INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Int. Agrophys., 2025, 39, 399-411
doi: 10.31545/intagr/204851

Morphophysiological adjustments and antioxidant defense in main tree species of southern China to simulated acid deposition**

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Received February 26, 2025; accepted May 9, 2025

Abstract. Acid deposition has become a significant issue due to increased pollution and forest degradation. To examine the response of acid deposition in Fuzhou, a city in Fujian Province, we investigated the physiology, morphology, osmotic adaptation, photosynthetic characteristics, and antioxidant defense of *Pinus massoniana, Cunninghamia lanceolata, Liquidambar formosana, Schima superba,* and *Phoebe zhennan*. Seedlings were subjected to pH levels of 2.5, 3.5, 4.5, and 5.5 in a greenhouse experiment. Plant height, diameter, and biomass increased in the control group (6.5); however, pH 4.5 and 5.5 resulted in tall and thin seedlings. Height and diameter decreased under increased acid deposition specifically at pH 2.5 and 3.5. Biochemical parameters and chlorophyll contents were reduced under low pH levels. The activities of catalase, peroxidase, superoxide dismutase, malonaldehyde, and proline increased initially under low pH, but decreased later due to high acidity, whereas soluble sugar and protein contents were inhibited under higher acidity levels (pH 2.5 and 3.5). The findings showed that the seedlings responded differently but more sensitively to acid deposition below pH 4.5. The exposure to less acidic deposition had a slight effect on the development of these tree seedlings. This trial aids in detecting bioindicators and resilient species to acid deposition existing in forests and urban areas.

Keywords: acid rain, photosynthesis, antioxidant enzyme, gas exchange, osmotic adjustment

1. INTRODUCTION

Acid deposition is recognized as one of the most serious environmental concerns (Gilani *et al.,* 2021). It causes significant damage to forest ecosystems in Scandinavian countries, the United States, Canada, and Europe (Grennfelt *et al*., 2020). To date Europe, northeast America and China have emerged as the three leading regions in the world impacted by acid deposition (Liu *et al*., 2022). Reports indicate that the losses caused by acid rain are alarming in several provinces of southern China (Chen *et al*., 2010). For instance, Sichuan province in southern China is being badly stricken by acid deposition (Zhang *et al*., 2021b). The pH of rainfall in southern China ranks among the lowest globally, placing the region third in terms of acid rain severity (Ren *et al*., 2024). Coastal cities are a major source of acid rain due to air pollution caused by industries, vehicles, and construction activities (Meng *et al*., 2019). This issue is predominantly caused by the use of coal for energy production culminating at addition of sulfate (SO_4^{2-}) (Cape, 1993). Fuzhou, the capital of Fujian Province, is substantially affected by acid deposition, with higher severity observed in spring and winter compared

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**This work was supported by Cross-Strait Collaborative Innovation Center of Soil and Water Conservation (K80ND8003) (2015-2027).

to autumn and summer, particularly in the southeastern, southwestern, western, and northwestern areas (Zheng *et al*., 2014). The acid deposition affected area has been reduced in recent years; however, this issue continues to restrict social development and economic growth of China (Ren *et al*., 2024). Despite the growing concern about acid deposition, research on its ecological consequences has lagged in China (Chang *et al*., 2022).

Environmental and ecosystem-level problems caused by both biotic and abiotic factors can disrupt plants in multiple ways (Yasin *et al*., 2023). Plants detect and respond to these changes by using defense mechanisms for their survival (Hasan *et al*., 2022). For example, tree leaching can alter nutrient cycling processes in forest ecosystems, resulting in physiological stress (Farooq *et al*., 2020). Similarly, acid deposition exacerbates the loss of foliage nutrients, thereby reducing tree biomass as well as vertical and radial growth (Fan and Wang, 2000). As a result, leaves often become desiccated and die, leading to a decline in both aboveground and belowground biomass (Huang *et al*., 2012). Studies have shown that acid deposition affects the process of photosynthesis (Dong *et al*., 2017).

This photosynthesis is eventually impacted by acid deposition due to destruction of chloroplast structure (Sun *et al*., 2012) and a decrease in the chlorophyll content (Du *et al*., 2017). Additionally, acid deposition can damage the leaf cuticle *e.g.* through the removal of wax layers, thereby exacerbating plant injury and disrupting metabolic processes (Diatta *et al*., 2021). This also enhances reactive oxygen species (ROS) and increases the malondialdehyde (MDA) content (Chen *et al*., 2006). The activity of antioxidant enzymes, such as superoxide dismutase, peroxidase, and catalase, initially rises in response to oxidative stress but may eventually decline under higher acidity, weakening the plant's ability to resist ROS (Rahman *et al*., 2021). Nevertheless, the enzyme activity lowers under increased acidity, decreasing plant resistance to stress (Ma *et al*., 2020). Previous findings have shown that acid deposition significantly impacted the physiology and antioxidant defense mechanisms of various tree seedlings, including *L. formosana* (Feng *et al*., 2002) and *P. massoniana* (Liu *et al*., 2018). Exposure to acid deposition particularly at pH 3.5 and below can result in noticeable phenotypic differences in the leaves of both broadleaved and coniferous tree species due to physiological damage, ultimately affecting the overall health of forest ecosystems (Chen *et al*., 2014). Similarly, another study revealed that the conifers forests are more vulnerable to acid deposition than the evergreen broadleaf forests (Hu *et al*., 2014). Acid deposition disrupts the enzymatic and non-enzymatic defense within plant cells, intensifying oxidative stress (Debnath *et al*., 2021). This oxidative stress can cause detrimental effects, such as reduced biomass and chlorophyll content as well as increased leaf dryness and mortality (Wei *et al*., 2017). Another study highlighted that populations of certain forest species have already declined due to acid deposition (Li *et al*., 2013). The levels of soluble proteins, sugars, and proline are important indicators of stress resistance under environmental hazards and are directly influenced by acid deposition (Hua *et al*., 2022). Proline accumulation is a key stress indicator in response to pathogens, heavy metals (Yasin *et al*., 2021), air pollution, nutrient deficiency (Noman *et al*., 2018), and increased sugar levels (Naheed *et al*., 2021). This study aimed to investigate the responses of trees under elevated acid deposition. A previous study showed that both conifers and broadleaved species showed structural damage after exposure to acid rain (Cape, 1993).

Chinese fir (*Cunninghamia lanceolata*) is a native species that accounts for approximately 25% of plantations in southern China (Farooq *et al*., 2019). Previous findings revealed that this conifer species was damaged by acid rain in seven provinces of southern China (Blanco *et al*., 2012). Masson pine (*Pinus massoniana*) is another indigenous species in southern China which is characterized by its durability and fast growth and is a pioneer species for reforestation and afforestation (Zhou *et al.,* 2020). Reports have indicated that *P. massoniana* is sensitive to acid deposition, with yield reductions of up to 43% when exposed to rain with a pH below 4.0 (Wu Gang, 1998; Farooq *et al*., 2021). *Phoebe zhennan* is a valuable species in China, prized for its highly durable wood, commonly known as "golden thread nanmu"(Zhu *et al*., 2022). As a result, the plantation area for this species has expanded rapidly. However, poor management practices have limited its production potential (Cheng *et al*., 2023). *Liquidambar formosana* is one of the ideal species for tree adjustment in plantations, and it is frequently chosen as an urban tree species (Wu *et al*., 2022). Several counties in Fujian Province have selected this species for experimental trials to enhance survival and germination rates (Gilani *et al*., 2023). *S. superba* is an evergreen broadleaf species found in subtropical forests in China (Chen *et al*., 2021). Earlier studies reported that its population size has declined throughout the last decade (Chen *et al*., 2013). Still, it is not evident whether its growth is influenced by acid deposition or by leaf injury (Yao *et al*., 2016). It was found that the population of *S. superba* and particularly *L. formosana* declined during the last few years (Liu *et al*., 2007). We hypothesized that acid rain can differentially affect photosynthetic attributes, lipid peroxidation, and antioxidant enzyme activity, thereby reducing biomass production in broadleaved species, pines, and conifers. To test this hypothesis, we conducted experiments with the following objectives: 1) to assess changes in gas exchange parameters under acid deposition; 2) to estimate the comparative activity of antioxidant enzymes and the extent of membrane damage due to acid rain; and 3) to record comparative biomass production in broadleaved species, pines, and conifers affected by acid rain. This trial aims to provide insight into the mechanisms by which acid deposition

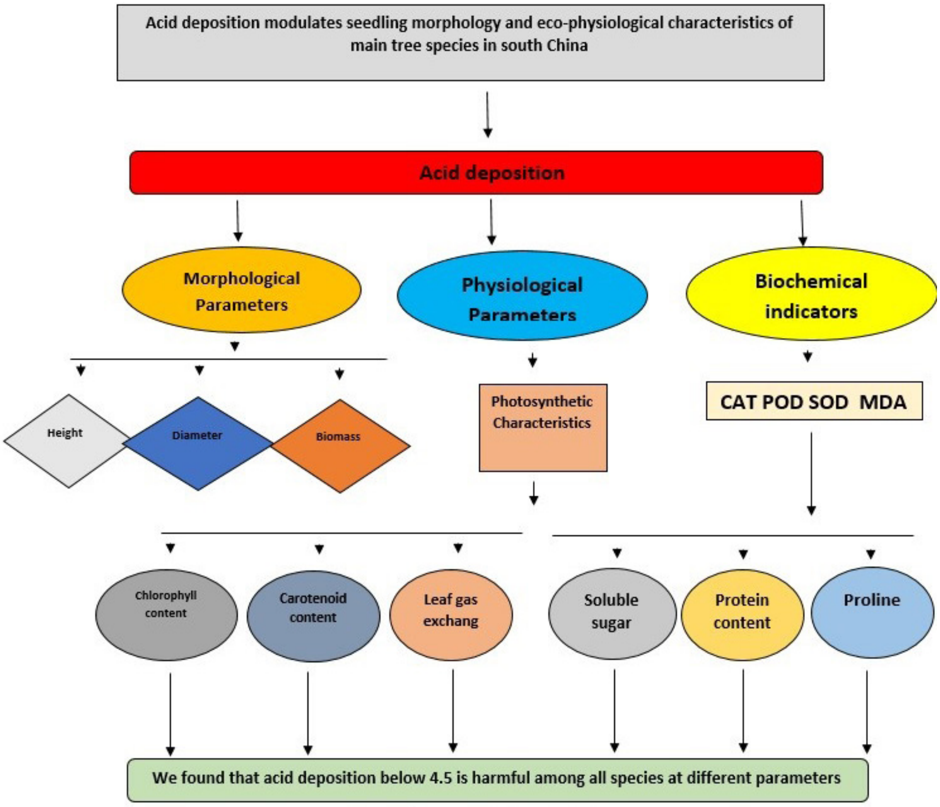

Fig. 1. Research layout.

affects these species (Fig. 1). We anticipate that the findings will have practical implications for the protection and management of forests exposed to acid deposition.

2. MATERIALS AND METHODS

2.1. Experimental material

A one-year pot experiment was carried out in the green house of Fujian Agriculture and Forestry University, Fuzhou, Fujian. The area lies in a subtropical region with high temperatures in summer. It also encounters typhoons originating from Pacific storms. The average annual deposition is 1400-2000 mm. One-year-old seedlings of *P. massoniana, C. lanceolata*, *L. formosana, S. superba*, and *P. zhennan* were shifted into 125 pots and arranged in a completely randomized design (CRD) with five replications for each treatment and 25 plants per species. Pots of a uniform size of 20 cm from the top and 15.5 cm from the bottom with a height of 16.5 cm) were established in a greenhouse. The seedlings were grown in commercial soil containing organic matter.

2.2. Acid rain solutions

The acid solution was prepared with 1 N H_2SO_4 and 1 N HNO_3 in the ratio of 10 to 1 by chemical equivalents, diluted with distilled water to attain solutions with pH values of 2.5, 3.5, 4.5, and 5.5. 500 ml of the solution was given to each pot once a week. This was determined according to the composition of rainfall by (Fan and Wang, 2000). Distilled water was used as the (CK) control group (pH 6.5). The seedlings were placed in a greenhouse which was covered with shade cloth to protect it from extreme temperature. The one-year trial began in August. The spray method was uniformly used throughout the experiment. The spray was applied at the top of the plants to distinguish the degree of stress due to different acidities. The sprayer contained acid resistant materials, the nozzle was made of stainless steel, and the water pipe was made of plastic.

2.3. Growth parameters

Seedling height (cm) was determined from the base to the top using a tape, and root collar diameter (mm) was determined using a vernier caliper (Mofunanya and Soonen, 2017). The seedlings were harvested and washed carefully for biomass measurement. Later, they were oven-dried at 65°C for 48 h. At the time of harvesting, the root/shoot ratio was measured by dividing the root with shoot of the plants.

2.4. Gas exchange measurements

Measurements were taken between 9:00-11:00 a.m. to ensure consistency. All measurements were recorded using a photosynthesis measurement system (LI-6400) at 1000 $\mu mol\ m^{-2}\ s^{-1}$ PPFD. Three similar leaves were chosen from the top of each seedling. We obtained three measurements and used the average as the result. The measurement included the transpiration rate (T_r), intercellular concentration (C_i), photosynthetic rate (P_n), and stomatal conductance (G_s).

2.5. Determination of biochemical and photosynthetic pigments

The concentrations of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) in leaf tissues were determined according to the methods described by (Lichtenthaler and Wellburn, 1983). Seedlings were either instantly used for testing or frozen in liquid nitrogen at -80°C. The pigment concentrations were determined using the following equations, based on the absorbance of the extract at 663, 645, and 470 nm, and expressed as mg g^{-1}:

$$Chlorophyll\ a\ content = \frac{(12.21D663 - 2.81D645)V}{1000W}, \tag{1}$$

$$Chlorophyll\ b\ content = \frac{(20.13D645 - 5.03D663)V}{1000W}, \tag{2}$$

$$Carotenoids = \frac{(1000D470 - 3.27C_a - 104C_b)}{229}\frac{V}{1000W}, \tag{3}$$

where: *D* is the absorbance of the supernatant at the specified wavelengths (nm), *V* is the extract volume, *W* is the sample weight, C_a represents chlorophyll *a*, C_b represents chlorophyll *b*.

2.6. Lipid peroxidation and enzymatic antioxidant measurement

The ELISA kit was used as stated by (Shanghai Enzyme-linked Biotechnology Co. Ltd). SOD was observed using Nitro blue tetrazolium chloride, POD with guaiacol, and CAT with UV-Vis Spectroscopy (Ahmed *et al*., 2013). The colorimetric method was employed to measure MDA contents (Hodges *et al*., 1999).

2.7. Total soluble protein and sugars

Soluble protein was measured with an earlier technique proposed by Bradford (1976), while soluble sugar was determined according to the protocol used by Yemm and Willis (1954). Proline in leaves was identified at 520 nm (Bates *et al*., 1973).

2.8. Statistical analysis

All data were analyzed using SPSS 20.0 and Statistics 8.1, while all figures were drawn by GraphPad Prism 8 Software. Two-way analysis was used and significance of differences between means was determined by applying Tukey HSD test at 5.

3. RESULTS

3.1. Growth parameters

The growth parameters, such as plant height and root collar diameter, decreased among all species and treatments. For example, the plant height in *C. lanceolata* decreased by 26.94, 21.26, 16.84, and 12.02% at pH 2.5, 3.5, 4.5, and 5.5. A more pronounced reduction in the plant height was observed in *Schima superba*, which showed decreases of 32.61, 27.63, 23.33, and 19.07% at the same pH levels, compared to the control group. Similarly, the root collar diameter decreased at all the pH levels, relative to the control. However, a greater reduction in the plant height was observed for *S. superba* at all the pH levels, which decreased by 32.61, 27.63, 23.33, and 19.07% at pH 2.5, 3.5, 4.5, and 5.5, respectively, compared to the control group. The root collar diameter decreased at all the pH levels, compared to the control; however, this reduction was higher at pH 2.5 in *C. lanceolata*, *L. formosana*, *P. zhennan*, and *S. superba*, compared to pH 3.5, pH 4.5, and pH 5.5, respectively (Table 1).

The leaf biomass in *C. lanceolata, P. massoniana, P. zhennan*, and *S. superba* decreased at pH 2.5 3.5, and 4.5; however, it increased in the studied species at pH 5.5.

Table 1. Effect of acid stress on plant height and root collar diameter of analyzed species (mean ± SE)

Tree parameter	Treatment	*C. lanceolata*	*L. formosana*	*P. massoniana*	*P. zhennan*	*S. superba*
Plant height (cm)	CK	45.24±3.67a	65.87±4.63a	41.28±4.01a	56.71±4.48a	48.38±3.73a
	2.5	33.05±1.69c	43.55±2.21c	23.48±1.62c	34.18±1.89c	32.60±2.17c
	3.5	35.62±1.89bc	45.33±3.30bc	25.41±1.69c	37.23±2.21c	35.01±2.51bc
	4.5	37.62±1.86b	48.37±3.37b	28.44±1.91bc	40.92±2.44bc	37.09±2.43b
	5.5	39.80±2.31ab	50.87±3.52b	33.37±2.77bc	44.74±2.69bc	39.15±2.90ab
Root collar diameter (mm)	CK	4.29±0.18a	5.10±0.23a	2.90±0.17a	4.96±0.47a	4.41±0.31a
	2.5	3.56±0.06c	4.24±0.06c	2.48±0.04c	3.74±0.26c	3.17±0.17c
	3.5	3.63±0.08c	4.47±0.10bc	2.44±0.08c	4.34±0.30bc	3.74±0.16bc
	4.5	3.73±0.08bc	4.81±0.22b	2.75±0.10b	4.44±0.30bc	3.83±0.26bc
	5.5	3.83±0.12b	4.85±0.23ab	2.52±0.11bc	4.64±0.36a	3.86±0.29b

Table 2. Leaf, stem, root biomass and root/shoot ratio of analyzed species in response to acid stress (mean ± SE)

Tree parameter	Treatment	*C. lanceolata*	*L. formosana*	*P. massoniana*	*P. zhennan*	*S. superba*
Leaf (g)	CK	17.35±1.15a	5.78±0.99a	16.35±1.25a	7.89±0.61b	7.45±0.48a
	2.5	9.12±0.35b	2.61±0.28b	9.32±0.39c	6.01±0.06b	4.03±0.48c
	3.5	13.47±1.14b	2.64±0.47b	14.47±1.17b	7.67±0.36b	5.43±0.74b
	4.5	10.59±0.67b	3.01±0.29b	11.59±0.69b	5.59±0.71c	5.11±0.29b
	5.5	17.10±1.18a	2.95±0.39b	18.10±1.28a	8.29±0.64a	6.68±0.67b
Stem (g)	CK	10.92±0.63a	7.41±0.51a	11.92±0.69a	8.38±0.37a	7.02±0.34a
	2.5	6.00±0.43b	5.71±1.06c	6.10±0.49b	5.89±0.61c	5.08±0.53b
	3.5	6.67±0.38b	6.64±0.24b	6.74±0.42b	6.94±0.24b	4.52±0.36c
	4.5	7.71±0.71b	6.69±0.28b	7.73±0.76b	7.96±0.71b	5.47±0.22b
	5.5	10.21±0.60a	6.82±0.96b	10.36±0.68a	7.76±0.47b	5.53±0.18b
Root (g)	CK	9.13±0.17a	6.66±0.41a	9.36±0.18a	12.93±1.55a	7.98±0.69a
	2.5	6.35±0.70b	3.46±0.08c	6.39±0.71c	8.69±0.61b	4.78±0.25c
	3.5	7.13±0.60b	5.95±0.32b	7.16±0.61b	9.31±0.59b	5.53±0.43b
	4.5	8.13±0.65b	5.49±0.44b	8.16±0.68b	10.26±0.84b	6.15±0.65a
	5.5	7.98±0.89b	6.62±1.40b	7.87±0.85b	12.57±0.81a	6.07±0.52a
Root/Shoot Ratio	CK	0.83±0.11b	0.89±0.13ab	0.78±0.09b	1.54±0.23b	1.13±0.25b
	2.5	1.05±0.06a	0.60±0.09c	1.04±0.14a	1.47±0.31b	0.94±0.20c
	3.5	1.06±0.09a	0.89±0.06ab	1.06±0.19a	1.34±0.19c	1.22±0.09a
	4.5	1.05±0.23a	0.82±0.12b	1.14±0.08a	1.28±0.11c	1.12±0.13b
	5.5	0.78±0.08b	0.97±0.27a	0.75±0.11b	1.61±0.29a	1.09±0.16b

In the case of *L. formosana*, the maximum leaf biomass (5.78 g) was computed in the control, and it decreased at all the pH levels with a minimum (2.61 g) at pH 2.5 (Table 2). The stem biomass decreased across all the species at all the pH levels, except at pH 5.5. The root biomass also declined in all the species under acid treatments, except at pH 4.5, where *Cunninghamia lanceolata*, *Pinus massoniana*, and *Phoebe zhennan* exhibited an increase, compared to the control group. The root/shoot ratio in *C. lanceolata, L. formosana*, and *P. massoniana* increased at pH 2.5, 3.5, and 4.5, except pH 5.5, where it decreased by 6.02% in *C. lanceolata,* 8.98% in *L. formosana*, and 3.84% in *P. massoniana*, respectively. The root/shoot ratio in *P. zhennan* decreased in all the treatments except pH 5.5, where it increased by 4.54%. Similarly, the root/shoot ratio in *S. superba* also showed a decreasing trend under all the pH treatments except pH 3.5, where it increased by 7.96%, compared to the control (Table 2).

3.2. Gas exchange measurements

The photosynthetic rate (P_n) decreased across all the treatments in all the studied species except *S. superba*. The P_n in *S. superba* increased by 9.82, 25.21, 26.41, and 45.4% at pH 2.5, 3.5, 4.5, and 5.5, respectively. Stomatal conductance (G_s) in *Cunninghamia lanceolata* increased under all the treatments except at pH 5.5, where it decreased by 16.03%, compared to the control. The G_s in *P. massoniana*, *L. formosana,* and *P. zhennan* was reduced at all the pH levels except pH 5.5, where it increased by 4.34% in *P. massoniana*, 10.97% in *L. formosana*, and 61.11% in *P. zhennan*. However, the G_s in *S. superba* showed an increasing trend at all the pH levels, compared to the control treatment. The intercellular CO_2 concentration (C_i) in *C. lanceolata* and *P. massoniana* increased across all pH levels. However, in *L. formosana*, C_i decreased by 15.37 and 41.1% at pH 2.5 and pH 3.5, respectively, while it increased by 1.81 and 36.23% at pH 4.5 and pH 5.5. Similarly, the C_i in *S. superba* increased by 17.95% at pH 3.5, compared with the control treatment. The transpiration rate (T_r) in *C. lanceolata* increased at all the pH levels except pH 5.5, where it decreased by 18.39%. The T_r in *P. massoniana, L. formosana*, and *P. zhennan* were lower at pH 2.5, 3.5, and 4.5; however, higher T_r was observed at pH 5.5. In the case of *S. superba*, a greater transpiration rate was observed at all the pH levels, compared with the control (Fig. 2).

3.3. Determination of biochemical and photosynthetic pigments

The chlorophyll contents in all the species decreased at all the pH levels (Table 3). The level of *chl a* in *C. lanceolata* decreased by 65.5, 58.2, 46.6, and 26.7% at pH 2.5, 3.5, 4.5, and 5.5. In the case of *S. superba*, it decreased by 64.25, 64.73, 71.49, and 68.11% at pH 2.5, 3.5, 4.5, and 5.5, respectively, compared to the control treatment. Similarly,

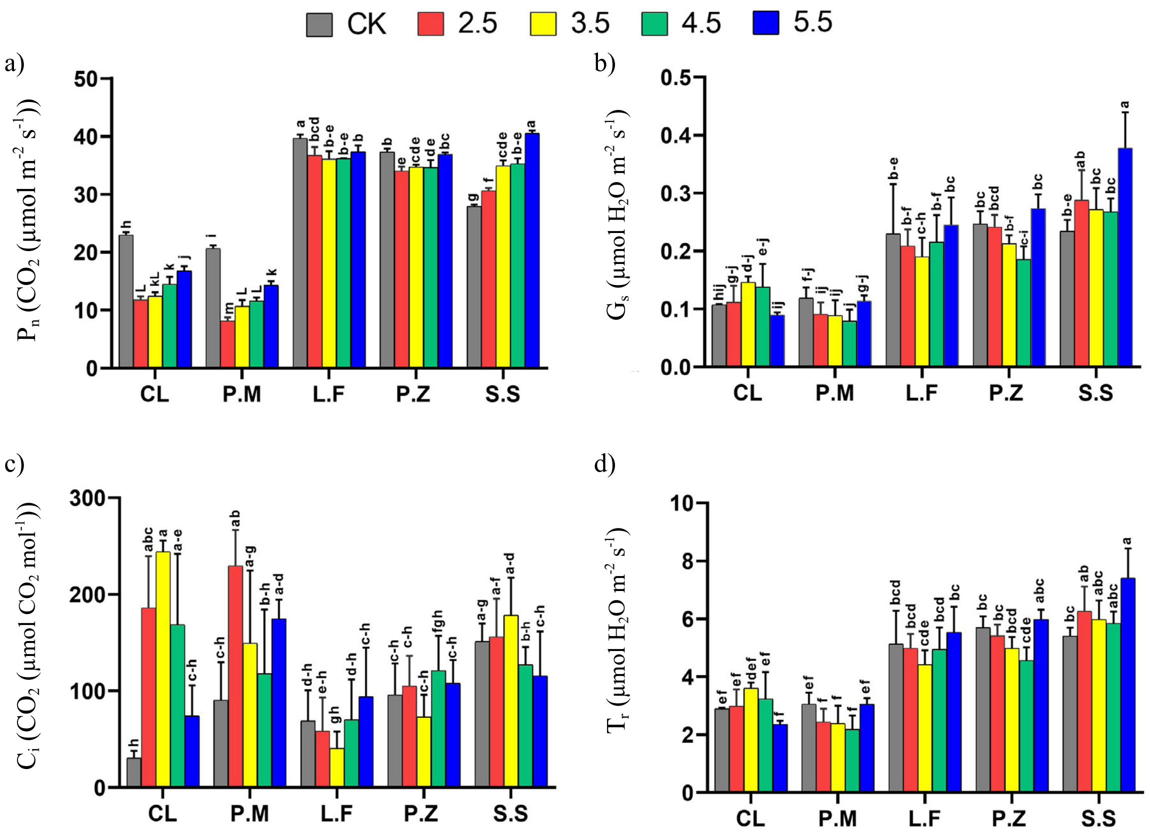

Fig. 2. a) Response of net photosynthetic rate (P_n) of photosynthetic photon flux (PPFD); b) stomatal conductance (G_s) to H_2O; c) intercellular (C_i) CO_2 concentration; and d) transpiration rate (T_r) to treatment with simulated acid deposition.

Table 3. Chlorophyll *a*, chlorophyll *b*, and carotenoid content in response to acid stress (mean ± SE)

Tree parameter	Treatment	*C. lanceolata*	*L. formosana*	*P. masoonian*	*P. zhennan*	*S. superba*
Chl *a* (mg g^{-1})	CK	2.32±0.25a	1.99±0.13a	1.74±0.08a	1.93±0.10a	2.07±0.13a
	2.5	0.80±0.07c	0.83±0.05c	0.43±0.04c	0.97±0.03c	0.74±0.06b
	3.5	0.97±0.09c	1.15±0.02b	0.56±0.01b	0.97±0.08c	0.73±0.13b
	4.5	1.24±0.04b	1.52±0.03b	0.71±0.05b	1.34±0.07b	0.59±0.10b
	5.5	1.70±0.04b	1.76±0.05b	0.99±0.11b	1.21±0.07b	0.66±0.09b
Chl *b* (mg g^{-1})	CK	1.69±0.04a	1.62±0.12a	1.27±0.05a	1.23±0.12a	1.55±0.11a
	2.5	0.41±0.06b	0.35±0.03b	0.10±0.01c	0.32±0.05b	0.32±0.03b
	3.5	0.39±0.04b	0.46±0.06	0.26±0.008b	0.22±0.05c	0.27±0.05b
	4.5	0.58±0.06b	0.30±0.05c	0.26±0.02b	0.21±0.06c	0.25±0.01c
	5.5	0.48±0.06b	0.42±0.06b	0.24±0.02b	0.48±0.07b	0.40±0.07b
Carotenoids (mg g^{-1})	CK	0.63±0.15a	0.89±0.12a	0.81±0.03a	0.62±0.09a	0.94±0.05a
	2.5	0.14±0.01c	0.17±0.02c	0.12±0.01c	0.27±0.02c	0.22±0.02b
	3.5	0.21±0.04b	0.25±0.02c	0.13±0.004c	0.30±0.04b	0.20±0.04b
	4.5	0.24±0.02b	0.45±0.03b	0.18±0.02b	0.45±0.03b	0.13±0.03c
	5.5	0.44±0.03a	0.50±0.04b	0.28±0.05b	0.28±0.03c	0.56±0.04b

the *chl b* content in all the studied species decreased at all the pH levels. Likewise, the carotenoid contents under acid deposition declined with the increase in acidity. The carotenoid contents decreased by 77.8, 66.7, 61.9, and 30.2% in *C. lanceolata* and by 76.59, 78.72, 86.17, and 40.42% in *S. superba* at pH 2.5, 3.5, 4.5, and 5.5, respectively, as presented in Table 3.

The influence of acid deposition on enzymatic antioxidants in all the studied species is presented in Fig. 3. The findings revealed that all the enzymatic activities in the seedlings of studied species decreased with the increase in acidity levels. The CAT activity in *C. lanceolata* and *L. formosana* increased at all the pH levels. In the case of *P. zhennan,* the CAT activity decreased across all the pH levels except pH 2.5 where it increased by 19.08%, compared to the control. However, CAT in *S. superba* increased by 11.97, 19.74, 31.6, and 28.03% at pH 2.5, 3.5, 4.5, and 5.5, respectively. Similar to CAT, the SOD activity also showed a decreasing trend with the increase in acidity among all the species. The lowest SOD values were computed in the control treatment, whereas the highest SOD activity was observed at pH 5.5. The POD value increased by 0.43 and 1.63% at pH 2.5 and 3.5 in *P. massoniana* but decreased by 50.53 and 67.85% at pH 4.5 and pH 5.5, respectively. Similarly, POD increased in all the other species at all the pH levels, compared to the control treatment, showing maximum POD values at pH 5.5 and minimum levels in the control group (Fig. 3). The MDA contents showed no significant variation in all the species under all the treatments. The MDA activity in *C. lanceolata, P. massoniana, L. formosana, P. zhennan*, and *S. superba* decreased at all the pH levels except pH 2.5, where it increased by 13.86, 13.26, 6.21, 4.24, and 8.3%, respectively.

In *C. lanceolata,* the soluble sugar content decreased by 44.25, 47.16 31.21, and 44.76% at pH 2.5, 3.5, 4.5, and 5.5, respectively, while the soluble sugar content in *S. superba* decreased at all the pH levels except pH 4.5, where it increased by 1.2%. The soluble sugar content in *L. formosana*, *P. massoniana*, and *P. zhennan* decreased at all the pH levels except pH 5.5, where it showed an increasing trend. The soluble protein content in all the species showed a decreasing trend with the increase in the acidity level (Table 4). The proline content in all the species was greater at pH 2.5, compared with all the other pH levels and the control. In *C. lanceolata*, the proline content increased at pH 2.5 but decreased by 24 and 28% at pH 4.5 and 5.5, respectively. Similarly, in *S. superba,* the proline

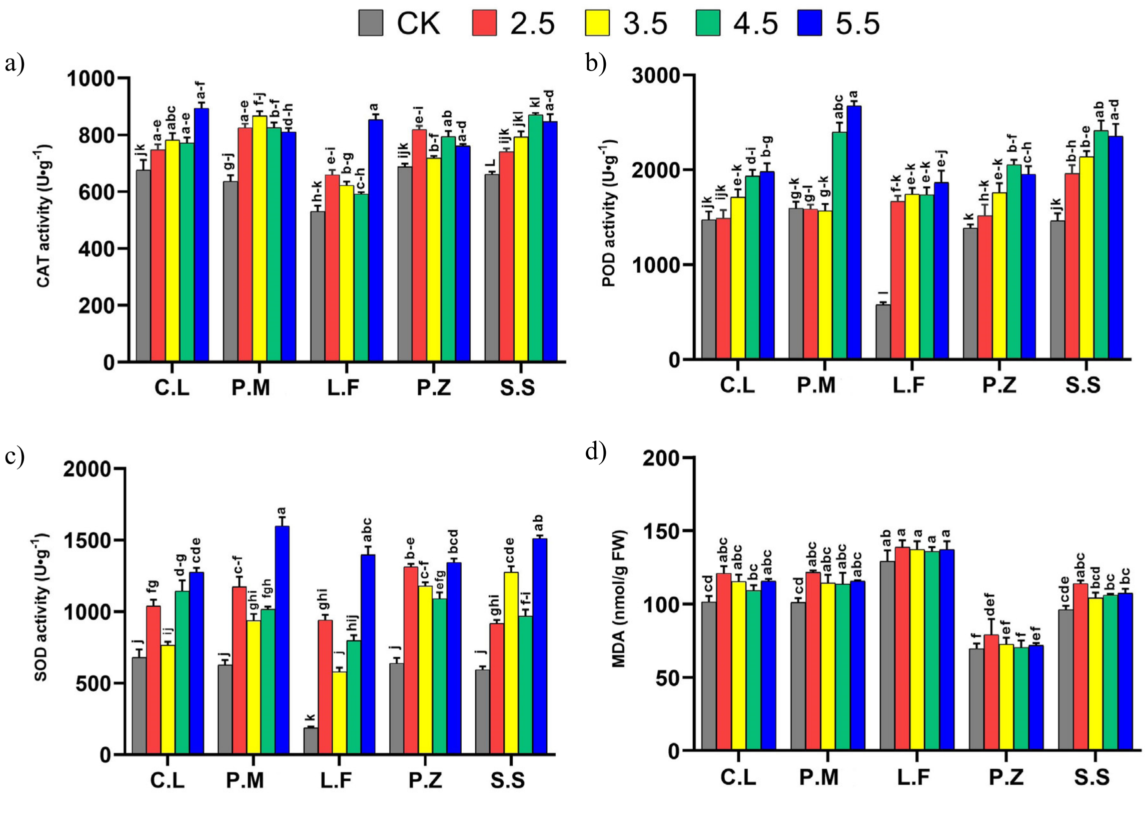

Fig. 3. Enzymatic antioxidants and lipid peroxidation under acid deposition treatments. Vertical bars indicate the standard error, the results are different at $p \leq 0.05$: a) CAT, b) POD, c) SOD, and d) MDA.

Table 4. Total soluble sugar, protein, and proline content in response to acid stress (mean ± SE)

Tree parameter	Treatment	*C. lanceolata*	*L. formosana*	*P. masooniana*	*P. zhennan*	*S. superba*
Soluble sugar (mg g^{-1} FW)	CK	5.83±1.07a	10.35±0.66a	5.34±1.29a	12.25±0.95a	4.99±1.11b
	2.5	3.25±0.28b	5.98±0.73b	2.96±0.20c	9.67±0.72b	4.25±0.84b
	3.5	3.08±0.31b	6.59±0.18b	3.66±0.23b	10.18±1.03b	4.94±0.89b
	4.5	4.01±0.02b	4.53±0.31b	3.12±0.19b	10.35±0.77b	5.05±1.05a
	5.5	3.22±0.07b	6.83±0.73b	3.67±0.17b	11.63±0.45a	4.54±1.09b
Protein (mg g^{-1} FW)	CK	4.92±0.06a	7.94±0.51a	7.17±0.14a	9.28±0.43a	8.69±0.07a
	2.5	1.84±0.03c	1.73±0.02c	2.55±0.06b	2.28±0.06c	2.06±0.05c
	3.5	2.07±0.06c	2.47±0.05c	2.68±0.21b	3.20±0.17b	3.24±0.23b
	4.5	2.24±0.03b	3.84±0.03b	3.33±0.34b	3.70±0.13b	3.25±0.39b
	5.5	3.85±0.08b	4.47±0.15b	4.92±0.06a	4.80±0.34a	3.89±0.34b
Proline (mg g^{-1} FW)	CK	0.25±0.03b	0.64±0.21b	0.19±0.01b	0.15±0.004b	0.22±0.007b
	2.5	0.59±0.04a	1.00±0.23a	0.25±0.03b	0.16±0.008b	0.24±0.009b
	3.5	0.33±0.04b	0.75±0.17b	0.19±0.009b	0.17±0.008b	0.36±0.04a
	4.5	0.19±0.01b	0.46±0.01b	0.24±0.02b	0.20±0.008a	0.21±0.008b
	5.5	0.18±0.005b	0.52±0.02b	0.24±0.03b	0.21±0.005a	0.18±0.02c

content increased by 9.9 and 63.6% at pH 2.5 and pH 3.5 but decreased by 4.5 and 18.1% at pH 4.5 and pH 5.5, as presented in Table 4.

4. DISCUSSION

The present study demonstrated that acid deposition exerts significant detrimental effects on plant growth, as evidenced by reductions in plant height, root collar diameter, and overall biomass. These findings are consistent with previous research by Tong and Liang (2005), who reported pronounced structural damage and visible foliar chlorosis in response to acid deposition at pH levels below 3.5. Such symptoms suggest that acid stress not only impairs aboveground growth but also disrupts physiological processes vital for plant development. Our findings are corroborated with earlier reports mentioning leaf damage in seedlings exposed to pH 3.0 for ten days (Eguagie *et al*., 2016), marginal necrosis (Bussotti *et al*., 1997), and curtailed photosynthesis (Flagler *et al*., 1994). All these symptoms collectively impaired plant height, stem growth, and tree biomass (Song *et al*., 2011). These cumulative findings strongly reinforce our conclusion that acid deposition at or below pH 3.5 significantly inhibits seedling growth and biomass accumulation. The severity of this inhibition suggests that seedlings exposed to such stress conditions may adopt specific tolerance strategies to mitigate damage. We argue that one primary strategy involves the selective abscission of damaged leaf regions, allowing the plant to preserve healthier tissue and limit further injury. A secondary mechanism may include the formation of protective scars on leaves, which could serve to isolate and contain the damage. Furthermore, our results revealed marked variation in root-to-shoot ratios across different pH treatments, indicating adaptive allocation of resources under stress. This trend aligns with earlier findings in spruce seedlings, where similar changes in biomass partitioning were observed under acid stress, supporting the notion of a broader, conserved physiological response among tree species (Bäck *et al*., 1995).

4.1. Gas exchange measurements

Photosynthetic rate (P_n) can be influenced by both stomatal and non-stomatal limitations. Stomatal constraints primarily involve the regulation of gas exchange through the opening and closing of stomata, whereas non-stomatal factors affect photosynthesis by altering mesophyll cell activity (Wang *et al*., 2014). Photosynthesis generates several organic compounds taking critical part in physiological function of plants (Xu *et al*., 2022). It was observed that the P_n value was reduced with a rise in acidity, which is consistent with our results for *C. lanceolata* and *P. massoniana* (Sun *et al*., 2016). One plausible explanation for this decline is the leaching of magnesium or hydrogen ions from chlorophyll, which may impair chloroplast function and hinder the synthesis of adenosine triphosphate (ATP), a key energy molecule in the photosynthetic process (Verbruggen and Hermans, 2013). The study found that *L. formosana*, *P. zhennan*, and *S. superba* maintained higher photosynthetic rates under acid stress, compared to *C. lanceolata* and *P. massoniana*, suggesting greater stomatal tolerance. Additionally, both stomatal and non-stomatal factors, especially enzyme activity, contribute to regulating photosynthesis in acidic conditions (Zhang *et al.*, 2021b). It is well agreed that stomata are vanguards in the process of photosynthesis and particularly influence P_n when G_s and C_i are lower. The decrease in G_s is in inverse relationship with C_i, which also reflects appropriate and essential dominance of non-stomatal factors (Velikova *et al.*, 1999). Our recorded data revealed that increased C_i reduced G_s in all the species and alleviated acid deposition by reducing transpiration loss. The difference in photosynthetic attributes after acid exposure can also be due to biological and morphological characteristics of species and their resistance to acid deposition (Zhang *et al.*, 2023). Plant species respond differentially to stress, which is a crucial factor in influencing the photosynthetic activity of any plant (Ahmad *et al.*, 2019). The G_s and T_r of *S. superba*, *P. zhennan,* and *L. formosana* were higher at pH 5.5, compared to the control group, revealing that mild stress enhanced the photosynthetic rate in these species. Acid deposition below pH 3.5 had harmful impacts on the photosynthesis activity, and our results agree with those obtained by Shu *et al.* (2019), who reported the same findings.

4.2. Estimation of biochemical attributes and photosynthetic pigments

Chlorophyll can be used as a sign of resistance to depict the eco physiological condition of plants (Zhou *et al.*, 2020). This study showed that the chlorophyll *a*, *b*, and carotenoid content in the five species were lower under different acid levels. Our study agrees with the findings of the study by Moharekar *et al.* (2003), who observed reduction in the chlorophyll content due to a rise in acid deposition stress. The chlorophyll content is frequently affected by strong acid rain (Kumaravelu and Ramanujam, 1998). The main cause of the decline in chlorophyll content might be the foliar leaching of nutrients due to acidity (Bussotti *et al.*, 1997).

The findings reported by Qiu and Liu (2002) demonstrated that elevated acidity levels (pH 2.5 and 3.5) led to a reduction in chlorophyll a content in *Dimocarpus longan* leaves, while chlorophyll *b* was comparatively less affected – an observation that aligns with our results. Similarly, other findings (Jian-Fu *et al.*, 2013) have also confirmed chlorophyll reduction by acid deposition. Carotenoids can protect chlorophylls by scavenging ROS through xanthophyll (Khalid *et al.*, 2018). Our findings revealed that the carotenoid content decreased below pH 3.5 and pH 2.5, compared to that in the control treatment plants; this is perhaps due to degradation of pigments (Zhang *et al.*, 2020). Antioxidant enzymes are produced by plants to deal with stress stimulated by abiotic and biotic stresses. We revealed that, contrary to the control plants, the acid deposition enhanced CAT and POD activities, which is in accordance with a study conducted by Wu *et al.* (2012).

The study found that the increased acid concentration elevated leaf POD activity, indicating heightened plant stress. While *P. zhennan* and *S. superba* managed hydrogen peroxide more effectively, *L. formosana* showed reduced CAT, POD, and SOD activity, supporting earlier findings that it is more sensitive to acid stress (Chen *et al.*, 2013). The initial increase followed by a decrease in enzyme activities observed in this study was attributed to prolonged exposure to acidity. Higher acidity impaired the plants' detoxification capacity, disrupting the balance between protective enzymes and antioxidant scavenging. MDA, an indicator of oxidative damage, significantly increased under the acid treatment, rising with the acidity levels. This trend suggests that acid deposition caused membrane damage, leading to the accumulation of reactive oxygen species (ROS) (Liu *et al.*, 2019). In our trial, MDA in both *P. zhennan* and *S. superba* was lower than in the other species, suggesting that these species displayed advanced antioxidant enzyme activities, mitigating the damage caused by ROS, compared to *C. lanceolata*, *P. massoniana*, and *L. formosana*. These findings are in agreement with those shown by Wyrwicka and Skłodowska (2006), Rajjou *et al.* (2012) and Chen *et al.* (2013), who observed the same results. These findings suggested that free radical production is enhanced by acid deposition and causes lipid peroxidation of membranes, resulting in increased MDA. This increase exhibits membrane injury, ROS activity, and oxidative stress (Montillet *et al.*, 2005). Plants with improved enzyme activity can defend their cells from ecological pressure (Noman *et al.*, 2015). It is most likely that the antioxidant compounds keep the defensive pathway active, and these seedlings produce enzymes under adapting stress. Soluble sugars are the primary osmoregulators which are significant in sugar detection in plants (Akhter *et al.*, 2022). The soluble sugar of the five species decreased under the acid deposition at all the pH levels. These sugars work as osmoprotectants in maintaining the pressure by stabilizing the cell membrane (Marcińska *et al.*, 2013). In the present study, *P. zhennan* was least affected by the acid deposition, compared to the other species. The increased sugar level can be viewed as an effective approach of *P. zhennan* to stop the damaging impact of acid stress (Foyer and Noctor, 2005). It can be concluded that acid deposition increases the demand for nutrients by lowering sugar synthesis (Bao *et al.*, 2019).

Soluble protein is a prominent osmotic plant regulator, as its increased content aids in balancing cells and its resistance to the damage caused by stress (Amini and Ehsanpour, 2005). The results showed a significant reduction in protein content under the acid stress (≤ pH 3.5), compared to the control treatment. A similar study on *Dimocarpus longan* found that stress at ≤ pH 3.5 also impacted leaf protein levels, indicating that acid deposition reduces primary respiratory pathways and plant energy (Pan *et al.*, 2015).

Similarly, if the acid stress is high, the activity of enzymes involved in the synthesis of plant proteins is reduced (Bao *et al*., 2019). Proline acts as an osmolyte that can protect cell dehydration as well a defensive agent and a (ROS) scavenger (Debnath *et al*., 2018). It increased under the acid stress in the five species. This indicates that proline was involved in osmotic regulation. A previous study also showed that acid stress prevents proline production in *Hypogymnia* (Kováčik *et al*., 2011). Our result agrees with those reported by Chen *et al*. (2013), who found that the proline level in *L. formosana, P. zhennan*, and *S. superba* increased under acid stress. A similar study also noted that the stress led to higher proline contents in *P. zhennan* leaves (Hu *et al*., 2015). Plants maintain cell shape and protect membranes by generating proline under high acidity, preventing lipid peroxidation and oxidative stress (Zhang *et al*., 2021a). Our findings are consistent with those obtained by Zhang *et al*. (2020), who examined tea trees. The observed changes in acid resistance among the species highlight the importance of developing strategies to mitigate damage, which is vital for the sustainability of forest ecosystems.

5. CONCLUSIONS

The present trial concludes that *P. massoniana, C. lanceolata, P. zhennan*, *L. formosana,* and *S. superba* were sensitive to acid deposition, showing varying response among different parameters. Seedling height, root collar diameter, and plant biomass decreased under the acid stress. The levels of chlorophyll *a* and chlorophyll *b* were lower than in the control. Seedlings used a protective mechanism to respond differently to stress under osmotic adjustment. The acid deposition significantly increased the antioxidant enzyme activities and aggravated membrane lipid peroxidation. This trial showed improved understanding of photosynthetic, antioxidant, and physiological tolerance of seedlings to numerous kinds of acid deposition. The current findings are useful for the estimation of plant sensitivity to stress and can be valuable for examining plants in acid stress conditions under changing climate. Moreover, the present study aids in identifying *P. zhennan*, and *S. superba* as bioindicators and resilient species, compared to the others; hence, extensive planting programs can be beneficial in urban and forest areas to clean the atmosphere and improve the quality of the air.

Conflict of interest. Authors declare no conflict of interest exists.

Author Contributions: Matoor Mohsin Gilani and Bo Liu conceived the idea; Matoor Mohsin Gilani and Xiangqing Ma designed the study. Matoor Mohsin Gilani, Muhammad Haroon U. Rashid and Muhammad Waqqas Khan Tarin performed the lab work, Matoor Mohsin Gilani and Ghulam Yasin analyzed the data. Muhammad Zubair, and Ali Noman discussed the results and discussion. Taimoor Hassan Farooq and Fahad Mohammed Alzuaibr revised the English language.

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