Effects of Simulated Acid Rain on Soil Enzyme Activity and Related Chemical Indexes in Woodlands

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Abstract: In order to explore the effects of different concentrations of acid rain on soil nutrient content and nutrient utilization efficiency, and to provide a basis for an improvement in acidified soil in acid rain regions, a year-long acid rain experiment was conducted in a typical evergreen broad-leaved forest and coniferous and broad-leaved mixed forest in Jinyun Mountain of Chongqing. Four pH treatments (pH 4.5, 4.0, 3.25, and 2.5) were established to simulate acid rain. The results showed that: (1) Acid rain promoted the accumulation of soil nutrients, and the contents of organic carbon (SOC), total nitrogen (TN), and hydrolyzed nitrogen (HN) significantly increased during the experiment ($p < 0.05$). (2) Soil SOC content was significantly positively correlated with acid rain concentration ($p < 0.01$), HN content was negatively correlated with acid rain concentration, and TN and total phosphorus (TP) contents were not significantly correlated with acid rain concentration. (3) The activities of soil sucrase, urease, and acid phosphatase were negatively correlated with acid rain concentration ($p < 0.01$) and the activity of soil cellulase was positively correlated with acid rain concentration ($p < 0.01$). (4) The enzyme activity changed differently, depending on the concentration of acid rain during the study period. (5) According to RDA analysis, soil total nitrogen content and hydrolyzed nitrogen content had significant effects on enzyme activity ($p < 0.05$). Conclusions: Acid rain did not significantly alter the overall soil nutrient content but reduced the available nutrient content and seriously inhibited enzyme activity—most notably, the soil enzymes involved in nutrient utilization efficiency.

Keywords: acid rain; acidified soil; enzyme activity

1. Introduction

Acid rain is the general term for atmospheric precipitation with a pH less than 5.6, and acid rain is the wet form of acid deposition [1]. Studies have shown that acid rain can affect tree growth and reduce the fine root biomass of trees [2,3]. Acid rain not only harms trees [4,5] but also harms forest ecosystems through changing the pH in soil and water sources [6–8]. Acid rain mainly reduces soil pH (i.e., acidifies the soil) or causes soil nutrients to be leached, which increases the activity of heavy metal elements and poisonous plants, thus, negatively affecting forest growth [8,9]. In recent years, with gradual reductions in acid rain, the adverse effects of soil acidification have become the focus of research [10,11].

Acid rain in China is mainly distributed in areas south of the Yangtze River and in southwest inland areas, and during the most serious instances, the acid rain concentration has even exceeded some heavy industrial areas in Europe [12]. Acid rain can enhance the leaching of soil cations, and the higher the acidity, the stronger the leaching of base ions [13]. Indeed, acid rain has been observed to drive obvious increases in the release intensity of heavy metal elements Cd, Cu, and Zn from polluted soil [14]. Similarly, higher concentrations of acid rain have been related to an increased release of Al$^{3+}$ from soil [15].
Other studies have found that acid rain can affect soil elemental cycling by affecting soil respiration [16]. A laboratory simulation showed that acid rain leaching could lead to soil nutrient loss [17]. However, some scholars observed acid rain to increase soil organic carbon content in field experiments. In addition, acid rain increased the stability of the carbon sequestration capacity of surface soil, and this phenomenon was more pronounced under the higher-concentration acid rain treatments [18,19]. High concentrations of acid rain have also been shown to affect the transformation of N and P elements in soil, reduce the content of active components, and inhibit the absorption of nutrients by plants [4,20]. These studies indicate that the mechanisms by which acid rain affects soil in an actual forest ecosystem are highly complex, and that measuring the change in soil nutrient contents does not fully reflect the effect of acid rain on soil.

All the complex biochemical processes in soil involve a variety of soil enzymes. At the same time, soils have the characteristics of high specificity and comprehensiveness. Each chemical reaction in soil is driven by specific soil enzymes [21]. Therefore, soil enzyme activity can be used as an integrated biological activity index of soil quality [22]. It has also been shown that soil enzymes are more susceptible to long-term acid rain than other soil indexes [23]. Therefore, in this study, we used the soil enzyme activity index to analyze changes in soil due to acid rain.

The Chongqing acid rain area is in the center of the second largest acid rain area in China. This acid rain area has the earliest occurrence time each year and experiences the most severe pollution [24]. An estimated 26% of the 6500 km$^2$ of pine forests in Wanzhou District of Chongqing has died and 55% has been seriously damaged. The mortality rate of Masson pine due to acid rain on Nanshan Mountain of Chongqing has reached 45% [25]. Under long-term erosion from acid rain, the average pH of the soil in the Chongqing acid rain area has decreased notably, now ranging between 3.3 and 4.0, which makes it a typical acidic soil [26]. Soil acidification has a serious effect on forest growth [27]. Laboratory simulations have demonstrated that acid rain reduces soil nutrient contents and inhibits soil enzyme activity [28]. However, research on the effects of acid rain on soil nutrient contents and enzyme activity in natural forests is still lacking. Therefore, this study examined the effects of acid rain on forest soil nutrient content, available nutrient content, and nutrient utilization efficiency, using a field-simulated acid rain experiment. This study provides a theoretical basis for the targeted improvement of forest soils under the influence of acid rain.

2. Materials and Methods
2.1. Overview of the Study Area

The study was conducted at Jinyun Mountain Forest Ecological Station (106°17′~106°24′ E, 29°41′~29°52′ N), beside the Wentang Gorge of Jialing River, Beibei District, Chongqing city (Figure 1). It is typical of acid rain areas in China. The total area of the ecological station is 76 km$^2$, with an altitude of 175~951.5 m.

The study area experiences a typical subtropical monsoon humid climate, with an annual rainfall of 1611.8 mm, annual relative humidity of 87%, and average annual temperature of 13.6 °C. The three Gorges reservoir area is rich in soil types, the yellow soil and paddy soil are the main types of jinyun Mountain, and there is less purple soil. Yellow soil occupies the largest area, accounting for about 1382.2 hm$^2$. Paddy soil occupies only about 40.9 hm$^2$. The soil layer in this area is deep and mainly developed from carbonaceous shale and argillaceous sandstone as parent materials. The soil pH is between 3.5 and 4.5, which is acidic soil with weak erosion resistance.

The main forest types were evergreen broad-leaved forest, bamboo forest, coniferous and broad-leaved mixed forest, warm coniferous forest, and evergreen broad-leaved shrub.
Soil sucrase activity was determined by the colorimetric assay with 3,5-dinitrosalicylic acid [30]. First, 5 g air-dried soil samples were placed in a 50 mL triangulated flask, and 10 mL of 1% starch solution was injected. This was followed by the addition of 10 mL of pH 5.6 phosphate-buffer solution and five drops of toluene, shaking, and storage in an incubator. The samples were then cultured at 37 °C for 24 h. After culture, the suspension was then diluted with deionized water to obtain simulated acid rain with pH 4.5, 4.0, 3.25, and 2.5. The simulated rainfall experiment was conducted from October 2016 to October 2017, during which the acid solutions were applied once every two weeks. All plots were treated with acid rain within a single day by means of uniform spraying with watering cans. As such, 8 L of acid rainwater was applied to each square meter, equal to 2 mm rainfall. The annual H+ load input to the natural state quadrat was 0.4292 kmol/ha, and the added H+ input into the pH 4.25, pH 4.0, pH 4.0, and pH 3.25 quadrats were 0.62, 0.11, and 0.04-times of that of natural rainfall, respectively [29]. In January 2017, April 2017, July 2017, and October 2017, a five-point sampling method was used to collect soil samples (0–10 cm) with a ring knife from each sample plot. The soil samples were thoroughly mixed in plastic bags and brought back to the laboratory for analysis. The soil samples collected from each plot were separated into two subsamples. One fresh soil subsample from each plot was sieved (2 mm) and stored at 4 °C and soil enzyme activity was measured within one week. The other soil subsamples were dried (natural air dry), ground, and sieved for the analysis of soil chemical properties. A 0.25 mm screen was used for soil pH, organic carbon, total nitrogen, and total phosphorus, and a 2 mm screen was used for soil for other indicators.

2.3. Index Measurement and Analysis Methods

The activities of the main enzymes involved in the C, N, and P cycles were determined by spectrophotometry [30–33]; these included cellulase, sucrase, urease, and acid phosphatase.

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was filtered. Next, 1 mL of filtrate was poured into a 50 mL volumetric flask. Two mL of 3,5-dinitrosalicylic acid solution was added and heated in a boiling water bath for 5 min; the solution was then moved to the volumetric flask to the running water to cool. After a constant volume of 50 mL was achieved, colorimetry was performed at 508 nm on a spectrophotometer. Glucose solution was used as the standard.

The activity of sucrase (Suc) was represented by the glucose content (mg) in 1 g of soil after 24 h. The formula for determining soil sucrase activity was as follows:

\[
\text{Suc} = a \times V \times n/m \tag{1}
\]

where \( a \) is the concentration of glucose obtained from the standard curve (mg/mL); \( V \) is the volume of the chromatic liquid (50 mL); \( n \) is the separation multiple; and \( m \) is the weight of the drying soil (g).

Soil cellulase activity was determined by a colorimetric assay with 3,5-dinitrosalicylic acid [31]. First, 10 g air-dried soil samples were placed in a 50 mL triangular flask, and 15 mL of toluene solution was injected and left to stand for 15 min after shaking. This was followed by the addition of 5 mL of pH 5.5 acetate buffer solution and 5 mL of 1% carboxymethyl cellulose solution, and storage in an incubator. The samples were then cultured at 37°C for 72 h. After culture, the suspension was filtered. Next, 1 mL of filtrate was poured into a 50 mL volumetric flask. Three mL of 3,5-dinitrosalicylic acid solution was added and heated in a boiling water bath for 5 min; the solution was then moved to the volumetric flask to the running water to cool. After a constant volume of 50 mL was achieved, colorimetry was performed at 540 nm on a spectrophotometer. Glucose solution was used as the standard.

The activity of cellulase (Cel) was represented by the glucose content (mg) in 1 g of soil after 72 h. The formula for determining soil sucrase activity was as follows:

\[
\text{Cel} = a \times V \times n/m \tag{2}
\]

where \( a \) is the concentration of glucose obtained from the standard curve (mg/mL); \( V \) is the volume of the chromatic liquid (50 mL); \( n \) is the separation multiple; and \( m \) is the weight of the drying soil (g).

Soil urease activity was determined by a colorimetric assay using sodium phenate–sodium hypochlorite [32]. First, 5 g air-dried soil samples were weighed in a 50 mL triangular flask, and 1 mL of toluene was added, followed by shaking until the contents were evenly mixed. After 15 min, 10 mL of 10% urea solution and citrate buffer solution was added, followed by shaking and incubation at 37°C for 24 h. After culture, the filtrate was filtered, and 1 mL of filtrate was added into a 50 mL volumetric flask. Next, 4 mL of sodium phenol solution and 3 mL of sodium hypochlorite solution were added and shaken well. After 20 min, the mixture was diluted to 50 mL mark and the spectrophotometer was colorimetric at 578 nm (the blue of indophenol remained stable). Urease activity was calculated by subtracting the absorbance value of the sample from the difference in the absorbance value of the control sample.

The activity of urease (Ure) was represented by the ammonia–nitrogen content (mg) in 1 g of soil after 24 h. The formula for determining soil urease activity was as follows:

\[
\text{Ure} = a \times V \times n/m \tag{3}
\]

where \( a \) is the concentration of ammonium–nitrogen obtained from the standard curve (mg/mL); \( V \) is the volume of the chromatic liquid (50 mL); \( n \) is the separation multiple; and \( m \) is the weight of the drying soil (g).

Soil phosphatase activity was determined by a colorimetric assay with disodium phenyl phosphate [33]. First, 5 g air-dried soil samples were placed in a 200 mL triangulation flask, and 2.5 mL of toluene was added. After shaking for 15 min, 20 mL of 0.5% benzene-disodium phosphate was added. After shaking, the samples were placed in an
incubator and cultured at 37 °C for 24 h. Next, 100 mL of 0.3% aluminum sulfate solution was added to the culture medium and filtered. Three mL of filtrate was then absorbed into 50 mL volumetric bottles, and 5 mL of buffer solution and four drops of chlorodibromo-p-benzoquinone imines reagent were added to each bottle. After color development, the solution was diluted to the scale, and the colorimetric determination was conducted 30 min later. The boric acid buffer was blue and colorimetric at 660 nm on the spectrophotometer.

The activity of phosphatase (Pho) was represented by the phenol content (mg) in 1 g of soil after 24 h. The formula for determining soil phosphatase activity was as follows:

\[
\text{Pho} = a \times V \times \frac{n}{m} \tag{4}
\]

where \(a\) is the concentration of phenol obtained from the standard curve (mg·mL\(^{-1}\)); \(V\) is the volume of the chromatic liquid (50 mL); \(n\) is the separation multiple; and \(m\) is the weight of the drying soil (g).

The pH of the soil was measured by the glass electrode method (soil–water ratio: 1:2.5). Organic carbon was determined by oxidizing with dichromate and titrating with ammonium ferrous sulfate [34]. Total nitrogen was determined by the Kjeldahl method [35]. Total phosphorus was determined after soil was digested with sulfuric acid and perchloric acid [36]. Hydrolyzed nitrogen was determined using the boric acid absorption and hydrochloric acid titration method [37]. The results of soil contents were calculated in terms of dry weight. All summary statistics of the original data were obtained using basic processing in Excel (mean, variance, etc.). SPSS and R were used to examine the effects of acid rain, and both were used to compare acid treatments over time. The activities of the four kinds of enzyme, the concentration of acid, time, and their interactions were included in the two-factor repeated measurements analysis of variance. The Canoco software was used to conduct a redundancy analysis of soil enzyme activity and environmental factors to further explore the impact of environmental factors on enzyme activity. Excel and Origin were used to assist in the mapping process.

### 3. Results

#### 3.1. Influence of Simulated Acid Rain on Soil pH in Two Woodland Areas

The soil pH and main nutrient contents in the evergreen broad-leaved forest and coniferous and broad-leaved mixed forest in January, April, July, and October, under four acid rain concentrations, are shown in Tables 1–4.

### Table 1. Soil chemical properties under different acid rain treatments in January.

| Forest Type                   | Chemical Element Content (mg g\(^{-1}\)) | pH 4.5            | pH 4.0            | pH 3.25           | pH 2.5            |
|-------------------------------|------------------------------------------|-------------------|-------------------|-------------------|-------------------|
|                               |                                           | SOC 16.03 ± 0.27\(d\) 18.02 ± 0.14\(e\) 20.19 ± 0.16\(b\) 21.04 ± 0.13\(a\) | 1.07 ± 0.09\(a\) 1.12 ± 0.10\(b\) 1.05 ± 0.13\(c\) 1.06 ± 0.07\(d\) | 0.56 ± 0.02\(d\) 0.72 ± 0.06\(a\) 0.61 ± 0.05\(b\) 0.59 ± 0.04\(c\) | 0.19 ± 0.00\(a\) 0.19 ± 0.00\(a\) 0.18 ± 0.00\(c\) 0.17 ± 0.00\(c\) | 3.71 ± 0.03\(e\) 3.66 ± 0.01\(e\) 3.53 ± 0.03\(d\) 3.48 ± 0.03\(d\) |
| Evergreen Broad-leaved Forest |                                          | SOC 13.11 ± 0.41\(b\) 15.36 ± 0.29\(a\) 16.02 ± 0.43\(ab\) 18.02 ± 0.21\(a\) | 0.86 ± 0.17\(a\) 0.95 ± 0.21\(a\) 1.08 ± 0.22\(a\) 1.01 ± 0.25\(a\) | 0.39 ± 0.04\(a\) 0.45 ± 0.04\(a\) 0.47 ± 0.02\(a\) 0.38 ± 0.08\(a\) | 0.17 ± 0.00\(a\) 0.17 ± 0.00\(a\) 0.15 ± 0.00\(b\) 0.14 ± 0.00\(c\) | 3.46 ± 0.05\(e\) 3.42 ± 0.02\(e\) 3.31 ± 0.04\(b\) 3.26 ± 0.04\(c\) |
| Mixed Forest                  |                                          |                  |                   |                   |                   |

Lowercase letters represent significant differences in soil nutrient contents under different acid rain treatments (\(p < 0.05\)) (data are mean ± standard error).
### Table 2. Soil chemical properties under different acid rain treatments in April.

| Forest Type                  | Chemical Element Content (mg g⁻¹) | Acid Rain Treatment Concentration | pH 4.5 | pH 4.0 | pH 3.25 | pH 2.5 |
|------------------------------|-----------------------------------|-----------------------------------|--------|--------|---------|--------|
|                              |                                   |                                   | SOC    | TN     | TP      | HN     | Soil pH |
| Evergreen Broad-leaved Forest|                                   |                                   | 16.52 ± 0.31 b | 1.17 ± 0.15 b | 0.48 ± 0.03 b | 0.22 ± 0.01 b | 3.93 ± 0.04 b |
| Mixed Forest                 |                                   |                                   | 19.02 ± 0.05 c | 1.45 ± 0.13 a | 0.57 ± 0.02 b | 0.24 ± 0.03 a | 4.02 ± 0.04 a |
|                              |                                   |                                   | 16.98 ± 0.19 b | 1.26 ± 0.03 b | 0.35 ± 0.01 c | 0.20 ± 0.00 a | 3.69 ± 0.04 a |

Lowercase letters represent significant differences in soil nutrient contents under different acid rain treatments (p < 0.05) (data are mean ± standard error).

### Table 3. Soil chemical properties under different acid rain treatments in July.

| Forest Type                  | Chemical Element Content (mg g⁻¹) | Acid Rain Treatment Concentration | pH 4.5 | pH 4.0 | pH 3.25 | pH 2.5 |
|------------------------------|-----------------------------------|-----------------------------------|--------|--------|---------|--------|
|                              |                                   |                                   | SOC    | TN     | TP      | HN     | Soil pH |
| Evergreen Broad-leaved Forest|                                   |                                   | 19.03 ± 0.22 c | 1.36 ± 0.19 a | 0.51 ± 0.01 b | 0.22 ± 2.06 a | 3.81 ± 0.22 b |
| Mixed Forest                 |                                   |                                   | 17.13 ± 0.59 b | 1.18 ± 0.04 c | 0.45 ± 0.02 a | 0.31 ± 0.00 b | 3.61 ± 0.22 b |
|                              |                                   |                                   | 25.27 ± 0.15 a | 1.49 ± 0.05 a | 0.40 ± 0.02 b | 0.16 ± 0.00 c | 3.47 ± 0.03 c |

Lowercase letters represent significant differences in soil nutrient contents under different acid rain treatments (p < 0.05) (data are mean ± standard error).

### Table 4. Soil chemical properties under different acid rain treatments in October.

| Forest Type                  | Chemical Element Content (mg g⁻¹) | Acid Rain Treatment Concentration | pH 4.5 | pH 4.0 | pH 3.25 | pH 2.5 |
|------------------------------|-----------------------------------|-----------------------------------|--------|--------|---------|--------|
|                              |                                   |                                   | SOC    | TN     | TP      | HN     | Soil pH |
| Evergreen Broad-leaved Forest|                                   |                                   | 23.03 ± 0.16 d | 2.16 ± 0.16 e | 0.56 ± 0.09 a | 0.23 ± 0.00 b | 3.65 ± 0.04 b |
| Mixed Forest                 |                                   |                                   | 25.16 ± 0.16 f | 1.74 ± 0.04 b | 0.63 ± 0.02 b | 0.22 ± 0.01 b | 3.62 ± 0.02 b |
|                              |                                   |                                   | 27.31 ± 0.11 b | 1.65 ± 0.04 b | 0.63 ± 0.02 a | 0.22 ± 0.01 b | 3.52 ± 1.05 a |

Lowercase letters represent significant differences in soil nutrient contents under different acid rain treatments (p < 0.05) (data are mean ± standard error).

After the simulated acid rain treatment, the soil pH in the evergreen broad-leaved forest was 3.3–4.0, while that in the conifer broad-leaved mixed forest was slightly lower at 3.1–3.8. The soil pH decreased with increasing simulated acid rain concentration, which demonstrated the effectiveness of the simulated acid rain treatments.

### 3.2. Effects of Simulated Acid Rain on the Soil Main Nutrients

As can be seen from Tables 1–4, the contents of organic carbon and hydrolyzed nitrogen showed significant differences (p < 0.05). The higher the acid rain concentration was, the higher the organic carbon content and the lower the hydrolyzed nitrogen content. There were no significant differences in the contents of total nitrogen and total phosphorus among treatments.
By comparing the soil nutrient contents in different periods from Tables 1–4, it can be found that the contents of organic carbon, total nitrogen, and hydrolyzed nitrogen showed a monotonical increasing trend during the test period, with the highest values appearing in October and the lowest in January. At the end of the test, the contents of organic carbon, total nitrogen, and hydrolyzed nitrogen had increased by 38%, 64%, and 19%, respectively, compared with the initial measurements in January. The total phosphorus content showed no obvious change, and there was no significant change in total phosphorus content during the experiment in any treatment.

These results showed that high concentrations of acid rain increased soil organic carbon content and decreased soil-available nitrogen (hydrolyzed nitrogen) content. Furthermore, soil organic carbon, total nitrogen, and hydrolyzed nitrogen contents accumulated over time.

3.3. Effects of Simulated Acid Rain on Soil Enzyme Activity

The measured values of soil sucrase, cellulase, urease, and acid phosphatase activities in January, April, July, and October, under four acid rain treatments, are shown in Figure 2. As can be seen, under the four acid rain treatments, both urease and acid phosphatase activity decreased with increasing acid concentration, and the change with concentration became more obvious later in the study period. The sucrase activity in the evergreen broad-leaved forest showed the same general pattern, but under pH 4.0 and pH 3.25 treatments, it was significantly higher than in the other two treatments before July. Cellulase activity increased with increasing acid rain concentration.

The activities of sucrase, urease, and acid phosphatase were higher in the evergreen broad-leaved forest than in the coniferous and broad-leaved mixed forest, while the opposite was true for the activity of cellulose. In evergreen broad-leaved forests and coniferous broad-leaved mixed forests, the relative ranges of sucrase activity were 36% and 63%, the relative ranges of cellulase activity were 42% and 54%, the relative ranges of urease activity were 31% and 50%, and the relative ranges of acid phosphatase activity were 15% and 20%, respectively. It can be seen that acid rain had larger impacts on the activities of sucrase, cellulose, and urease than the activity of acid phosphatase. Meanwhile, the impact of acid rain on the activities of enzymes in the coniferous broad-leaved mixed forest were stronger than in the evergreen broad-leaved forest.

3.4. Soil Enzyme Activities during the Study Period

According to two-factor repeated measure ANOVA (Tables 5–8), acid rain treatment, time, and their interaction all had significant effects on soil enzyme activity ($p < 0.01$). The comparison of the soil enzyme activities in different periods in Figure 2 reveals that the activities of sucrase, cellulase, and urease all showed overall increasing trends during the study period, with higher activities at the end of the experiment than their initial values. In contrast, the changes in acid phosphatase activity were not obvious, and its activity at the end of the experiment was similar to its initial value.
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Table 5. ANOVA of soil sucrase activity.

| Forest Type                  | Source | SS    | df  | MS    | F-Ratio | $p$   |
|------------------------------|--------|-------|-----|-------|---------|-------|
| Evergreen Broad-leaved Forest| SAR    | 694.693 | 3.00 | 231.564 | 173.207 | <0.01 |
|                              | Times  | 3181.478 | 3.00 | 1060.493 | 793.233 | <0.01 |
|                              | Times × SAR | 1406.392 | 9.00 | 156.266  | 116.885 | <0.01 |
|                              | Error  | 64.172  | 48.00 | 1.337   |         |       |
| Mixed Forest                 | SAR    | 1688.466 | 3.00 | 562.822  | 463.743 | <0.01 |
|                              | Times  | 1505.531 | 3.00 | 501.844  | 413.499 | <0.01 |
|                              | Times × SAR | 1813.012 | 9.00 | 201.446  | 165.983 | <0.01 |
|                              | Error  | 58.255  | 48.00 | 1.214   |         |       |

“Times” represents time differences and “SAR” represents simulated acid rain treatments.

Figure 2. Soil enzyme activities in evergreen broad-leaved forest and coniferous broad-leaved mixed forest under different concentrations of acid rain (different lowercase letters indicate significant differences among soil enzyme activities under different acid rain treatments in the same period ($p < 0.05$)).
Table 6. ANOVA of soil cellulase activity.

| Forest type               | Source     | SS    | df  | MS    | F-Ratio   | p    |
|---------------------------|------------|-------|-----|-------|-----------|------|
| Evergreen Broad-leaved Forest | SAR       | 45.290 | 3.00| 15.097| 62.857    | <0.01|
|                           | Times      | 250.647| 3.00| 83.549| 347.864   | <0.01|
|                           | Times × SAR| 90.237 | 9.00| 10.026| 41.746    | <0.01|
|                           | Error      | 11.528 | 48.00| 0.240 |           |      |
| Mixed Forest              | SAR        | 34.165 | 3.00| 11.388| 475.412   | <0.01|
|                           | Times      | 19.653 | 3.00| 6.551 | 273.479   | <0.01|
|                           | Times × SAR| 4.372  | 9.00| 0.486 | 20.277    | <0.01|
|                           | Error      | 1.150  | 48.00| 0.024 |           |      |

“Times” represents time differences and “SAR” represents simulated acid rain treatments.

Table 7. ANOVA of soil urease activity.

| Forest type               | Source     | SS    | df  | MS    | F-Ratio  | p    |
|---------------------------|------------|-------|-----|-------|----------|------|
| Evergreen Broad-leaved Forest | SAR       | 0.144 | 3.00| 0.048| 223.286 | <0.01|
|                           | Times      | 0.407 | 3.00| 0.136| 629.647 | <0.01|
|                           | Times × SAR| 0.141 | 9.00| 0.016| 72.988  | <0.01|
|                           | Error      | 0.010 | 48.00| 0.000|         |      |
| Mixed Forest              | SAR        | 0.194 | 3.00| 0.065| 315.100 | <0.01|
|                           | Times      | 0.581 | 3.00| 0.194| 946.308 | <0.01|
|                           | Times × SAR| 0.027 | 9.00| 0.003| 14.392  | <0.01|
|                           | Error      | 0.010 | 48.00| 0.000|         |      |

“Times” represents time difference and “SAR” represents simulated acid rain treatment.

Table 8. ANOVA of soil acid phosphatase activity.

| Forest type               | Source     | SS    | df  | MS    | F-Ratio  | p    |
|---------------------------|------------|-------|-----|-------|----------|------|
| Evergreen Broad-leaved Forest | SAR       | 0.094 | 3.00| 0.031| 170.315 | <0.01|
|                           | Times      | 0.105 | 3.00| 0.035| 190.243 | <0.01|
|                           | Times × SAR| 0.127 | 9.00| 0.014| 76.728  | <0.01|
|                           | Error      | 0.009 | 48.00| 0.000|         |      |
| Mixed Forest              | SAR        | 0.122 | 3.00| 0.041| 165.524 | <0.01|
|                           | Times      | 0.044 | 3.00| 0.015| 60.103  | <0.01|
|                           | Times × SAR| 0.040 | 9.00| 0.004| 18.252  | <0.01|
|                           | Error      | 0.012 | 48.00| 0.000|         |      |

“Times” represents time differences and “SAR” represents simulated acid rain treatments.

In the broad-leaved forest, sucrase activity increased by 122%, 100%, and 68% from April to July under the pH 4.5, pH 4.0, and pH 3.25 simulated acid rain treatments, respectively. However, in the mixed forest, sucrase activity increased by 81%, 97%, and 112% under those same three acid rain treatments, respectively. In both forests, sucrase activity continued to increase from July to October in the pH 4.5 acid rain treatment, while in the pH 4.0 and pH 3.25 acid rain treatments, sucrase activity significantly decreased. Overall, cellulase activity showed an increasing trend during the test period. However, from April to July, cellulase activity in the evergreen broad-leaved forest under the pH 4.5 and pH 4.0 treatments and in the conifer broad-leaved forest under the pH 4.0 treatment decreased by 23%, 39%, and 10%, respectively. From July to October, under the different acid rain treatments, urease activity in the evergreen broad-leaved forest increased by 34%, 21%, 34%, and 88% and by 66%, 95%, 62%, and 135% in the coniferous broad-leaved forest, respectively. Acid phosphatase activity fluctuated during the test period and exhibited no obvious increasing or decreasing trend. The mean monthly changes in acid phosphatase activity were only 13% and 9% in the evergreen broad-leaved forest and coniferous broad-leaved mixed forest, respectively.
3.5. RDA Analysis of the Relationship between Soil Enzyme Activity and Soil Physical and Chemical Properties

In order to comprehensively explore the mechanisms underlying the change in soil enzyme activity in the study area, an RDA gradient analysis was conducted by combining the respective enzyme activity data and environmental factor data from the two woodlands (Figure 3a,b). The RDA analysis showed this.

![RDA analysis of soil enzyme activities and environmental factors in the evergreen broad-leaved forest.](b)

**Figure 3.** (a) RDA analysis of soil enzyme activities and environmental factors in the evergreen broad-leaved forest. (b) RDA analysis of soil enzyme activities and environmental factors in the coniferous and broad-leaved mixed forest (pH: soil pH, SOC: soil organic carbon content, TN: soil total nitrogen content, TP: soil total phosphorus content, NH: soil hydrolyzed nitrogen content).
In the evergreen broad-leaved forest, the first sequence axis and the second sequence axis of the RDA explained 54% and 17% of the variability, respectively, and they combined to explain 72%, which was statistically significant. Total nitrogen content and hydrolyzed nitrogen content both had significant effects on soil enzyme activity ($p < 0.01$), and separately, they explained 44% and 36% of the variability, respectively. The activities of sucrase and acid phosphatase were strongly correlated with pH and HN. Cellulase activity was strongly correlated with TN and SOC. Urease activity was strongly correlated with TN and HN.

In the coniferous and broadleaf mixed forest, the first sequence axis and the second sequence axis explained 47% and 25% of the variability, respectively, and their cumulative explanatory power was 72%, which was statistically significant. The hydrolyzed nitrogen content, soil pH, soil organic carbon content, and total nitrogen content all had significant effects on soil enzyme activity ($p < 0.01$), explaining 43%, 35%, 31%, and 23% of the variation, respectively. Sucrase activity was strongly correlated with pH and HN. Cellulase activity was strongly correlated with SOC. Urease activity was strongly correlated with pH and HN. Acid phosphatase activity was strongly correlated with HN and pH.

4. Discussion
4.1. Variation Characteristics of Soil Enzyme Activity

Sucrose is catalyzed by the enzyme sucrase. Hydrolyzed to glucose and D-D-fructose, two kinds of reducing sugar, the hydrolysis of cellulose to glucose monomers process is, in the cellulase enzyme, a complex enzyme system under the action of different enzymes; urease can in enzymatic soil urea hydrolyze into ammonia, the soil phosphatase is a kind of catalytic organophosphorus compound ore, which leads to an inorganic phosphorus enzyme. Therefore, we measured soil cellulase, sucrase, urease, and acid phosphatase, respectively, to reflect the conversion or recycling efficiency of soil main nutrient elements C, N, and P.

Sucrase activity was higher in the pH 4.0 and pH 3.25 acid rain treatments than the other acid rain treatments in the early and middle stages of the experiment, but its activity decreased with increasing acid rain concentration in the later stage. This indicated that sucrase activity is promoted by acid rain in the short term but inhibited in the long term under the lower-concentration acid rain treatment, while it is continuously inhibited under higher-concentration acid rain treatments. This was consistent with the observations of Wang et al. on sucrase activity in acidic red soil [38]. Long-term acid deposition inhibits the enzymatic activity of organic carbon decomposition [39,40]. Furthermore, the increase in soil temperature from April to July may also have promoted the increase in soil sucrase activity [41].

Cellulase activity increased with acid rain concentration. This was inconsistent with the observations of He et al., wherein soil cellulase activity around Cyclobalanopsis seedlings was inhibited by increasing the acid rain concentration [42]. This may have been because there was a lot of litter in our study area, and soil acidification leads to an increase in macromolecular organic matter that does not decompose well, including cellulose. Therefore, microorganisms that can decompose cellulose in soil litter flourished and more cellulase was produced. Although acid rain inhibited microbial activity, that inhibition did not completely offset the promotion of cellulase [43,44].

Urease activity decreased with increasing acid rain concentration because acid rain can inhibit the ammoniating microbial activity in soil and reduce the mineralization rate of soil organic nitrogen, which also reduces soil urease activity [45]. Pinghua Zhang et al. found that urease activity increased gradually with decreasing soil pH but decreased sharply after dropping to 4.3 [46]. Here, the soil pH was lower than 4.3 throughout the study area, so it had already been acidified by acid rain. During this long-term study, the urease activities in the middle period (April–July) and the late period (July–October) were significantly different. In addition to acid rain, urease activity was effectively inhibited by rainfall [47]. The study area was located in a forest region of Southwest China that
experiences abundant rainfall in June and July, which continuously inhibits soil urease activity [48,49]. Furthermore, the continuous input of leaf litter in autumn stimulates the activity of microorganisms and enhances nitrification processes in nitrogen cycling, such as ammonification, leading to increased urease activity [50,51].

The activity of acid phosphatase decreased with increasing acid rain concentration in our study, which was consistent with the results of previous studies [52]. However, there was no significant difference in enzyme activity under different acid rain concentrations. Soil pH is a key factor affecting acid phosphatase activity [53,54]. Soil pH = 5.5 has been shown to be the optimal pH for acid phosphatase [55], but the pH of the soil at the test site on Jinyun Mountain, Chongqing, ranged between 3.26 and 4.03 [26]. At the same time, the soil showed severe phosphorus deficiencies, which meant that acid phosphatase activity was severely inhibited.

The activities of soil sucrase, urease, and acid phosphatase in the evergreen broad-leaved forest were higher than those in the coniferous and broad-leaved mixed forest, while the activity of soil cellulose was lower. This was consistent with previous studies [56–58]. This is because the soil nutrient content in an evergreen broad-leaved forest is richer, which is conducive to microbial activities [59], while the coniferous species, such as the Masson pine in the coniferous and broad-leaved mixed forest, contain more lignin and cellulose, which microbes find difficult to degrade [60]. Therefore, the activities of sucrase, urease, and acid phosphatase were relatively high. Cellulose-degrading microorganisms were more active in the conifer and broadleaf mixed forests, leading to higher cellulase activity [47]. This phenomenon also indicated that the conversion and recycling capacity of soil nutrients in evergreen broad-leaved forests is higher than in coniferous and broad-leaved mixed forests [61]. Plant roots and soil microorganisms continuously release soil enzymes, which resulted in an increasing trend of soil enzyme activity during the test period.

4.2. Relationships between Soil Nutrients and the Associated Enzymes

In this study, the concentrations of soil main nutrients and related enzyme activities were measured. In terms of soil nutrient content, we measured soil organic carbon content, total nitrogen content, and total phosphorus content, respectively. Meanwhile, we measured the nitrogen that can be absorbed by crops during the growth period, namely alkali-hydrolyzed nitrogen, to reflect the changes in main nutrient elements C, N, P, and nitrogen concentration in soil.

The soil pH in the evergreen broad-leaved forest was higher than in the evergreen broad-leaved forest because it contained more organic carbon, which can effectively buffer the acidification of soil by acid rain [62,63]. Furthermore, the decomposition of litter in the coniferous and broad-leaved mixed forest will produce more acidic substances, which will exacerbate the acidification of the soil. The higher the concentration of the simulated acid rain, the higher the soil organic carbon content and the lower the hydrolyzed nitrogen content. Under normal circumstances, soil acidification caused by acid rain will reduce soil organic matter content in three ways: leaching of soil organic matter [64,65], changing the structure of clay minerals in soil [66], and promoting the redox of organic matter [67,68]; the more acidic the acid rain, the more pronounced the inhibition effect will be on organic matter. However, in this test, organic carbon content increased with the concentration of the acid rain because the sample area was rich in litter content, which becomes soil organic matter as it is decomposed [69–72]. At the same time, highly concentrated acid rain inhibited invertase activity, which caused a reduction in nutrient consumption within the soil [73]. Finally, the higher the acid rain concentration, the higher the soil organic carbon content. Meanwhile, the higher the acid rain concentration, the stronger the inhibition of enzyme activities related to the decomposition of nitrogenous substances (e.g., urease, etc.) and microbial activities [23]. Therefore, the content of hydrolyzed nitrogen decreased with increasing acid rain concentration. As shown in Section 3.2, the higher the concentration of acid rain, the stronger the inhibitory effect on soil sucrase and urease activities, which
also supported this observation. There were no significant differences among the acid rain treatments in terms of total nitrogen content in this experiment. This was because, while the addition of acid rain destroyed the nitrogen transformation and cycling processes by inhibiting urease activity, the input of NO3- in the acid rain and the change in temperature conditions both affected the nitrogen contents, resulting in no significant change in total nitrogen contents under different acid rain treatments [73,74]. Acid rain concentration also had no significant effect on total phosphorus content, which was related to the decrease in acid phosphatase activity [75].

Acid rain had an accumulative effect on soil nutrient contents over time in the two forestlands, and the average soil nutrient contents in the later stage of the experiment were higher than the initial values. Acid rain changed the decomposition rate of litter, mainly by affecting the activities of enzymes related to decomposition, thereby affecting the soil nutrient content [76]. Wang’s study showed that acid rain inhibited the decomposition of litter, and the inhibitory effect was greater in the coniferous forest than the broadleaved forest [77]. Although acid rain can inhibit the decomposition of litter, it cannot completely prevent increases in soil nutrient content caused by the decomposition of litter. Meanwhile, the inhibition of various enzymes by acid rain will inhibit the consumption rates of nutrients in soil [55], which will ultimately lead to the accumulation of soil nutrients under the action of acid rain. The increases in organic matter and total nitrogen were significantly larger than those of hydrolyzed nitrogen, and the increases in organic matter and total nitrogen were greater at higher acid rain concentrations, which demonstrated that higher-concentration acid rain will have a greater effect on soil nutrient conversion efficiency. In addition, the nutrient contents in the evergreen broad-leaved forest were higher than those in the coniferous and broad-leaved mixed forest. Compared with the coniferous and broad-leaved mixed forest, litter decomposition in the evergreen broad-leaved forest was faster, microbial activity was stronger, and soil structure, water storage, and fertilizer retention capacity were better [78]. Therefore, the nutrient content in the evergreen broad-leaved forest was higher than that in the coniferous and broad-leaved mixed forest.

Acid rain not only changes soil nutrient content by affecting soil enzyme activity, it also affects soil enzyme activity by affecting soil nutrient content. RDA analysis showed that the contents of total nitrogen and hydrolyzed nitrogen in the simulated experiment had large impacts on soil enzyme activity, while the pH, total phosphorus, and organic carbon in the soil had no significant impact on soil enzyme activity. This indicated that soil pH in this acid rain area was already low and had a serious phosphorus deficiency due to the long-term acid rain erosion. Therefore, it was difficult for the external environmental disturbance (i.e., our treatments) to further affect pH and phosphorus.

In summary, acid rain reduced soil nutrient conversion efficiency by inhibiting soil enzyme activities, resulting in the accumulation of soil nutrients. Simultaneously, acid rain reduced the nutrient availability in the soil, which was not conducive to the absorption and utilization of nutrients by plants and microorganisms and, thus, would adversely affect the growth of trees.

5. Conclusions

During the test period, the acid rain treatments increased soil organic carbon and total nitrogen contents, which facilitated a reduction in the nitrogen (hydrolyzed nitrogen) available to be absorbed by plants. Meanwhile, acid rain inhibited the activities of soil sucrase, urease, and acid phosphatase, and promoted the activity of soil cellulase. The higher the concentration of acid rain, the more damaging it is to the soil. The acid rain affected soil enzyme activity, mainly by changing soil pH, organic carbon content, total nitrogen content, and hydrolyzed nitrogen content. In addition, soil temperature also had a strong influence on enzyme activity. Furthermore, rather than supplementing soil with nutrients, remediation projects in acid rain areas should focus on the regulation of soil pH and improvement in enzyme activity.
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