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Effects of Acid Rain Stress on the Physiological and Biochemical Characteristics of Three Plant Species

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Abstract: The physiological and biochemical indicators of plants reflect the plant's adaptation to environmental changes and provide information for the planting and management of acid-resistant tree species. To analyze the responses of typical tree species to recent changes in acid rain conditions in Jinyun Mountain, Chongqing, we focused on three representative tree species in the Jinyun Mountain area of Chongqing: *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora*. A mixed acid rain experiment with five gradients of natural rainfall (NR) and pH values of 7.0, 4.5, 3.5, and 2.5 was conducted in May 2021. The changes in physiological and biochemical indicators (net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, light saturation point, light compensation point, apparent quantum efficiency, dark respiration rate, soluble sugar, starch, soluble protein, proline, malondialdehyde, and antioxidant enzyme activity) were determined. The results show the following: 1. Compared with other treatments, NR and slightly acidic rain increased the relative chlorophyll content in plant seedlings. 2. The synthesis of soluble sugars, starches, and soluble proteins was inhibited to different degrees in the three species under acid rain stress at pH $\leq$ 3.5. 3. The enzyme activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) initially increased and then decreased with an increase in acidity. 4. Acid rain treatments with pH $\leq$ 4.5 reduced the net photosynthetic rate (Pn) of plants; the higher the acidity, the lower the Pn. Conclusion: A comprehensive comparison of the indicators revealed that NR and mild acid rain enhanced the plant seedlings' physiological and biochemical characteristics. A pH of 3.5 was the threshold where acid rain had an adverse effect on *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora*. The high indicator values for NR indicate that these tree species have adapted to current conditions in the Jinyun Mountain area of Chongqing. This study provides new information for selecting tree species adapted to the acid rain environment in Jinyun Mountain, Chongqing.

Keywords: acid rain; photosynthetic characteristics; antioxidant enzyme activity

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1. Introduction

Atmospheric acid deposition has become a significant problem affecting the environment in China since the 1980s due to fossil fuel combustion and vehicle emissions related to increasing industrialization and urbanization [1,2]. China has promulgated and implemented several measures in recent years to reduce air pollution [3]. According to the bulletin on the status of the Chinese environment, the acid rain conditions have improved. The pH of acid rain has increased, and the frequency of acid rain has decreased annually [4]. However, long-term acid rain still poses an environmental hazard. The pH of forest soils in China has decreased from 5.64 to 5.08 from 1980 to 2019, and long-term acid rain has caused substantial soil acidification, adversely affecting forest ecosystems [5].

Chongqing is substantially affected by acid rain [6], and our study area, Jinyun Mountain in Chongqing, has significant soil acidification. According to the Acid Deposition

Monitoring Network in East Asia (EAET), the pH of the acid rain in Jinyun Mountain in 2021 was 5.05, and that of the surface soil was 4.2 [7]. In the past, acid rain occurred primarily due to sulfur emissions from coal burning [8]. However, SO_2 emissions in the air have decreased significantly in recent years due to improvements in the energy structure. In contrast, NO_X emissions from vehicle exhausts have increased significantly, affecting the sulfur-to-nitrogen ratio in acid rain and causing a shift from sulfuric acid rain to mixed acid rain [9]. The nitrogen content in the mixed acid rain may alter the growing environment of plants, affecting terrestrial ecosystems.

Plants convert light energy into chemical energy using photosynthesis, a fundamental physiological process required for plant growth and development [10]. The photosynthetic capacity of plants can evaluate the effect of environmental stress on plants [11,12]. Studies have shown that acid rain affects photosynthesis [13], causing morphological damage to plant leaves [14], damaging the waxy structure and epidermal cells of leaves, and leading to stomatal deformation and Mg^{2+} leaching [15–17]. Acid rain also alters the structural integrity and electron transfer efficiency of the photosystem II (PSII) [18], disrupts the chloroplast structure [19], and reduces the chlorophyll content [20], ultimately reducing the net photosynthetic rate (Pn) of plants. However, the effects of acid rain on plants are not always negative. Acid rain may be beneficial to the Pn of leaves because NO_3^- in acid rain provides plant nutrients, increasing the Pn [13,21]. Acid rain stress elevates the number of reactive oxygen species (ROS) and induces cell membrane peroxidation, increasing the malondialdehyde (MDA) content. The activity of antioxidant enzymes (superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)) in plants increases during acid rain stress and affects the plant's ability to resist ROS. However, the activity of antioxidant enzymes decreases with an increase in the acidity of acid rain, reducing the plant's acid rain resistance [22,23]. Therefore, high-acidity acid rain may affect the defense mechanisms of plants. The contents of proline, soluble sugars, starch, and soluble protein reflect the degree of plant stress because these substances provide stress resistance under adverse conditions, and their stress resistance is closely related to the acidity of acid rain [24]. Acid rain can also indirectly affect the growth and development of plants by affecting the soil. Some scholars found that acid rain accelerated the loss of salt-based ions from the soil and intensified the precipitation of Al^{3+} and H^+ from the soil [25], affecting plant growth and development. Others have observed that acid rain increased soil nutrients and improved plant growth [26]. In summary, the acidity of acid rain is critical in the plant response to acid rain; therefore, it is necessary to investigate the response of plant physiological and biochemical characteristics to acid rain with different acidity levels.

Some scholars have analyzed the response of forest ecosystems to acid rain in the Jinyun Mountain area, but these studies had some shortcomings, such as the focus on a single plant species and the difficulty of simulating the field environment in laboratory experiments [25,27]. Therefore, we investigate the response of three typical tree species (*Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora*) under acid rain stress. The experimental site was Jinyun Mountain in Chongqing. According to the acid rain characteristics of Jinyun Mountain, we simulated acid rain with different pH values to evaluate the physiological and biochemical characteristics of plants and other indicators. We hypothesized the following: (1) the physiological and biochemical characteristics of plant seedlings differ for different acidity levels of acid rain, and acidic rain slightly enhances these characteristics of plant seedlings; (2) acid rain affects the photosynthesis of plants by influencing their biochemical mechanisms.

2. Materials and Methods

2.1. Study Area

The study area is located at the Jinyun Mountain Forest Ecosystem Research Station (106°23′18″ E, 29°50′11″ N) (Figure 1) on the western bank of Wentang Gorge, one of three small gorges of the Jialing River in the Beibei District, Chongqing. The area has a typical subtropical humid monsoon climate, with an average annual rainfall of 1611.8 mm, average annual evaporation of 777.1 mm, an average annual temperature of 13.6 °C, and annual average relative humidity of 87%. The regional forest cover is 96.6%, and evergreen broad-leaved forest, warm coniferous forest, coniferous and broad-leaved mixed forest, deciduous forest, evergreen broad-leaved scrub, and bamboo forest are the main vegetation types. The dominant species include *Pinus massoniana*, *Cinnamomum camphora*, *Cunninghamia lanceolata*, *Symplocos setchuensis Brand*, *Gordonia acuminata*, and *Phyllostachys edulis*. The soil is rich, and the dominant soil types are Cambisols, Luvisols and Anthrosols [28]. The Cambisols have the widest distribution (13.8 km^2). The pH range of the soil is 4.5–5.5 in Jinyun Mountain, indicating acidic soil.

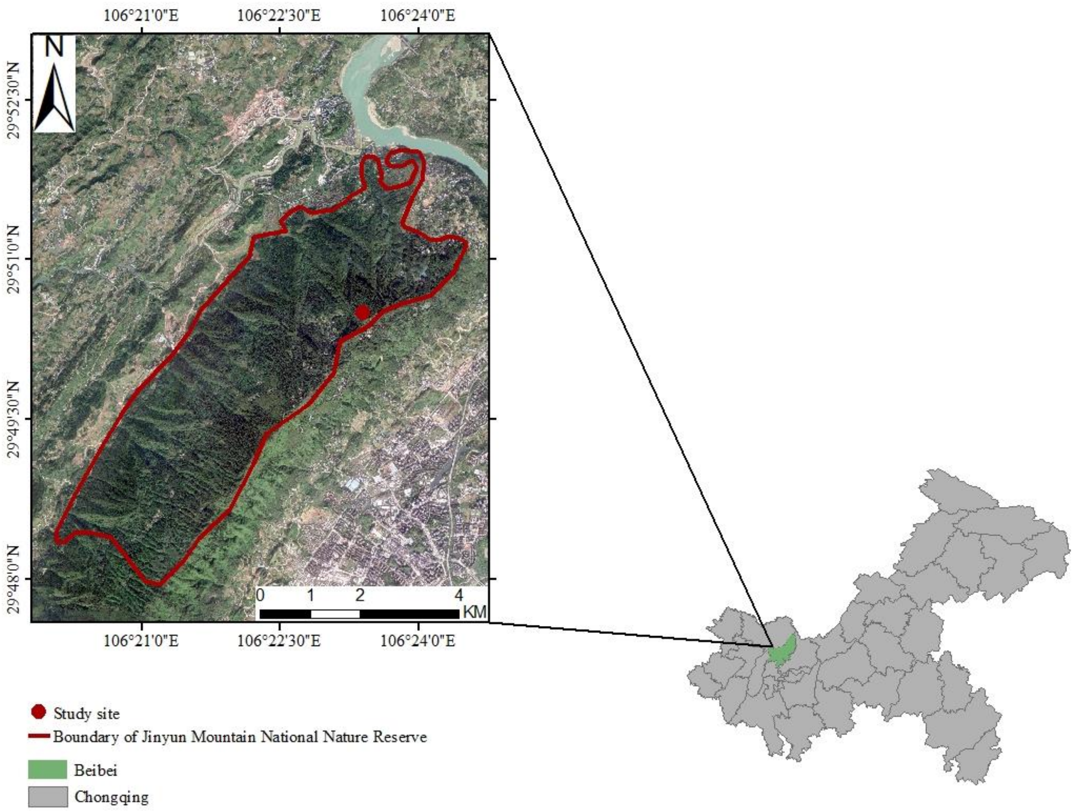

Figure 1. Geographical location of the study area.

2.2. Experimental Materials

The experiment simulated the natural environment of Jinyun Mountain, including light, temperature, and humidity. A temporary rain shelter consisting of stainless steel with a sliding roof was built at the experimental site. During the experiment, in case of rain, the pH 4.5, 3.5, 2.5 and 7.0 (CK) treatments were placed in a rain shelter, using the shelter to shield the natural rainfall, and the ventilation around the shelter was unobstructed to ensure that the plant growth could receive environmental conditions other than natural rainfall, while natural rainfall (NR) was placed in the open environment to receive the natural rainfall drenching. The plant species selected for the experiment were the dominant tree species in the Jinyun Mountain area, the conifer *Pinus massoniana*, the broad-leaved tree *Cinnamomum camphora*, and the bamboo *Phyllostachys edulis*. Their basic information is listed in Table 1.

On 14 March 2021, one-year-old embryonic seedlings of the same period in good growing conditions were transplanted into plastic pots with a height of 30 cm, 28 cm in base diameter, and 30 cm in caliber. The soil in the pots was Cambisols (pH = 5.37 ± 0.19) obtained from Jinyun Mountain, Chongqing, with the same weight and firmness.

Table 1. Basic situation of seedlings.

Latin Name	Average Plant Height (cm)	Average Ground Diameter (mm)	Source
Pinus massoniana	21.83 ± 4.87	4.40 ± 0.91	Chongqing
Phyllostachys edulis	32.29 ± 14.91	2.13 ± 0.63	Chongqing
Cinnamomum camphora	33.99 ± 3.87	4.21 ± 1.21	Chongqing

2.3. Experimental Design

2.3.1. Acid Rain Solutions

According to the ion components in the rain at the Jinyun Mountain site, the ratio of SO_4^{2-} to NO_3^- was 0.75 in 2019 [25], indicating mixed acid rain. Therefore, a mixed acid rain experiment was conducted. The mother liquor was prepared according to the molar ratio of H_2SO_4:HNO_3 = 1:1 and diluted with distilled water to obtain acid rain solutions with pH values of 4.5, 3.5, and 2.5. Distilled water was used as the control group (CK), and natural rainfall (NR) treatments were used (16 rainfall events were implemented during the experiment to obtain pH values ranging from 5.42 to 5.83), which comprised 5 treatments. A total of 10 replicates were performed for each treatment, and 1 pot per replicate was used for a total of 150 pots of seedlings for the 3 tree species. Each treatment area of the seedlings covered 4.16 m^2. The details of the experimental design are shown in Figure 2.

Figure 2. Experimental setup and simulated acid rain device.

2.3.2. Spray Application

The seedlings were placed in the temporary stainless steel rain shelter for 1.5 months to slow down their growth. The seedlings were watered normally with distilled water, were weeded but received no fertilizer, and their growth state was monitored closely to prevent pests. The acid rain simulation experiment began in May when the seedlings were well established. Table 2 lists the spraying volume, which was based on the multi-year monthly surface rainfall and patterns in Jinyun Mountain [25]. The seedlings were sprayed once a week for four months. The seedlings in the NR treatment received irrigation from natural rainfall. The average monthly rainfall at the test site was 193.35 mm from May to 1 September 2021 (source: National Positioning Observation and Research Station of Forest Ecosystems in the Three Gorges Reservoir Area, Jinyun Mountain, Chongqing). The spray was applied to the top of the plants to distinguish the degree of stress resulting from different acidities and simulate the natural rainfall pattern. The sprayer consisted of acid-resistant materials, the nozzle was made of stainless steel, the water pipe was plastic, and the pump was a 45 W micro diaphragm pump. The spraying method was consistent throughout the test.

Table 2. Monthly rainfall spraying.

Month	May	June	July	August
Spraying volume (mm)	175	206	233	179

Spraying volume: determined by multi-year monthly surface rainfall and patterns in Jinyun Mountain. Spray the plants monthly with acid rain according to the spraying volume in this table.

2.4. Sample Collection and Indices

2.4.1. Determination of Photosynthetic Physiological Indicators

A windless and sunny morning (8:00–11:00 am) on 3 September 2021 was selected for measuring the photosynthetic parameters using a Li-6400 portable photosynthesis meter with a red and blue light source (Li-COR). The effective photosynthetic radiation intensity was 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 20, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 13 gradients based on the results of a pre-experiment and the solar radiation data from the Jinyun Mountain Nature Reserve in Beibei District, Chongqing, China. The leaf chamber temperature was 25 °C, the CO_2 concentration was 400 $\mu mol \cdot mol^{-1}$, the gas flow rate was 500 $\mu mol \cdot s^{-1}$, and the relative humidity was 50%. Measurements were made from 8:00 to 11:00 a.m. on three healthy plants in each treatment. Three similar and mature, healthy leaves on the main tips were selected from each plant, and 5–7 needles were selected each time and placed side by side in the leaf chamber. We obtained three measurements and used the average as the result. During each measurement, the physiological indices were measured after 2 min of adaptation at each photosynthetically active radiation (PAR) intensity. The indices included the Pn, stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr).

The corrected right-angle hyperbolic model proposed by Ye (2007) (Equation (1)) was used for curve fitting of the optical response [29]. The maximum Pn (Pmax), light saturation point (LSP), light compensation point (LCP), apparent quantum efficiency (AQY), and dark respiration rate (Rd) were calculated using Equations (2) and (3).

The model is expressed as follows:

$$\mathrm{Pn}(\mathrm{I}) = \alpha \frac{1-\beta \mathrm{I}}{1+\gamma \mathrm{I}} \mathrm{I} - \mathrm{Rd} \quad (1)$$

where β is the correction factor (light suppression term), α is the initial slope of the photoresponse curve, i.e., the slope at point I = 0. The factor γ (light saturation term) is equal to the ratio of the initial slope of the photoresponse curve to Pmax, i.e., $\gamma = \alpha/\mathrm{Pmax}$, where I is the effective photosynthetic radiation, Pn is the net photosynthetic rate, and Rd is the dark respiration rate.

The Pmax was calculated as follows:

$$\mathrm{Pmax} = \alpha\left(\frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\gamma}\right)^2 - \mathrm{Rd} \quad (2)$$

The LSP was calculated as follows:

$$\mathrm{LSP} = \frac{\sqrt{\frac{\beta+\gamma}{\beta}} - 1}{\gamma} \quad (3)$$

where LSP is the saturated light intensity of the plant, and Pmax is the maximum net photosynthetic rate corresponding to the saturated light intensity of the plant. The LCP, AQY, and Rd were obtained by model fitting.

2.4.2. Determination of Biochemical Indicators

The relative chlorophyll content of the plant leaves was determined on 1 May and 4 September 2021, using a SPAD-502 chlorophyll meter. The leaves were placed inside the device, avoiding the leaf veins during the measurement. We selected 3–5 needles from the coniferous species and clamped them for the measurement. Three plants were randomly selected for each treatment; three mature leaves on the branch tips were selected from each plant, and the average value was used.

At the end of the acid rain treatment on 6 September, three plants of approximately the same size were randomly selected for each treatment. We used 3–5 mature and fully expanded leaves of each plant and removed the main leaf veins to determine the physiological and biochemical indices. Each measurement was repeated three times. The soluble sugar and starch contents were analyzed by the anthrone colorimetric method [30]. The soluble protein contents were determined by colorimetry using Kemas Brilliant Blue G-250 [31]. The proline content was determined by the acidic ninhydrin method [32], the MDA content was obtained from the thiobarbituric acid method [33], and the POD content was measured by guaiacol. The SOD content was obtained by nitroblue tetrazolium (NBT) photochemical reduction, and the CAT and ascorbate peroxidase (APX) contents were determined by ultraviolet (UV) spectrophotometry [34].

2.5. Data Analysis

SPSS and Excel were used for the statistical analysis of the data. The data in the graphs are the means ± standard deviations ($n = 3$). The significant differences ($p < 0.05$) between the mean values of the indicators for different acid rain gradients were determined by Duncan's test. Origin was used to create the graphs. Canoco software was used for the redundancy analysis (RDA) and principal component analysis (PCA). RDA was used to derive the relationship between the photosynthetic characteristics and the biochemical indicators of the plants, and PCA was used to determine the differences in photosynthetic and biochemical characteristics between different plant species.

3. Results

3.1. Effect of Acid Rain Stress on Photosynthetic Physiological Characteristics of Plants

The Pn, Gs, and Tr of *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora* under acid rain stress showed a linear increase with an increase in the PAR when PAR was $\leq$ 200 μmol·m^{-2}·s^{-1}. When PAR > 200 μmol·m^{-2}·s^{-1}, the rate of increase decreased until the values stabilized. The trend of Ci was the opposite of that of Pn, Gs, and Tr. When PAR $\leq$ 200 μmol·m^{-2}·s^{-1}, the Ci decreased linearly, and when PAR > 200 μmol·m^{-2}·s^{-1}, Ci decreased slowly until it stabilized. The Gs was lower, and the Ci was higher in the pH 2.5 treatment than in the CK for all three species. The extent of inhibition of the plants in the same acid rain treatment followed the order *Pinus massoniana* > *Cinnamomum camphora* > *Phyllostachys edulis* (Figure 3).

The Pmax of *Pinus massoniana* and *Phyllostachys edulis* decreased with decreasing pH under acid rain stress. A higher pH (NR and pH 4.5) resulted in a higher Pmax, whereas a lower pH (3.5 and 2.5) resulted in a lower Pmax. The LSP, LCP, AQY, and Rd of the three species under acid rain stress did not exhibit specific trends (Table 3).

Table 3. Characteristic parameters of light response curves of three plant species.

Tree Species	Treatment	LSP	LCP	Pmax	AQY	Rd
A	CK	1654.67 ± 281.22 a	20.09 ± 8.11 a	26.43 ± 4.15 b	0.063 ± 0.028 b	1.83 ± 0.42 a
	NR	1055.07 ± 81.79 b	13.87 ± 1.15 a	33.40 ± 1.62 a	0.119 ± 0.011 a	2.39 ± 0.58 a
	pH 4.5	1182.22 ± 128.21 ab	18.25 ± 6.59 a	26.60 ± 4.48 b	0.059 ± 0.030 b	1.38 ± 0.66 a
	pH3.5	1271.32 ± 334.57 ab	10.85 ± 1.12 a	23.87 ± 0.01 b	0.091 ± 0.013 ab	1.63 ± 0.53 a
	pH2.5	1357.80 ± 558.47 ab	11.54 ± 1.17 a	22.22 ± 1.90 b	0.069 ± 0.010 b	1.30 ± 0.33 a

Table 3. *Cont.*

Tree Species	Treatment	LSP	LCP	Pmax	AQY	Rd
B	CK	895.00 ± 90.57 b	15.47 ± 5.61 ab	8.89 ± 0.12 bc	0.037 ± 0.001 a	1.51 ± 0.70 a
	NR	1315.03 ± 51.06 a	8.13 ± 4.03 b	10.93 ± 0.19 a	0.025 ± 0.012 a	0.21 ± 0.08 b
	pH4.5	1086.57 ± 53.49 ab	4.47 ± 4.51 b	9.33 ± 1.01 ab	0.024 ± 0.011 a	0.26 ± 0.04 b
	pH3.5	1146.23 ± 33.93 ab	21.13 ± 9.10 a	7.17 ± 0.01 c	0.023 ± 0.007 a	1.34 ± 0.74 ab
	pH2.5	1135.21 ± 244.48 ab	7.04 ± 3.66 b	7.52 ± 1.01 c	0.022 ± 0.011 a	0.31 ± 0.21 b
C	CK	884.32 ± 201.88 b	10.73 ± 4.54 b	14.20 ± 0.73 a	0.059 ± 0.009 a	1.13 ± 0.39 ab
	NR	960.64 ± 96.07 b	21.57 ± 7.79 ab	12.07 ± 0.68 abc	0.045 ± 0.007 ab	1.37 ± 0.34 ab
	pH4.5	1452.34 ± 90.28 a	29.25 ± 11.49 ab	13.37 ± 0.02 ab	0.037 ± 0.016 ab	1.63 ± 0.16 a
	pH3.5	847.07 ± 141.31 b	11.62 ± 2.86 b	10.18 ± 0.01 bc	0.044 ± 0.005 ab	0.94 ± 0.08 b
	pH2.5	759.05 ± 42.98 b	35.04 ± 12.61 a	8.84 ± 1.56 c	0.029 ± 0.015 b	1.71 ± 0.01 a

A: *Pinus massoniana*; B: *Phyllostachys edulis*; C: *Cinnamomum camphora*. CK: the control group, NR: natural rainfall group, Pmax: the maximum Pn, LSP: light saturation point, LCP: light compensation point, AQY: apparent quantum efficiency, Rd: dark respiration rate. Different lowercase letters indicate significant differences ($p < 0.05$).

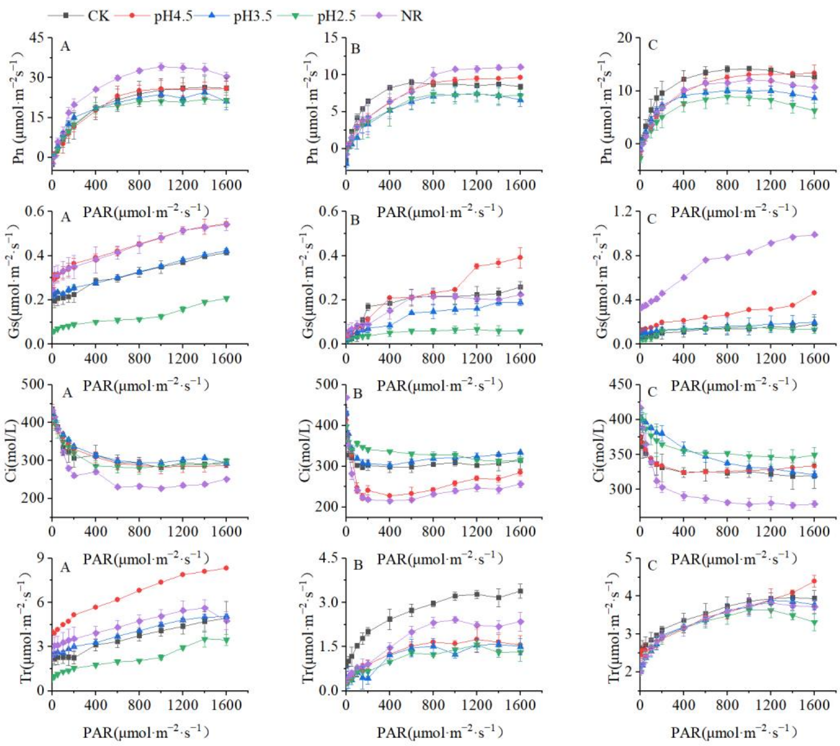

Figure 3. Response of Pn, Gs, Ci, and Tr to light intensity of three plant species under acid rain stress. (A): *Pinus massoniana*; (B): *Phyllostachys edulis*; (C): *Cinnamomum camphora*. The vertical bars represent the standard error (n = 3).

3.2. Effects of Acid Rain Stress on Plant Biochemical Characteristics

The biochemical indices of *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora* are shown in Figures 4 and 5.

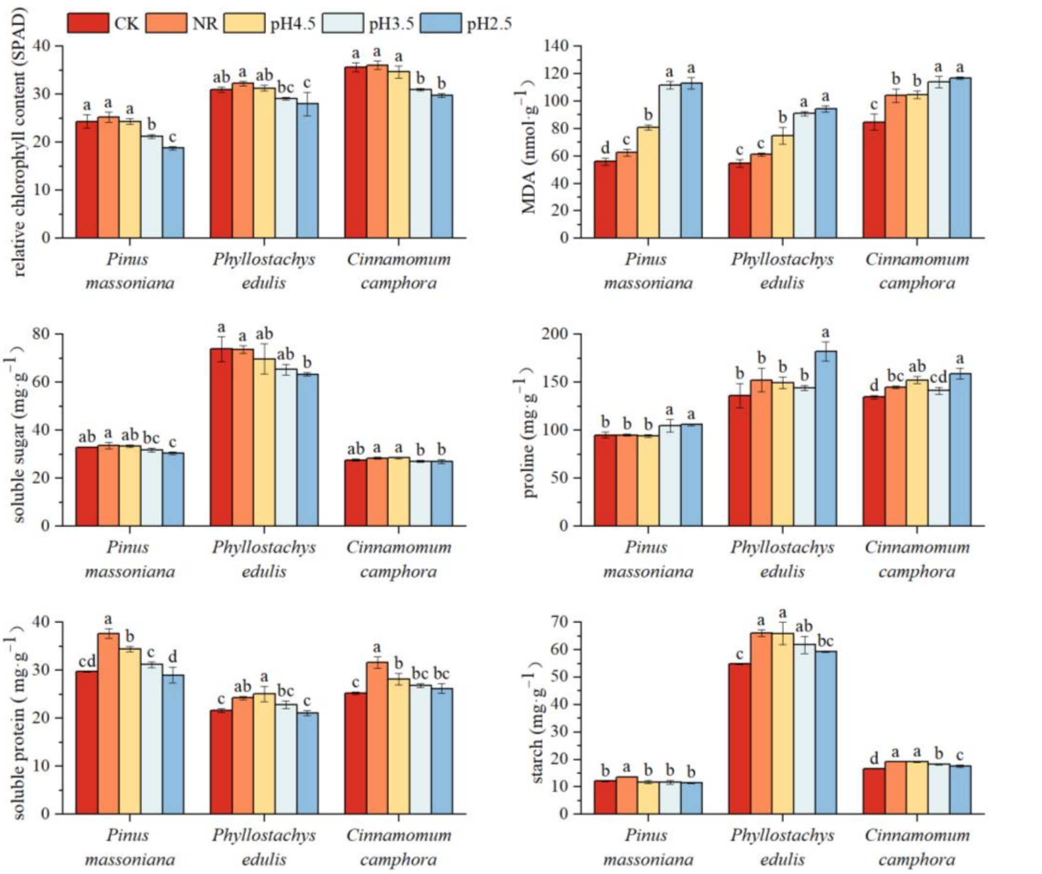

Figure 4. Biochemical indices of three plant species under acid rain stress. MDA: malondialdehyde content of plants, proline: proline content of plants, soluble protein: soluble protein content of plants, starch: starch content of plants, soluble sugar: soluble sugars content of plants, relative chl: relative chlorophyll content of plants. CK: the control group, NR: natural rainfall group. The vertical bars represent the standard error (n = 3). Different lowercase letters indicate significant differences ($p < 0.05$).

The relative chlorophyll content of the three species was higher in the NR than in the CK but decreased with increasing acidity when pH $\leq$ 3.5, reaching a minimum at pH 2.5. The chlorophyll contents of *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora* were 22.85%, 9.69%, and 16.38% lower, respectively, than in the CK. The MDA content showed the opposite trend as the relative chlorophyll content. The soluble sugar and starch contents of all three species decreased with an increase in acidity, and the proline content of all three species reached its maximum at pH 2.5. The proline content of *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora* were 11.63%, 34.13%, and 18.10% higher, respectively, than in the CK. The soluble protein content of *Phyllostachys edulis* increased first and then decreased, whereas that of *Pinus massoniana* and *Cinnamomum camphora* decreased with an increase in acidity.

Overall, the SOD, POD, CAT, and APX activities of the seedlings of the three plant species increased followed by a decrease under different acid rain treatments as the acidity increased, but there were differences in the pH at the inflection point of the enzyme activity. The CAT and SOD activities of *Pinus massoniana* peaked at pH 4.5 and were 97.12% and 43.98% higher, respectively, than those of the CK. The POD activity of *Pinus massoniana* was significantly higher at pH 4.5 and NR than for the CK. The POD activity was inhibited at pH $\leq$ 3.5. The APX activity of the three plant species was significantly higher at pH 4.5 than for the CK and reached the maximum at pH 3.5. The APX activities of *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora* were 48.48%, 87.5%, and 75.56% higher, respectively, than those of the CK.

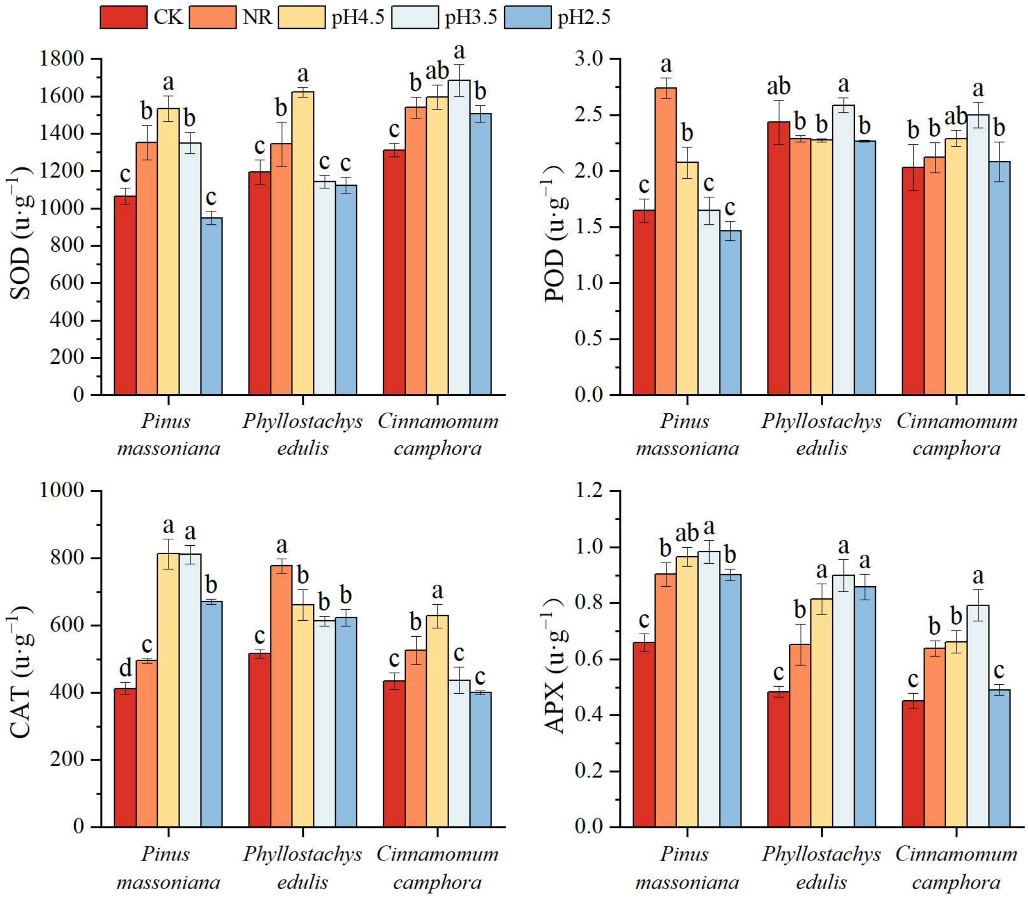

Figure 5. Antioxidant enzyme activities of three plant species under acid rain stress. SOD: superoxide dismutase activity content of plants, POD: peroxidase activity content of plants, CAT: catalase activity content of plants, APX: ascorbic acid peroxidase activity content of plants. CK: the control group, NR: natural rainfall group. The vertical bars represent the standard error ($n = 3$). Different lowercase letters indicate significant differences ($p < 0.05$).

3.3. RDA Results of Photosynthetic and Biochemical Indices of Plant Seedlings under Acid Rain Stress

The RDA results of the photosynthetic and biochemical indices of the three plant seedlings are shown in Figure 6. The interpretation rates of the first and second axes were 59.80% and 13.68%, respectively, and the cumulative interpretation rate was 73.48%, which was statistically significant. Table 4 indicates that the contents of the soluble protein, MDA, and relative chlorophyll had significant effects on the photosynthetic indices of the plant seedlings ($p < 0.01$), explaining 47.3%, 11.0%, and 9.0% of the variation, respectively. Pn was strongly correlated with the soluble protein content and pH, Ci was strongly correlated with the relative chlorophyll content, POD activities, and MDA content, and Gs and Tr were strongly correlated with the soluble protein content and pH. The PCA results (Figure 7) of the photosynthetic and biochemical indices showed that the first and second sequence axes explained 62.55% and 16.44%, respectively. The distances between different species indicated significant differences in the indices between different plant species.

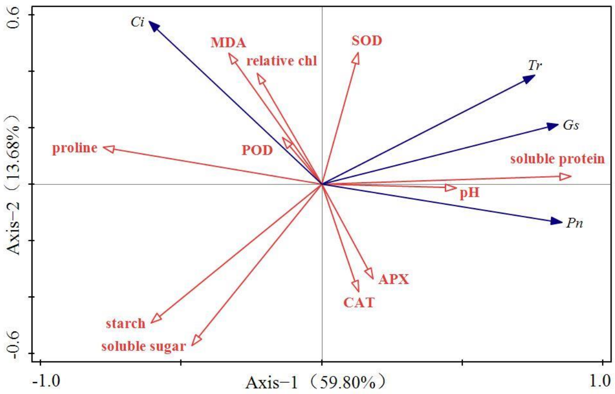

Figure 6. RDA results of photosynthetic and biochemical characteristics of plants. Pn: net photosynthetic rate, Ci: intercellular CO_2 concentration, Gs: stomatal conductance, Tr: transpiration rate, MDA: malondialdehyde content of plants, SOD: superoxide dismutase activity content of plants, POD: peroxidase activity content of plants, CAT: catalase activity content of plants, APX: ascorbic acid peroxidase activity content of plants, relative chl: relative chlorophyll content of plants. The red arrow is the explanatory variable and the blue arrow is the response variable. The angle between the two arrows indicates the correlation between the two indicators, the smaller the angle, the higher the correlation.

Table 4. Contribution rates of biochemical indexes to photosynthetic characteristics.

Name	Explains %	Contribution %	pseudo-F	*p*
Soluble protein	47.3	56.1	38.6	0.002
MDA	11.0	13.1	11.1	0.002
Relative chlorophyll	9.0	10.7	11.3	0.002

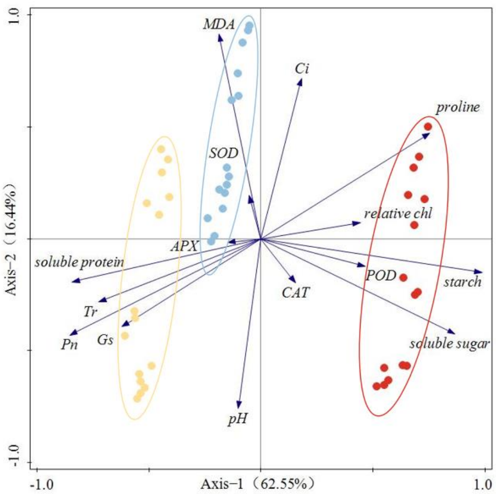

Figure 7. PCA results of photosynthetic and biochemical characteristics of plants. Yellow corresponds to *Pinus massoniana*, red corresponds to *Phyllostachys edulis*, and blue corresponds to *Cinnamomum camphora*. The length of the arrow indicates the magnitude of the indicator. The angle between the two arrows indicates the correlation between the two indicators, the smaller the angle, the higher the correlation.

4. Discussion

4.1. Effects of Acid Rain Stress on Photosynthetic Physiological Characteristics of Three Plant Species

Photosynthesis produces various organic substances, such as starch, for plant growth and development. It is a critical physiological function required for plant growth and development [35]. The light response curve reflects the correlation between the photosynthetic rate and the light intensity, and the photosynthetic parameters are indicators of the plant's photosynthetic capacity. The Pn of the three species increased linearly with an increase in the PAR at PAR $\leq$ 0–200 μmol·m^{-2}·s^{-1}, indicating that the most important environmental factor affecting the Pn of the plants was the PAR. When PAR > 200 μmol·m^{-2}·s^{-1}, the rate of increase in Pn of the three species decreased, and nonlinear growth occurred, followed by stabilization (PAR > 1200 μmol·m^{-2}·s^{-1}). Under these conditions, other factors than PAR also influenced the Pn of the plants. Photosynthesis saturation occurred when the Pn did not increase with an increase in PAR. It has been shown that the Pn of plants decreased with an increase in acidity [36], consistent with our results for *Pinus massoniana*. The reason may be the reduction of the chlorophyll content caused by Mg^{2+} leaching or the H^+ in acid rain [37,38]. Excessive accumulation of H^+ causes impaired membrane permeability, uncoupled electron transport, and insufficient accumulation of ATP and NADPH [38]. Overall, the Pn, Tr, and Gs of *Pinus massoniana* were higher in the NR and pH 4.5 treatments than in the CK, indicating that mild stress increased the photosynthetic rate of plants. However, as the acidity increased, the Pn, Tr, and Gs of the three species were low in the pH 2.5 treatment, indicating that high acidity had adverse effects on the photosynthetic activity of the three species. Our results are in agreement with those of Shu et al. (2019) [39]. The reason is that acid rain enters the plant leaves and damages the cuticle. The plant protects the cell membrane and chloroplasts from damage by reducing the Pn, Tr, and Gs [40].

Stomatal and non-stomatal restrictions can reduce the Pn of plants. Stomatal factors affect the Pn by regulating the stomatal opening, whereas non-stomatal factors influence the Pn by regulating the photosynthetic activity of the mesophyll cells [41]. Some scholars believe stomata are critical in controlling the Pn when the Gs and Ci are low. However, as the Gs decreases, the Ci increases, and non-stomatal factors are dominant [38]. The Gs was lower, and the Ci was higher in the pH 2.5 treatment than in the CK for all three species. Therefore, non-stomatal factors were crucial in reducing the Pn of plants at high acidity. The responses of the non-stomatal factors may be related to photosynthetic pigments and enzyme activities [42]. Acid rain stress has different effects on different plant species. Studies have shown that coniferous tree species are more vulnerable to acid rain than broad-leaved tree species [43]. In this study, the Pn of *Phyllostachys edulis* showed the smallest response to acid rain, followed by *Cinnamomum camphora* and *Pinus massoniana*. This result may be due to the differences in the species' abilities to recover from and resist acid rain due to their morphological, structural, and biological characteristics [44]. Plants with a thinner leaf epidermis and higher leaf water content are more vulnerable to acid rain [44]. Leaves with a thicker cuticle layer absorb water slower, and a thicker cuticle layer per unit leaf surface contains more chlorophyll, resulting in higher photosynthetic capacity [43,45,46]. Bamboo plants grow rapidly and have a high assimilation capacity, which is closely related to the photosynthetic pathway [47]. Different plant species respond differently to different acidities of acid rain, and environmental stress is a vital factor affecting the photosynthetic capacity of plants [48].

4.2. Effects of Acid Rain Stress on Biochemical Characteristics of the Tree Species

Plants accumulate organic matter for osmoregulation by metabolic regulation to alleviate osmotic stress caused by the environment [49]. This study showed that the relative chlorophyll content of the three species was significantly lower at pH $\leq$ 3.5. This result is in accordance with that of Moharekar et al. (2003), who found that high acid rain stress significantly decreased the plant chlorophyll content due to an increase in oxidative stress [50]. However, the relative chlorophyll content of the three species in this study was higher under NR, possibly because low-acidity acid rain provided N required for chlorophyll synthesis [51]. A higher chlorophyll content increases the photosynthetic capacity [52], improving plant growth. This result is in agreement with the first hypothesis that the physiological and biochemical characteristics of plant seedlings respond differently to different concentrations of acid rain, and that slightly acidic rainfall (NR) can promote the growth of plant's physiological and biochemical indicators. Our study found that the MDA content of the three species showed an increasing trend with an increase in acidity, indicating that acid rain damaged the cell membrane of leaves, causing ROS accumulation and lipid peroxidation of the cell membrane [53]. Moreover, the MDA content showed a negative correlation with the relative chlorophyll content, indicating that MDA produced by peroxidation of the cell membranes inhibited chlorophyll synthesis and hindered plant growth [54].

The soluble protein content reflects the metabolism and aging of plants because it is a critical osmoregulatory substance and nutrient [55]. The results of this study showed that the soluble protein content of the three species was higher under slightly acidic rain stress than in the CK and decreased with an increase in acidity. This result demonstrates the resistance of the plant seedlings to acid rain stress and indicates that *Pinus massoniana*, *Phyllostachys edulis*, and *Phyllostachys edulis* show adaptation to acid rain. When the acidity was very high, the activity of enzymes that synthesize plant proteins decreased, reducing the soluble protein content [56]. The soluble sugar content of the three species decreased with a reduction in the pH of the acid rain, and the effect was the most pronounced for *Phyllostachys edulis*. The results of this study were the same as those of Debnath et al. (2018), who investigated the response of tomatoes to acid rain stress [34]. It can be concluded that acid rain stress damaged the cell membranes, increased permeability, and improved the requirement of plant seedlings for nutrients and energy, reducing soluble sugar synthesis [57]. In this study, the proline content increased with an increase in acidity and was higher in all acid rain treatments than in the CK, indicating that proline was involved in the osmotic regulation of plants under acid rain stress. Plants protect cell membranes and maintain the cell shape by producing proline in response to acid rain stress, improving their resistance and preventing oxidative stress and lipid peroxidation [58]. This result is consistent with that of Zhang et al. (2021), who analyzed tea trees under simulated acid rain [58]. Environmental stress increases the synthesis pathway, such as glutamine synthesis of proline or the synthesis of amino acids that can be converted into proline [59].

Acid rain stress can increase the number of ROS, which can affect plant growth and development. The enzyme antioxidant defense system (CAT, SOD, POD, and APX) is crucial for maintaining cell homeostasis and ensuring the normal growth and development of plants. It provides an essential mechanism for cells to cope with oxidative stress [60]. In this study, the enzyme activities of CAT, SOD, POD, and APX initially increased and then decreased with an increase in acidity. The higher the acidity, the stronger the inhibitory effect. Debnath et al. (2018) also obtained the same result in a study of tomatoes [34]. It may be that the high acidity reduced the detoxification capacity of plants, changing the dynamic balance between the protective enzyme system and the scavenging effect of the antioxidants. As a result, the ROS accumulated, causing oxidative damage and affecting the plants' normal growth and development [61]. These results demonstrate that plants have a defense capacity, and their antioxidant enzyme defense system deals with external stresses. However, the plants may suffer damage when the external stresses exceed the plant's defense capacity [24]. In this study, the production and scavenging of ROS were out of balance at a pH of 3.5, and the scavenging capacity of the antioxidant enzyme system was exceeded, resulting in the production of large amounts of MAD due to peroxidation of cell membrane lipids [62]. Overall, plant growth and development under environmental stress depend on the antioxidant defense mechanisms of plants [34].

4.3. Relationship between Photosynthetic and Biochemical Characteristics of Plant Seedlings under Acid Rain Stress

As the soluble protein content in the plant leaves increased, the enzyme activity required for photosynthesis increased, improving the photosynthetic capacity and increasing the accumulation of photosynthetic products and dry matter [63]. The MDA content reflects the level of peroxidation in the cell membranes and the strength of the response to stress conditions, affecting plant photosynthesis [54]. The level of chlorophyll content was strongly associated with the physiological properties of the plant leaves, affecting photosynthesis [46]. The results of the RDA in this study showed that the soluble protein, MDA, and relative chlorophyll contents of plants substantially affected the photosynthetic characteristics, which supports our second hypothesis that acid rain affects photosynthesis by influencing the plants' biochemical mechanisms. Previous studies have shown that the decrease in the Pn of plants due to acid rain was related to the decrease in the leaf chlorophyll content [64]. Acid rain leaches from plant leaves, leading to Mg^{2+} leaching [17]. Mg is a critical component of plant chlorophyll and is required for photosynthesis. A reduction in Mg^{2+} inhibits chlorophyll synthesis and reduces the chlorophyll content. It also causes the production of ROS, which damages the photosynthetic apparatus and increase the synthesis of antioxidant enzymes, ultimately affecting photosynthesis [37]. Within a certain range, the higher the chlorophyll content, the stronger the photosynthetic capacity [52], which was demonstrated by the relative chlorophyll content and Pn in the NR treatment (Figures 3 and 4). We found that an increase in the soluble protein content under slightly acidic conditions alleviated acid rain stress, improving the photosynthetic capacity of plants. MDA, an end product of cell membrane lipid peroxidation, inhibits the formation of chlorophyll; therefore, the soluble protein, MDA, and relative chlorophyll contents were closely related to the photosynthetic capacity of plants.

This study has some limitations. The 4-month (May to August 2021) simulated acid rain experiment with potted seedlings was relatively short, considering the life span of the species. Further studies are needed to determine how the response indicators of the plants will change and whether their adaptability will increase or decrease as the treatment time and the age of the plants increase.

5. Conclusions

This study investigated the effects of acid rain with different acidity levels on the physiological and biochemical characteristics of *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora*. The responses of the three species to acid rain stress were variable. The NR and low acidity treatments enhanced the physiological and biochemical characteristics of the plants. Non-stomatal factors were dominant in reducing the Pn of plants under higher acidity conditions. The antioxidant enzyme activities increased when the plants were first exposed to acid rain stress. However, as the acidity increased, the antioxidant enzyme activity level decreased, and inhibition occurred. The simulated acid rain affected plant photosynthesis by influencing biochemical mechanisms. The soluble protein, MDA, and relative chlorophyll contents were the dominant factors affecting plant photosynthesis. A comprehensive comparison of the indicators revealed that a pH of 3.5 acid rain might be the threshold where acid rain has an adverse effect on *Pinus massoniana*, *Phyllostachys edulis*, and *Cinnamomum camphora*. The high values of the indicators under NR indicate that the plants in the Jinyun Mountain area of Chongqing have adapted to the current conditions.

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