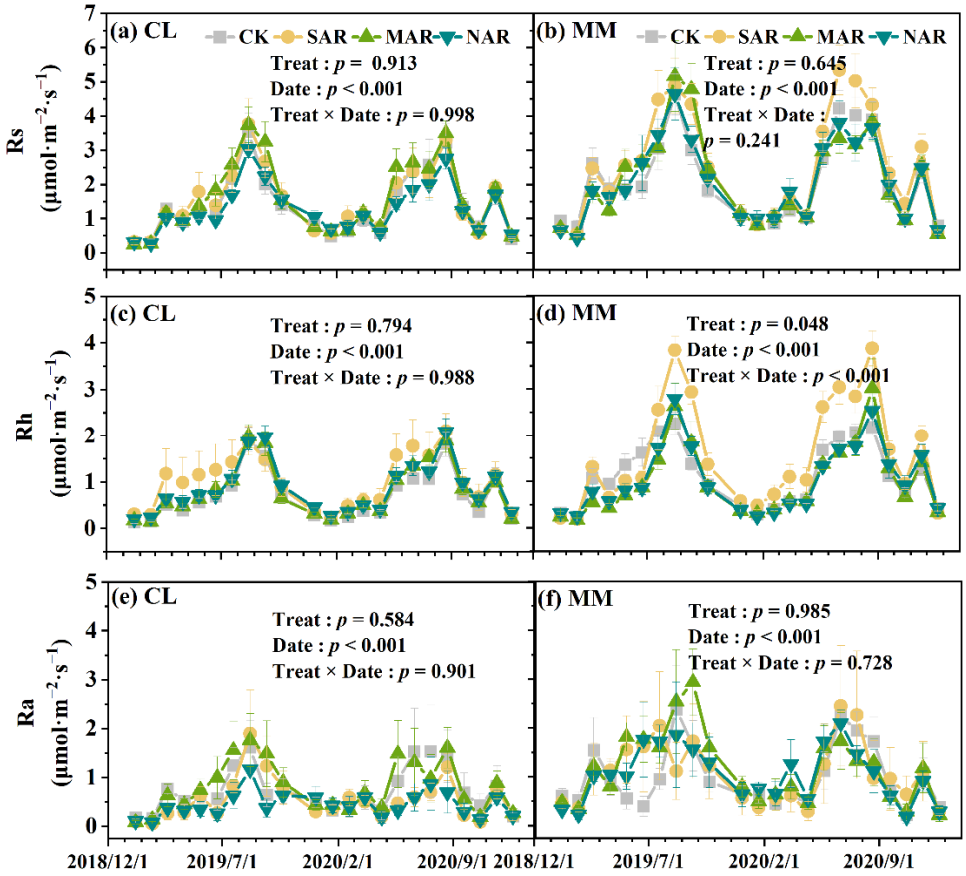

Figure 3. Seasonal variations in soil respiration (Rs) (**a**,**b**), soil heterotrophic respiration (Rh) (**c**,**d**), and soil autotrophic respiration (Ra) (**e**,**f**) under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations from January 2019 to December 2020. Vertical bars represent standard errors (n = 4).

In the CL plantation, acid rain did not significantly affect the annual mean Rs, Rh, and Ra in 2019 and 2020 (Figure 4a,c,e). Similarly, there was no obvious response of the annual mean Rs and Ra to acid rain treatments in the MM plantation ($p > 0.05$, Figure 4b,f). However, we found that acid rain significantly affected the annual mean Rh, and no interaction effect between acid rain treatment and year was observed in the MM plantation ($p < 0.01$, Figure 4d). Multiple comparisons showed that SAR, but not MAR and NAR, significantly increased Rh in the MM plantation. Compared with CK, SAR resulted in 24.20% and 54.96% increases in Rh in 2019 and 2020, respectively. We also found that the contribution of Rh to Rs showed no response to all acid rain types in both plantations (Table 1).

3.3. Temperature Sensitivity of Rs and Its Components

In the CL and MM plantations, soil temperature was exponentially and significantly correlated with Rs, Rh, and Ra in all the acid rain treatments (Figure 5). Compared with Ra, the soil temperature could explain greater variation in Rs and Rh. The Q_{10} values of Rs, Rh, and Ra varied from 2.08 to 2.68, from 2.16 to 2.70, and from 1.83 to 2.64, respectively, regardless of the acid rain treatments and plantations. No obvious difference in the Q_{10} of Rs, Rh, and Ra was observed among the different acid rain treatments in both plantations.

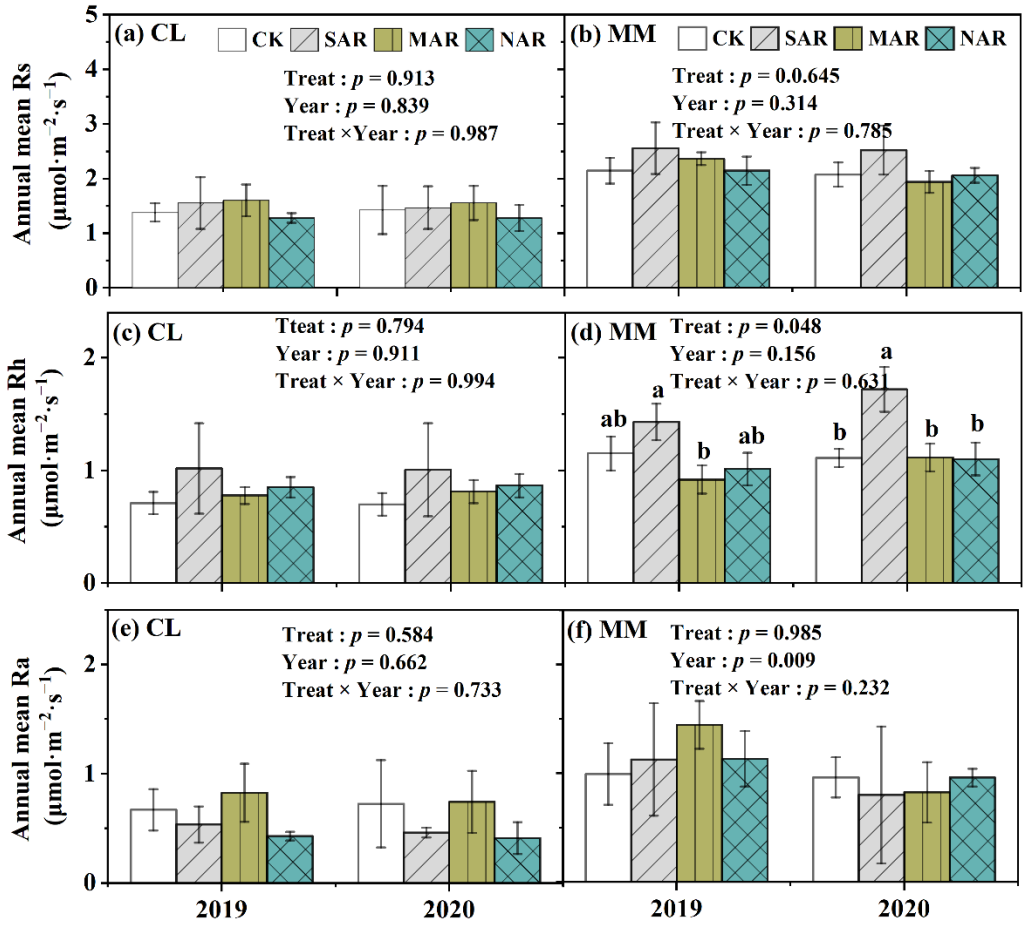

Figure 4. Variations in the annual mean soil respiration (Rs) (**a**,**b**), soil heterotrophic respiration (Rh) (**c**,**d**), and soil autotrophic respiration (Ra) (**e**,**f**) under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations. Vertical bars represent standard errors (n = 4). Means with different letters in common are significantly different ($p < 0.05$). The absence of letters indicates no significant differences among acid rain treatments.

Table 1. Effects of acid rain on the contribution of Rh to Rs (Rh/Rs) in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations. Data are the mean ± standard error (n = 4). Means followed by a common letter are not significantly different among different acid rain treatments ($p < 0.05$).

Plantation	Treatment			
	CK	SAR	MAR	NAR
CL	0.57 ± 0.15 a	0.63 ± 0.10 a	0.55 ± 0.10 a	0.67 ± 0.02 a
MM	0.55 ± 0.08 a	0.72 ± 0.19 a	0.49 ± 0.08 a	0.51 ± 0.05 a

3.4. Soil Properties, Root Biomass, Microbial Community and Enzyme Activities

The soil pH was not significantly different among the SAR, MAR, NAR, and CK treatments in the CL and MM plantations ($p > 0.05$, Table 2). The type of acid rain had no significant effect on the soil inorganic nitrogen, available phosphorus, and available sulfur levels in the two plantations regardless of trenching, except for the inorganic nitrogen content in the trenched subplot in the CL plantation (Table 2). The fine root biomass was also unaffected by different acid rain treatments in the two plantations ($p > 0.05$, Figure 6).

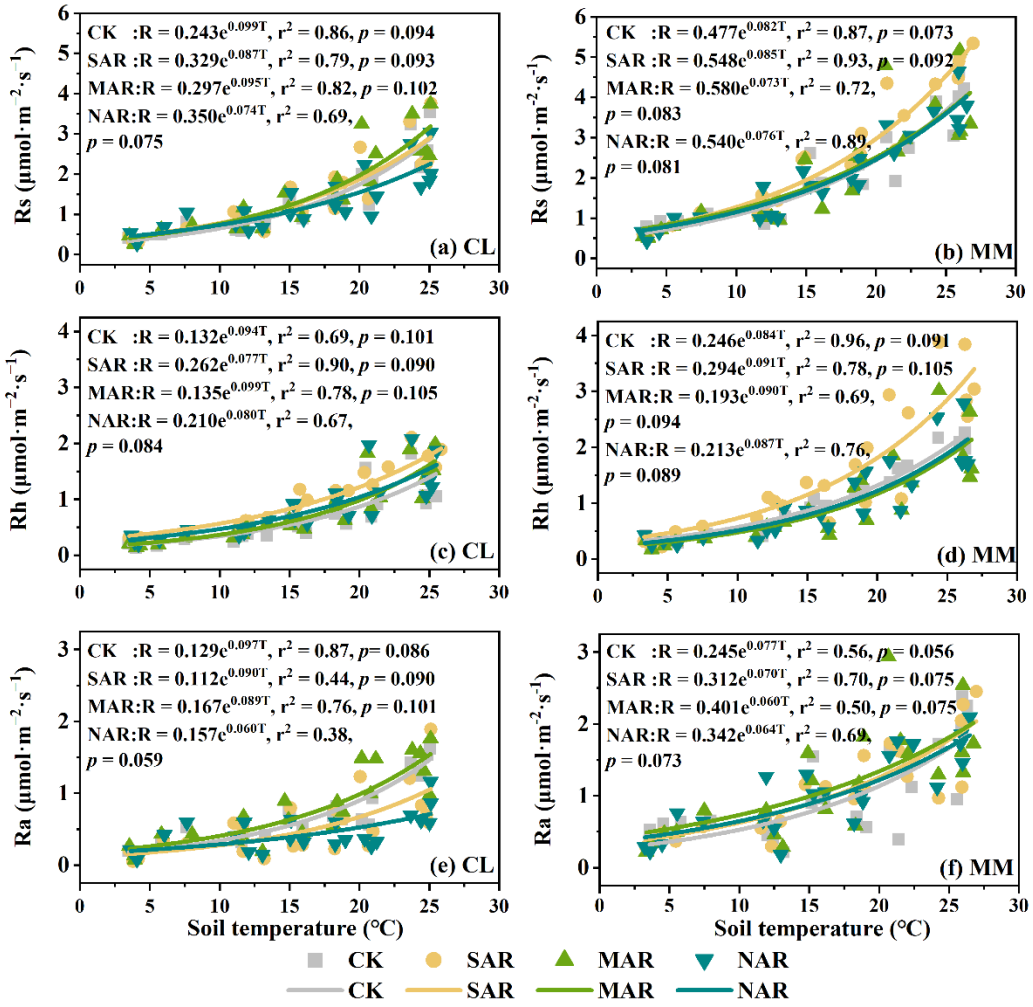

Figure 5. Relationship between Rs (**a**,**b**), Rh (**c**,**d**), Ra (**e**,**f**), and soil temperature under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations.

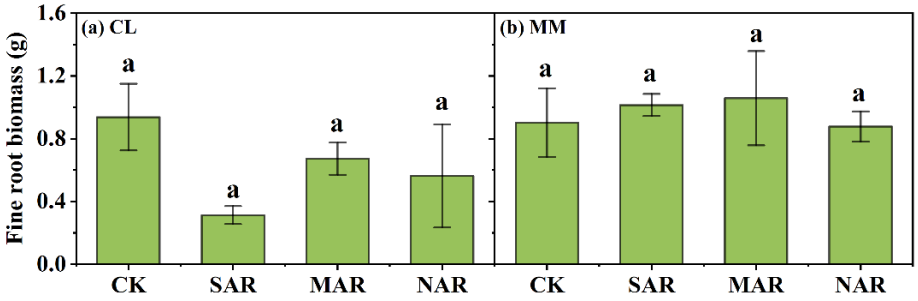

Figure 6. Fine root biomass under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (**a**), and *Michelia macclurei* Dandy (**b**) plantations. Vertical bars represent standard errors (n = 4). Means followed by a common letter are not significantly different among different acid rain treatments ($p < 0.05$).

No significant differences in total soil microbial biomass and microbial community structure (G+/G− or fungi/bacteria) were observed among the different acid rain types in the two plantations ($p > 0.05$, Table 3). Different types of acid rain significantly increased soil LAP, AP, and PHO activities in the trenched subplots of the CL plantation, but not in the MM plantation. The activities of LAP, AP, and PHO were increased by 322.3%, 66.0% and 74.9%, respectively, due to SAR addition in the CL plantation ($p < 0.05$, Table 3). The BG and

URE activities did not respond to different types of acid rain regardless of trenching in the CL and MM plantations. Moreover, acid rain, especially NAR, reduced the NAG activity.

Table 2. Variations in soil properties under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations. Data are the mean ± standard error (n = 4). Means with different letters in common are significantly different ($p < 0.05$). The absence of letters indicates no significant differences among acid rain treatments.

Plantation		Treatment	pH	Inorganic N (mg·g^{-1})	Availability P (mg·kg^{-1})	Availability S (mg·kg^{-1})
CL	Untrenched	CK	4.16 ± 0.04	13.68 ± 0.82	1.09 ± 0.12	25.44 ± 1.21
		SAR	4.10 ± 0.05	14.63 ± 0.76	1.26 ± 0.08	24.80 ± 1.83
		MAR	4.03 ± 0.08	14.57 ± 0.62	1.30 ± 0.14	26.76 ± 1.32
		NAR	4.14 ± 0.15	16.24 ± 0.51	1.34 ± 0.11	25.32 ± 1.61
	Trenched	CK	4.11 ± 0.04	11.51 ± 0.90 b	1.05 ± 0.30	25.49 ± 1.49
		SAR	4.06 ± 0.06	14.09 ± 0.64 a	1.07 ± 0.06	24.28 ± 2.72
		MAR	4.00 ± 0.05	13.36 ± 0.41 ab	0.93 ± 0.04	26.95 ± 0.75
		NAR	4.11 ± 0.06	12.00 ± 0.30 b	1.05 ± 0.07	26.21 ± 0.55
MM	Untrenched	CK	4.20 ± 0.02	10.69 ± 1.06	1.30 ± 0.09	24.90 ± 2.06
		SAR	4.19 ± 0.03	9.50 ± 0.97	1.05 ± 0.05	26.10 ± 1.12
		MAR	4.31 ± 0.10	12.56 ± 1.60	1.20 ± 0.13	23.74 ± 2.36
		NAR	4.18 ± 0.07	12.58 ± 1.36	1.24 ± 0.16	27.66 ± 0.27
	Trenched	CK	4.28 ± 0.05	8.22 ± 0.97	0.95 ± 0.09	25.35 ± 1.28
		SAR	4.20 ± 0.02	7.55 ± 0.78	0.97 ± 0.06	27.62 ± 0.76
		MAR	4.32 ± 0.08	10.15 ± 0.74	0.99 ± 0.06	27.08 ± 1.17
		NAR	4.19 ± 0.04	9.47 ± 0.44	1.06 ± 0.11	28.16 ± 0.58

Table 3. Variations in soil microbial PLFAs under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations. Data are the mean ± standard error (n = 4). The absence of letters indicates no significant differences among acid rain treatments.

Plantation		Treatment	Total Biomass (nmol·g^{-1})	Fungi (nmol·g^{-1})	G+ (nmol·g^{-1})	G− (nmol·g^{-1})	G+/G−	Fungi/ Bacterial
CL	Untrenched	CK	30.83 ± 6.01	0.50 ± 0.08	8.54 ± 1.66	8.95 ± 1.70	0.98 ± 0.11	0.03 ± 0.00
		SAR	21.08 ± 6.61	0.39 ± 0.08	5.47 ± 1.79	6.61 ± 2.32	0.87 ± 0.06	0.04 ± 0.01
		MAR	28.89 ± 0.88	0.97 ± 0.39	7.20 ± 0.40	9.06 ± 0.68	0.80 ± 0.05	0.06 ± 0.02
		NAR	34.15 ± 14.00	0.42 ± 0.06	5.80 ± 1.55	7.61 ± 2.52	0.86 ± 0.13	0.04 ± 0.01
	Trenched	CK	21.86 ± 3.11	0.30 ± 0.05	6.29 ± 1.16	6.24 ± 1.13	1.07 ± 0.24	0.02 ± 0.01
		SAR	24.79 ± 7.08	0.39 ± 0.11	6.87 ± 1.61	7.83 ± 2.81	1.05 ± 0.18	0.03 ± 0.01
		MAR	24.70 ± 3.05	0.35 ± 0.06	7.59 ± 0.51	7.43 ± 1.23	1.07 ± 0.11	0.02 ± 0.00
		NAR	52.84 ± 29.63	0.31 ± 0.05	6.63 ± 0.49	6.02 ± 0.37	1.10 ± 0.07	0.02 ± 0.00
MM	Untrenched	CK	35.16 ± 6.50	0.79 ± 0.14	9.38 ± 1.37	11.87 ± 2.82	0.84 ± 0.08	0.04 ± 0.00
		SAR	35.06 ± 5.76	0.97 ± 0.17	9.36 ± 1.29	11.15 ± 2.17	0.87 ± 0.05	0.05 ± 0.00
		MAR	47.14 ± 7.64	1.01 ± 0.24	12.53 ± 2.47	16.17 ± 5.59	0.78 ± 0.08	0.04 ± 0.01
		NAR	51.79 ± 5.61	1.00 ± 0.14	14.56 ± 1.56	16.56 ± 1.94	0.89 ± 0.05	0.03 ± 0.00
	Trenched	CK	33.03 + 1.24	0.81 ± 0.06	8.82 ± 0.88	11.02 + 0.56	0.81 ± 0.10	0.04 ± 0.00
		SAR	33.66 ± 5.34	0.84 ± 0.09	8.87 ± 1.05	10.80 + 2.01	0.86 ± 0.07	0.04 ± 0.00
		MAR	42.35 ± 8.77	1.06 ± 0.21	11.23 ± 2.45	13.91 + 3.07	0.82 ± 0.08	0.04 ± 0.01
		NAR	38.20 ± 6.17	0.90 ± 0.16	10.13 ± 1.79	11.52 + 2.15	0.90 ± 0.07	0.04 ± 0.00

4. Discussion

We found that different types of simulated acid rain did not significantly change the Rs in the CL and MM plantations (Figures 3 and 4). This finding is inconsistent with the results of a meta-analysis that reported a 14.7% reduction in Rs due to acid rain [20]. Many studies have demonstrated that acid rain has negative effects on Rs, which may be attributed to the soil acidification induced by acid rain [3,4]. In addition to the direct input of H^+, SO_4^{2-} and NO_3^- from acid rain may also influence the soil pH value to a certain extent [20]. In fact, SO_4^{2-} and NO_3^- inputs from acid rain can be exchanged with the hydroxyl groups (OH^-) of soil particles, and it was shown that SO_4^{2-} is more easily absorbed by soil particles, while NO_3^- is prone to leach [18,19]. Thus, the reduction in soil pH induced by NAR is more obvious than that induced by SAR due to their different adsorption mechanisms [47,48]. However, we found no difference in soil pH between NAR and SAR, or even between the control and all of the acid rain treatments (Table 2). The lack of change in soil pH between treatments may be partly attributable to the low initial soil pH (from 4.11 to 4.28) in our study. Obviously, the soil in our study was in the Al buffering stage, considering the soil pH remained above 4.0. Therefore, acid rain will hardly change soil pH as long as the soil still contains A1 oxides and/or hydroxides [49]. On the other hand, the fact that acid rain did not change soil pH may be due to the short duration of the experimental treatment. Liang et al. [50] also found no significant change in soil pH in the short-term acid rain treatment. Therefore, the absence of changes in Rs in the CL and MM plantations may largely be explained by a negligible soil acidification response.

Another possible reason for the absence of changes in Rs is the complementary effect of Rh and Ra [28,45]. In particular, the N input from NAR can be considered a nutrient that promotes root growth and thus stimulates Ra [51]. Therefore, comparatively lower Rh in NAR may be compensated by the higher Ra caused by N fertilization, resulting in invariable Rs [13]. Contrary to these studies, we did not observe the fertilization effect of NAR on Ra under NAR treatment (Figure 3), as evident by the unchanged root biomass (Figure 6). Our experiment was conducted in a subtropical region; these regions are generally considered to be nitrogen-saturated rather than nitrogen-limited [52]. Therefore, the nitrogen input from NAR did not promote root growth given that nitrogen was not the limiting factor. These results indicated that the lack of changes in Rs under acid rain treatments in the present study should not be attributed to the complementary effects of Ra and Rh. Similar to our results, Chen et al. [16] reported no complementarity effect between Rh and Ra after the simulated acid rain conditions.

SAR could only stimulate Rh in the MM (broad-leaved) plantation rather than in the CL (coniferous) plantation (Figure 4). The soil acid buffering capacity of coniferous forests was previously confirmed to be lower than that of evergreen broad-leaved forests [53]. The higher acid buffering capacity of the broad-leaved forests' soil leads to the elimination of acid substances; therefore, the impact on soil microorganisms is weaker than of that in coniferous forests [54]. As shown in the report by Oulehle et al. [53], Rs in coniferous forests was significantly decreased, but was unaffected in broad-leaved forests under acid rain conditions. However, the difference in the acid buffering capacities between coniferous and broad-leaved forests may insufficiently explain our result that SAR promoted Rh in the broad-leaved plantation and had no effect in the coniferous plantation.

In general, the soil microbial community and enzyme activities are closely related to Rh [55]. We found that SAR increased the activity of N-related and P-related enzymes in the CL plantation, as evident by the results of an increase in LAP and AP activities and a decrease in the ratio of C and N enzyme activities (Table 4). These results indicated that the stoichiometry of the resource supply mismatches that of the microbial requirements due to the input of SO_4^{2-} from SAR; as such, microbial functional may be limited by N and P nutrients [56,57]. We also detected that the activity of PHO in the CL plantation was enhanced by SAR, suggesting that substrates for Rh may shift from labile to recalcitrant [58]. However, in our study, the soil microbial biomass and community structure in the CL plantation were not influenced by SAR. In addition, our results showed that SAR had no

effect on enzyme activity and soil microorganisms in the MM plantation (Table 4), which revealed that the microbial activities were not limited by N and P nutrients and by substrate availability under the SAR treatment. Therefore, the increased Rh in the MM plantation could not be explained by the nutrient availability of soil microorganisms. We speculate that microbial carbon use efficiency was reduced by the input of S from acid rain, resulting in the increase in Rh in the MM plantation [59,60]. However, the microbial carbon use efficiency was not measured in the present study, and should be verified in future research.

Table 4. Variations in soil enzyme activities under different types of acid rain in *Cunninghamia lanceolata* (Lamb.) Hook. (CL) and *Michelia macclurei* Dandy (MM) plantations. Data are the mean ± standard error (n = 4). Means with different letters in common are significantly different ($p < 0.05$). The absence of letters indicates no significant differences among acid rain treatments.

Plantation		Treatment	BG ($\mu mol \cdot h^{-1} \cdot g^{-1}$)	NAG ($\mu mol \cdot h^{-1} \cdot g^{-1}$)	LAP ($\mu mol \cdot h^{-1} \cdot g^{-1}$)	AP ($\mu mol \cdot h^{-1} \cdot g^{-1}$)	PHO ($\mu mol \cdot h^{-1} \cdot g^{-1}$)	URE ($\mu g \cdot g^{-1}$)
CL	Untrenched	CK	811.84 ± 166.00	369.18 ± 177.75	455.36 ± 128.62	2313.62 ± 500.23	3261.57 ± 549.25 b	156.70 ± 20.75
		SAR	848.12 ± 59.14	353.30 ± 105.68	384.58 ± 103.11	1686.90 ± 307.12	4256.55 ± 567.32 ab	220.55 ± 54.31
		MAR	791.95 ± 93.12	333.88 ± 163.71	530.20 ± 275.20	2651.00 ± 741.47	4746.67 ± 760.78 ab	214.35 ± 58.01
		NAR	901.98 ± 114.44	412.97 ± 102.22	254.00 ± 21.13	2121.30 ± 202.19	5646.23 ± 779.41 a	265.37 ± 50.72
	Trenched	CK	874.62 ± 96.21	354.27 ± 149.39	156.26 ± 37.97 b	1944.38 ± 183.65 b	2822.85 ± 264.60 b	136.75 ± 47.89
		SAR	871.60 ± 42.27	357.26 ± 109.92	659.87 ± 144.11 a	3227.34 ± 376.87 a	4937.89 ± 941.18 a	261.50 ± 71.84
		MAR	820.48 ± 76.42	332.52 ± 144.43	341.46 ± 93.88 b	2004.47 ± 216.54 b	3024.80 ± 124.09 b	193.71 ± 25.67
		NAR	808.04 ± 75.18	335.48 ± 150.61	412.28 ± 92.50 ab	2707.51 ± 321.10 ab	4069.72 ± 643.20 ab	215.11 ± 45.96
MM	Untrenched	CK	1119.50 ± 85.49	859.97 ± 43.32 a	612.56 ± 290.74	3899.86 ± 715.74	5708.89 ± 438.11	379.14 ± 66.70
		SAR	951.59 ± 37.41	758.34 ± 40.40 ab	387.46 ± 68.06	3081.71 ± 418.84	4564.59 ± 491.18	335.07 ± 76.02
		MAR	918.49 ± 61.27	753.55 ± 57.73 ab	425.14 ± 52.94	4215.41 ± 825.04	6180.58 ± 1246.20	349.31 ± 97.69
		NAR	947.19 ± 137.39	688.74 ± 44.45 b	234.21 ± 50.91	2846.32 ± 470.58	4991.83 ± 972.13	221.48 ± 72.89
	Trenched	CK	1255.61 ± 148.54	949.82 ± 71.29	1013.56 ± 131.14 a	3622.70 ± 381.73	3482.39 ± 570.14	319.77 ± 67.85
		SAR	1064.37 ± 286.63	826.51 ± 141.20	708.32 ± 313.91 ab	3948.73 ± 760.23	3697.56 ± 804.21	354.35 ± 77.61
		MAR	1072.72 ± 121.11	781.20 ± 104.45	318.44 ± 153.13 b	3907.05 ± 770.03	3020.00 ± 323.02	286.89 ± 100.31
		NAR	980.64 ± 137.42	748.93 ± 130.62	403.78 ± 88.67 b	3612.26 ± 694.84	3876.82 ± 623.44	255.92 ± 65.81

The fitted Q_{10} values of Rs and their components in the present study ranged from 1.82 to 2.68, and were within the range of reported values in evergreen coniferous forests and evergreen broad-leaved forests (from 1.37 to 3.05) [61]. However, the fitted Q_{10} values were not influenced by the SAR, MAR, or NAR treatments (Figure 5). Previous studies reported a higher temperature sensitivity of Ra compared to Rh due to the rapid growth of fine roots in the high temperature period [26,62], meaning that the Q_{10} value of Rs changes if the ratio of Rh or Ra to Rs is altered by environment conditions. However, in our study, no significant differences in Rh/Rs were found among all the treatments (Table 1), which may be one of the reasons for the absence of change in the Q_{10} of Rs. In addition, variations in soil moisture induced by simulated acid rain could affect the Q_{10} of Rs, because the temperature sensitivity of respiration is dependent on soil moisture [63,64]. In fact, acid rain may be toxic to fine roots, which in turn affects plant water uptake and increases soil moisture [65]. However, we did not find evidence of a reduction in fine root biomass (Figure 6) or increase in soil moisture (Figure 2) in any of the acid rain treatments, indirectly suggesting that the Q_{10} of Rs was not regulated by soil moisture induced by acid rain.

5. Conclusions

In this study, acid rain had no significant effect on Rs in the CL and MM plantations. The negligible response of Rs to simulated acid rain could be mainly attributed to the unchanged soil pH value, not to the compensation of Ra and Rh. The Rh increased significantly due to SAR addition in the MM, but not in the CL, plantation indicating that the response of Rh to SAR varied in different forest ecosystems. Interestingly, MAR and NAR did not significantly affect Rh in either plantation. Moreover, the temperature

sensitivities of Rs and its components were not influenced by different types of acid rain. Our work highlights the specific ecosystem effects of acid rain on Rs and that the transition from SAR to NAR should be considered when assessing soil CO_2 emission into the atmosphere in the context of acid rain pollution.

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