Ecotoxicology and Environmental Safety 160 (2018) 154–161

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of sulfuric, nitric, and mixed acid rain on Chinese fir sapling growth in Southern China

Xin Liu[a], Zhiyuan Fu[a], Bo Zhang[b], Lu Zhai[c], Miaojing Meng[a], Jie Lin[a], Jiayao Zhuang[a], G. Geoff Wang[d], Jinchi Zhang[a,*]

[a] Co-Innovation Center for Sustainable Forestry in Southern China, Jiangsu Province Key Laboratory of Soil and Water Conservation and Ecological Restoration, Nanjing Forestry University, 159 Longpan Road, Nanjing, Jiangsu 210037, China
[b] Department of Biology, University of Miami, Coral Gables, FL 33124, USA
[c] Southeast Environmental Research Center, Florida International University, Miami, FL 33199, USA
[d] Department of Forestry and Environmental Conservation, Clemson University, Clemson, South Carolina 29634, USA

ARTICLE INFO

Keywords:
Chinese fir
Growth rate
Chlorophyll
Acid rain
SO_4^{2-}/NO_3^- ratio

ABSTRACT

The influence of acid rain on plant growth includes direct effects on foliage as well as indirect soil-mediated effects that cause a reduction in root growth. In addition, the concentration of NO_3^- in acid rain increases along with the rapid growth of nitrogen deposition. In this study, we investigated the impact of simulated acid rain with different SO_4^{2-}/NO_3^- (S/N) ratios, which were 1:0, 5:1, 1:1, 1:5 and 0:1, on Chinese fir sapling growth from March 2015 to April 2016. Results showed that Chinese fir sapling height growth rate (HGR) and basal diameter growth rate (DGR) decreased as acid rain pH decreased, and also decreased as the percentage of NO_3^- increased in acid rain. Acid rain pH significantly decreased the Chlorophyll a (Chla) and Chlorophyll b (Chlb) content, and Chla and Chlb contents with acid rain S/N 1:5 were significantly lower than those with S/N 1:0 at pH 2.5. The chlorophyll fluorescence parameters, maximal efficiency of Photosystem II photochemistry (Fv/Fm) and non-photochemical quenching coefficient (NPQ), with most acid rain treatments were significantly lower than those with CK treatments. Root activities first increased and then decreased as acid rain pH decreased, when acid rain S/N ratios were 1:1, 1:5 and 0:1. Redundancy discriminant analysis (RDA) showed that the Chinese fir DGR and HGR had positive correlations with Chla, Chlb, Fv/Fm ratio, root activity, catalase and superoxide dismutase activities in roots under the stress of acid rain with different pH and S/N ratios. The structural equation modelling (SEM) results showed that acid rain NO_3^- concentration and pH had stronger direct effects on Chinese fir sapling HGR and DGR, and the direct effects of acid rain NO_3^- concentration and pH on HGR were lower than those on DGR. Our results suggest that the ratio of SO_4^{2-} to NO_3^- in acid rain is an important factor which could affect the sustainable development of monoculture Chinese fir plantations in southern China.

1. Introduction

With the rapid growth of the population and economy, China has established the world's largest area of tree plantations during the past few decades (Tang et al., 2016). Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook), an economically valuable conifer with good wood quality, high yield, and multiple uses, is a mainly indigenous tree species that occupies approximately 25% of plantations in subtropical areas of southern China (Duan et al., 2016; Li et al., 2017; Ma et al., 2017). However, owing to the increasing seriousness of acid rain in southern China, the total Chinese fir plantation areas damaged by acid rain in seven provinces of South China were estimated to be 4.913×10^5 ha (Fan and Wang, 2000; Blanco et al., 2012).

The southern region of China, where precipitation in the late 1980s was found to have average pH's between 3.5 and 4.8, has become the third region in the world seriously affected by acid rain (Fan and Wang, 2000; Singh and Agrawal, 2008; Sun et al., 2016). SO_4^{2-} was the dominant anion in precipitation as the majority of energy is generated from coal combustion (Liang et al., 2016; Zhang et al., 2017). According to the data derived from the State Environmental Protection Administration of China, total SO_2 emissions in China has been increasing compared to the mid-1990s (Tu et al., 2005). The effects of acid rain on plants can be determined by the altering of the biochemical and physiological processes, such as chlorosis and necrosis, nutrient loss from leaves, variation of several enzyme activities (Yu et al., 2002; Singh and Agrawal, 2008; Du et al., 2017). Cape (1993) reported that

* Corresponding author.
E-mail address: zhang8811@njfu.edu.cn (J. Zhang).

https://doi.org/10.1016/j.ecoenv.2018.04.071
Received 6 February 2018; Received in revised form 26 April 2018; Accepted 29 April 2018
0147-6513/

both conifers and broadleaved tree saplings showed subtle changes in the structural characteristics of leaf surfaces after exposure to acid rain at pH 3.5. Liao and Chen (1992) found that acid rain with pH 2.0 can significantly inhibit the fine root growth of Chinese fir. Ramlall et al. (2015) reported that acid rain with pH 3.0 caused leaf tip necrosis, abnormal bilobed leaf tips, leaf necrotic spots and chlorosis, and reduced leaf chlorophyll concentration and root biomass.

Since the late 1990s, China has been implementing flue gas desulfurization and phasing out small inefficient units in the power sector (Chan and Yao, 2008), so sulfate ion (SO_4^{2-}) in acid rain has decreased significantly (Lv et al., 2014). However, the amount of motor vehicle traffic has increased rapidly in China, and NO_x is emitted into atmosphere through tailpipes (Liu et al., 2018). Therefore, acid rain pollution is gradually changing from sulfuric acid dominated rain to nitric acid dominated rain (Niu et al., 2014). Combining the acidification effects of sulfate ion and nitrate ion, the benefits of SO_2 reduction would almost be negated by increased N emissions (Zhao et al., 2009). Prior studies found that the inhibitory effects of nitric acid rain on litter decomposition, soil microbial biomass, and most enzyme activities were more significant than those of sulfuric acid rain in subtropical forests of China (Lv et al., 2014; Liu et al., 2017). Liu et al. (2018) reported that fine-root element contents and antioxidant enzyme activities were significantly affected by the acid rain SO_4^{2-}/NO_3^- ratio. However, there is still a lack of information on the effects of acid rain with different SO_4^{2-}/NO_3^- ratios on Chinese fir growth. This fundamental knowledge would be useful for making informed management decisions to promote sustainable development of monoculture Chinese fir plantations in southern China.

To explore the effects of acid rain with different ratios of SO_4^{2-} to NO_3^- on Chinese fir sapling growth, we established a series of pot experiments. Our primary objective was to discuss the impacts of increasing acid rain NO_3^- concentration and decreasing acid rain pH relative to control in terms of their effects on Chinese fir chlorophyll content, chlorophyll fluorescence parameters, root activity and antioxidant enzymes activities, which are sensitive indicators of forest productivity. Based on the previous studies and reports, we hypothesized that acid rain would depress the Chinese fir sapling growth, and the inhibitory effects would increase with acid rain NO_3^- concentration increases.

2. Materials and methods

2.1. Plant material and treatments

The study was conducted in the intelligent greenhouse of Xiashu Ecological Station of Nanjing Forestry University (31°7′ N, 119°12′ E), Jiangsu Province, China, from March 2015 to April 2016. One-year old Chinese fir saplings of uniform height were selected as our research object. The average height and ground diameter were 27.15 ± 1.33 cm and 5.03 ± 0.28 cm, respectively. The saplings were transplanted in plastic flowerpots (25 cm height × 20 cm diameter) with yellow brown clay soil collected from plantations nearby. The soil pH was 6.31 ± 0.01. These saplings had two months for recover after they were transplanted. During recover period, we watered them with distilled water.

After two months, eighty saplings with healthy growth and uniform height were selected for the simulated acid rain treatments. Five stock solutions of acid rain were prepared by mixing 0.5 mol L^{-1} H_2SO_4 and 0.5 mol L^{-1} HNO_3 at molar ratios of 1:0, 5:1, 1:1, 1:5 and 0:1. The experiment consisted of 16 treatments: CK (distilled water, pH = 7.0), SAR treatments with 1:0 for SO_4^{2-}/NO_3^- (S1 pH = 4.5, S2 pH = 3.5, S3 pH = 2.5), SAR treatments with 5:1 for SO_4^{2-}/NO_3^- (S4 pH = 4.5, S5 pH = 3.5, S6 pH = 2.5), SAR treatments with 1:1 for SO_4^{2-}/NO_3^- (S7 pH = 4.5, S8 pH = 3.5, S9 pH = 2.5), SAR treatments with 1:5 for SO_4^{2-}/NO_3^- (S10 pH = 4.5, S11 pH = 3.5, S12 pH = 2.5), SAR treatments with 0:1 for SO_4^{2-}/NO_3^- (S13 pH = 4.5, S14 pH = 3.5, S15 pH = 2.5). The total amount of simulated acid rain was 670.38 mm based the annual average precipitation (1117.29 mm) and acid rain frequency (60%) (Liu et al., 2017). The monthly volume of simulated acid rain applied to every flowerpot was 1754.16 ml, which was calculated by the monthly amount of acid rain (55.865 mm) and the area of flowerpot (314 cm^2). Each sapling was sprayed four times a month from May 2015 to April 2016, and each time with 438.54 ml of solution.

2.2. Growth measurement

Sapling height was measured using a tape rule from the base of the stem to the terminal bud. Stem basal diameter was measured by a Vernier caliper at the base of stem (Guo et al., 2016). Sapling height and stem basal diameter measurements were taken in April 30, 2015 and April 30, 2016, respectively. The relative growth rate of height (HGR) and basal diameter (DGR) were calculated using the following equations (Mofunanya and Soonen, 2017):

$$HGR = (H_2 - H_1)/H_1 \tag{1}$$

$$DGR = (D_2 - D_1)/D_1 \tag{2}$$

where H_1 is initial sapling height (cm), H_2 is final sapling height (cm), D_1 is initial basal diameter (mm), D_2 is final basal diameter (mm).

2.3. Chlorophyll content

The chlorophyll content was measured according to the procedure described by Gassama et al. (2015). The amount of 0.1 g of leaf was homogenized in a 10 ml mixture with acetone and ethyl alcohol (1:1, v/v) for 10 h in a darkroom, and then was centrifuged at 2500 rpm for 20 min and supernatant was extracted. About 2.5 ml of samples were pipetted into microfuge and the chlorophyll content was measured by using scanning spectrophotometer UV–VIS. The samples were read at wavelength of 663 and 645 nm.

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence (Fs) measurements were performed according to the method of Osório et al. (2013) and Ying et al. (2014) using a chlorophyll fluorescence imager (CF Imager, Technologica, UK). Prior to the measurement of Fo and Fm (minimum and maximum fluorescence), the leaves were dark-adapted for 30 min and then the light-adapted parameters of Fs, Fo′ and Fm′ were determined after applying actinic light [500 μmol m^{-2} s^{-1}] to the leaves for light adaptation. The maximal efficiency of Photosystem II (PSII) photochemistry (Fv/Fm = (Fm-Fo)/Fm), actual PSII efficiency (Fv′/Fm′), the effective efficiency of PSII photochemistry (ΦPSII), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) were calculated using equations according to Wang et al. (2017).

2.5. Root activity

Root activity was measured using the TTC (triphenyl tetrazolium chloride) method (Zhang et al., 2015) and expressed as the deoxidization ability (μg g^{-1} h^{-1}). Dehydrogenase was expressed as the deoxidized TTC quantity, which was an index of root activity. Ten milliliter solutions of equal quantities of TTC (0.4%) and phosphate buffer were added to root samples (0.5 g) and kept in the dark at 37 °C for 2 h. The reaction was stopped with 1 mol L^{-1} H_2SO_4. The roots were ground and transferred into a tube with ethyl acetate to a total volume of 10 ml. The solution was measured at the absorbance of 485 nm using a scanning spectrophotometer UV–VIS.

2.6. Antioxidant enzymes activities

Prior to determination of antioxidant enzyme activities, a crude enzyme extract was prepared by homogenizing 2–3 g leaf or fine root tissues with 5 ml of an ice-cold phosphate buffer (50 mM, pH 7.8). The homogenate was centrifuged at 15,000 g for 20 min. All steps in the preparation of enzyme extract were carried out at 4 °C. The supernatant was used as the crude extract for the assay of activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and the activities of the enzymes were expressed as unit mg protein^{-1} min^{-1} (Khan et al., 2017; Liu et al., 2018). Protein content (Pro) was determined according to Teisseire and Guy (2000) using bovine albumin for calibration.

2.7. Statistical analyses

The Duncan test was used when one-way ANOVA (SPSS Inc., Chicago, Ill., USA) showed that acid rain treatment effects on Chinese fir growth properties were significant. Two-way ANOVA was used to test the main effects and interactions of acid rain pH, S/N ratios on growth rates, foliar and roots properties by SPSS 19.0. Redundancy discriminant analysis (RDA) was performed to reveal the relationships between the acid rain pH, S/N ratios, growth rate, foliar traits and root traits by using Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA). Structural equation modelling (SEM) was used to investigate how acid rain S/N ratio and pH affected Chinese fir growth rates, foliar and root properties in the short-term (one year). The model was used to test whether acid rain S/N ratio and pH influenced the growth rate directly or indirectly through modifying foliar characteristics and/or root properties. SEM analyses were performed using AMOS 24.0 (SPSS Inc., Chicago, Ill., USA).

3. Results

3.1. Growth rate

Sapling height growth rate (HGR) first increased and then decreased as acid rain pH decreased (Fig. 1A). However, no significant differences of growth of height were found among different acid rain pH values with the same S/N ratios. In contrast, the growth rate of sapling basal diameter (DGR) significantly decreased as acid rain pH decreased (Fig. 1B). In addition, there were significant differences for HGR ($p < 0.01$) and DGR ($p < 0.001$) among acid rain S/N ratios in this study. S13 and S15 significantly decreased HGR compared to CK treatments ($p < 0.05$). DGR with CK treatment were significantly higher than those with acid rain treatments ($p < 0.05$), except for weaker acid rain treatments with S1, S4, S5 and S7 treatments.

3.2. Chlorophyll content

The contents of both chlorophyll a (Chla) and chlorophyll b (Chlb) with stronger acid rain treatments (pH= 3.5, 2.5) were significantly lower than those with CK treatments ($p < 0.05$, Fig. 2A,B). In addition, the contents of Chla and Chlb decreased as acid rain pH decreased ($p < 0.001$) and NO_3^- concentration increased in this study. There were significant differences for Chlb among acid rain S/N ratios ($p < 0.01$). Finally, statistically significant acid rain pH and the interaction of acid rain S/N ratio and pH influencing the ratio of Chla to Chlb were found in our study ($p < 0.05$).

3.3. Chlorophyll fluorescence

The chlorophyll fluorescence parameters, the Fv/Fm ratio, Fv'/Fm' ratio, qP, NPQ and ΦPSII, varied with the acid rain S/N ratio and pH doses compared with the control (Table 1). Two-way ANOVA revealed that the interaction of acid rain S/N ratio and pH significantly affected these chlorophyll fluorescence parameters in our study. Fv/Fm ratios with CK treatments were significantly higher than those with acid rain treatments ($p < 0.05$) and decreased with NO_3^- concentration increased in acid rain ($p < 0.001$). However, there were no significant differences for Fv'/Fm' ratio, qP and ΦPSII among acid rain pH values. In addition, acid rain S/N ratio significantly influenced Fv'/Fm' ratio, NPQ and ΦPSII in our study.

3.4. Root activity

Two-way ANOVA revealed that acid rain S/N ratio, pH, and their interaction significantly affected root activity (Fig. 3). When the SO_4^{2-} concentrations in acid rain were higher than NO_3^- (S1, S2, S3, S4, S5, S6), TTC values decreased as acid rain pH decreased. When acid rain S/

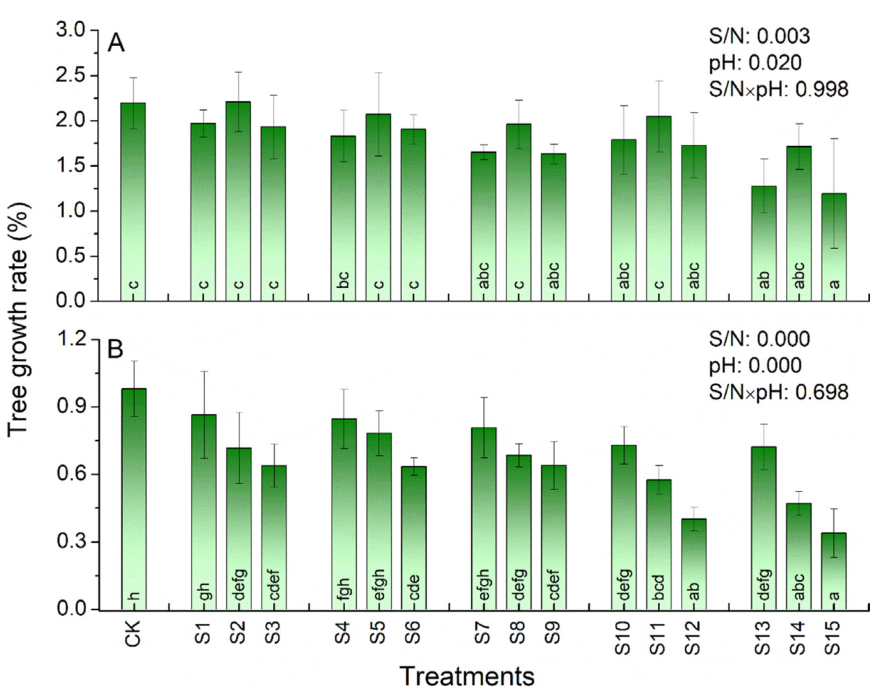

Fig. 1. Changes of the tree growth rates under different simulated acid rain treatments. A, tree height growth rate; B, basal diameter growth rate. The experimental treatments are: CK = control check; S1 = pH 4.5, S:N 1:0; S2 = pH 3.5, S:N 1:0; S3 = pH 2.5, S:N 1:0; S4 = pH 4.5, S:N 5:1; S5 = pH 3.5, S:N 5:1; S6 = pH 2.5, S:N 5:1; S7 = pH 4.5, S:N 1:1; S8 = pH 3.5, S:N 1:1; S9 = pH 2.5, S:N 1:1; S10 = pH 4.5, S:N 1:5; S11 = pH 3.5, S:N 1:5; S12 = pH 2.5, S:N 1:5; S13 = pH 4.5, S:N 1:0; S14 = pH 3.5, S:N 1:0; S15 = pH 2.5, S:N 1:0. Different letters indicate significant difference ($p < 0.05$) among different acid rain acidity with the same acid rain S/N ratio and same season based on one-way ANOVA, followed by a Duncan test. S/N, acid rain S/N ratio; pH, acid rain pH. Two-way ANOVA was applied to indicate significant difference among variances.

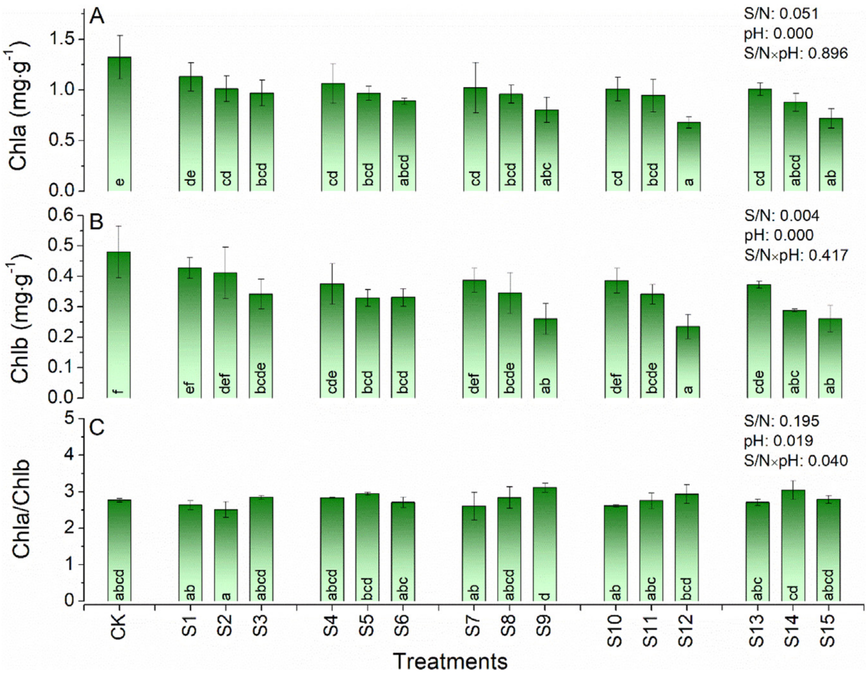

Fig. 2. Changes of the content of chlorophyll in leaf under different simulated acid rain treatments. A, chlorophyll A; B, chlorophyll B; C, ratio of chlorophyll A to chlorophyll B. The experimental treatments are: CK = control check; S1 = pH 4.5, S:N 1:0; S2 = pH 3.5, S:N 1:0; S3 = pH 2.5, S:N 1:0; S4 = pH 4.5, S:N 5:1; S5 = pH 3.5, S:N 5:1; S6 = pH 2.5, S:N 5:1; S7 = pH 4.5, S:N 1:1; S8 = pH 3.5, S:N 1:1; S9 = pH 2.5, S:N 1:1; S10 = pH 4.5, S:N 1:5; S11 = pH 3.5, S:N 1:5; S12 = pH 2.5, S:N 1:5; S13 = pH 4.5, S:N 1:0; S14 = pH 3.5, S:N 1:0; S15 = pH 2.5, S:N 1:0. Different letters indicate significant difference ($p < 0.05$) among different acid rain acidity with the same acid rain S/N ratio and same season based on one-way ANOVA, followed by a Duncan test. S/N, acid rain S/N ratio; pH, acid rain pH. Two-way ANOVA was applied to indicate significant difference among variances.

N ratios were 1:1, 1:5 and 0:1, TTC values first increased (pH = 3.5) and then decreased (pH = 2.5) as acid rain pH decreased.

3.5. Autioxidant enzyme activity

Sulfuric acid rain (S/N 1:0, 5:1) significantly increased foliar SOD activity compared to CK treatment (Fig. 4A). When acid rain S/N ratio was 1:1, there were no significant differences between acid rain treatments and CK. As NO_3^- concentration increased in acid rain, weaker acid rain significantly increased foliar SOD activity ($p < 0.05$). In contrast, root SOD activity with acid rain treatment (S9, S10, S12, S13, S14) was significantly lower than that with CK (Fig. 4B). In leaves, POD activity significantly increased as acid rain pH decreased ($p < 0.05$) (Fig. 4C). However, there was no significant difference for POD activity in roots among acid rain pH ($p = 0.816$) (Fig. 4D). Stronger acid rain (pH = 3.5 and 2.5) significantly increased CAT activity in leaves ($p < 0.05$) (Fig. 4E). However, as acid rain NO_3^- concentration increased, CAT activities in roots with acid rain treatments (S8, S9, S10, S14 and S15) were significantly lower than those with CK (Fig. 4F). In addition, two-way ANOVA revealed that acid rain S/N ratio and the interaction of acid rain S/N and pH significantly affected autioxidant enzyme activity (Fig. 4).

Table 1
Chlorophyll fluorescence images of maximum PSII photo-chemical efficiency (Fv/Fm), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) in Chinese fir leaf after addition of different acid rain treatments.

Treatments		Fv/Fm	Fv′/Fm′	qP	NPQ	ΦPSII
CK		0.884 ± 0.010^{f}	0.655 ± 0.022^{cde}	0.635 ± 0.045^{d}	1.963 ± 0.152^{f}	0.375 ± 0.040^{b}
1:0	S1	0.855 ± 0.009^{de}	0.582 ± 0.065^{a}	0.631 ± 0.067^{cd}	1.849 ± 0.124^{ef}	0.359 ± 0.004^{ab}
	S2	0.866 ± 0.019^{e}	0.659 ± 0.016^{cde}	0.570 ± 0.031^{ab}	1.605 ± 0.071^{bcde}	0.370 ± 0.012^{ab}
	S3	0.854 ± 0.021^{de}	0.650 ± 0.025^{bcd}	0.571 ± 0.016^{ab}	1.647 ± 0.090^{bcdef}	0.367 ± 0.025^{ab}
5:1	S4	0.854 ± 0.002^{de}	0.707 ± 0.008^{ef}	0.575 ± 0.007^{abc}	1.638 ± 0.200^{bcdef}	0.402 ± 0.008^{cd}
	S5	0.821 ± 0.003^{c}	0.615 ± 0.066^{abc}	0.598 ± 0.054^{abcd}	1.816 ± 0.179^{def}	0.359 ± 0.008^{ab}
	S6	0.809 ± 0.006^{abc}	0.627 ± 0.020^{abc}	0.603 ± 0.023^{abcd}	1.598 ± 0.186^{bcde}	0.372 ± 0.004^{b}
1:1	S7	0.841 ± 0.006^{d}	0.649 ± 0.033^{bcd}	0.579 ± 0.001^{abcd}	1.580 ± 0.089^{bcde}	0.372 ± 0.005^{b}
	S8	0.805 ± 0.002^{abc}	0.654 ± 0.020^{cde}	0.593 ± 0.010^{abcd}	1.764 ± 0.118^{cdef}	0.384 ± 0.004^{bc}
	S9	0.821 ± 0.004^{c}	0.713 ± 0.018^{f}	0.581 ± 0.023^{abcd}	1.897 ± 0.251^{ef}	0.410 ± 0.010^{d}
1:5	S10	0.813 ± 0.012^{abc}	0.657 ± 0.009^{cde}	0.583 ± 0.010^{abcd}	1.455 ± 0.001^{abc}	0.381 ± 0.002^{bc}
	S11	0.818 ± 0.005^{c}	0.690 ± 0.013^{def}	0.549 ± 0.001^{a}	1.165 ± 0.186^{a}	0.374 ± 0.006^{b}
	S12	0.796 ± 0.003^{ab}	0.605 ± 0.017^{abc}	0.623 ± 0.027^{bcd}	1.831 ± 0.223^{def}	0.374 ± 0.006^{b}
0:1	S13	0.814 ± 0.010^{bc}	0.653 ± 0.006^{cde}	0.552 ± 0.001^{a}	1.500 ± 0.039^{bcd}	0.356 ± 0.005^{ab}
	S14	0.793 ± 0.018^{a}	0.635 ± 0.009^{abcd}	0.589 ± 0.033^{abcd}	1.389 ± 0.376^{ab}	0.370 ± 0.026^{ab}
	S15	0.803 ± 0.014^{abc}	0.597 ± 0.010^{ab}	0.582 ± 0.019^{abcd}	1.951 ± 0.193^{f}	0.343 ± 0.006^{a}
S/N		0.000^{***}	0.022^{*}	0.696	0.037^{*}	0.000^{***}
pH		0.000^{***}	0.450	0.495	0.003^{**}	0.793
S/N × pH		0.001^{**}	0.000^{***}	0.016^{*}	0.002^{**}	0.000^{***}

Note: The experimental treatments are: CK = control check; S1 = pH 4.5, S:N 1:0; S2 = pH 3.5, S:N 1:0; S3 = pH 2.5, S:N 1:0; S4 = pH 4.5, S:N 5:1; S5 = pH 3.5, S:N 5:1; S6 = pH 2.5, S:N 5:1; S7 = pH 4.5, S:N 1:1; S8 = pH 3.5, S:N 1:1; S9 = pH 2.5, S:N 1:1; S10 = pH 4.5, S:N 1:5; S11 = pH 3.5, S:N 1:5; S12 = pH 2.5, S:N 1:5; S13 = pH 4.5, S:N 1:0; S14 = pH 3.5, S:N 1:0; S15 = pH 2.5, S:N 1:0. S/N, acid rain S/N ratio; pH, acid rain pH. Different letters indicate significant difference ($p < 0.05$) among different acid rain treatments on one-way ANOVA, followed by a Duncan test. Two-way ANOVA was applied to indicate significant differences among variances. ***indicates significant difference at $p < 0.001$; ** indicates significant difference at $p < 0.01$; * indicates significant difference at $p < 0.05$.

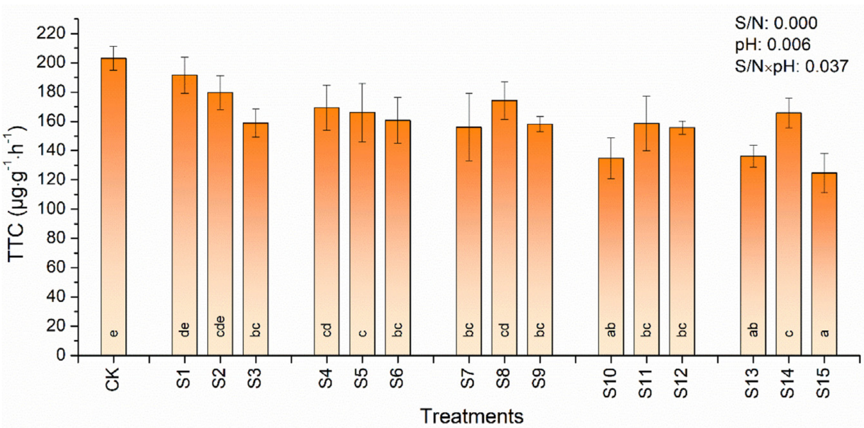

Fig. 3. Changes of the root activity (TTC) under different simulated acid rain treatments. S/N, acid rain S/N ratio; pH, acid rain pH. The experimental treatments are: CK = control check; S1 = pH 4.5, S:N 1:0; S2 = pH 3.5, S:N 1:0; S3 = pH 2.5, S:N 1:0; S4 = pH 4.5, S:N 5:1; S5 =;pH 3.5, S:N 5:1; S6 = pH 2.5, S:N 5:1; S7 = pH 4.5, S:N 1:1; S8 = pH 3.5, S:N 1:1; S9 = pH 2.5, S:N 1:1; S10 = pH 4.5, S:N 1:5; S11 = pH 3.5, S:N 1:5; S12 = pH 2.5, S:N 1:5; S13 = pH 4.5, S:N 1:0; S14 = pH 3.5, S:N 1:0; S15 = pH 2.5, S:N 1:0. Different letters indicate significant difference ($p < 0.05$) among different acid rain acidity with the same acid rain S/N ratio and same season based on one-way ANOVA, followed by a Duncan test. Two-way ANOVA was applied to indicate significant difference among variances.

3.6. Linking sapling growth rates and Chl content, Chl fluorescence, root activity and autioxidant enzyme activities

In the RDA of sapling growth rates with acid rain, leaf and root properties as the explanatory variables Axis 1 accounted for 48.69% of the variation in the dataset, with 10.23% of the variation accounted for by Axis 2 (Fig. 5). High HGR with high TTC, SOD_r, CAT_r, Fv/Fm, Chla and Chlb were found at the right-hand end of the ordination plots and were associated with lower acid rain NO_3^- concentration and POD_r. DGR, acid rain pH, Chla, Chlb, CAT_r, and Fv/Fm ratio increased along the y-axis, whereas the CAT_f and POD_f decreased.

3.7. SEM results

Fig. 6 shows the structural equation modelling (SEM) as estimated by AMOS. Each of the observed variables is displayed in a rectangle (Liu et al., 2018). The χ^2 test showed that the model generated $\chi^2 = 19.358$, df = 17, and p = 0.308 (> 0.05). The goodness-of-fit index (GFI) was 0.924 (> 0.900), and the root mean square error of approximation (RMSEA) was 0.056 (< 0.080). The direct effects of acid rain NO_3^- concentration (−0.70, $p < 0.001$) and pH (0.63, $p < 0.001$) on sapling DGR were significant. However, the direct effects of acid rain NO_3^- concentration (−0.34, $p < 0.05$) and pH (−0.29, $p < 0.05$) on HGR were lower than those on DGR. Both acid rain NO_3^- concentration and pH also altered DGR (0.17 and −0.01, respectively) and HGR (−0.14 and 0.31, respectively) indirectly through changes in the properties of roots and leaves. Therefore, the total effects of acid rain NO_3^- concentration and pH on DGR were − 0.53 and 0.62, respectively, and the total effects on HGR were − 0.48 and 0.02.

4. Discussion

Rapid economic growth, increasing fossil fuel energy consumption and the rapidly growing numbers of motor vehicles have resulted in

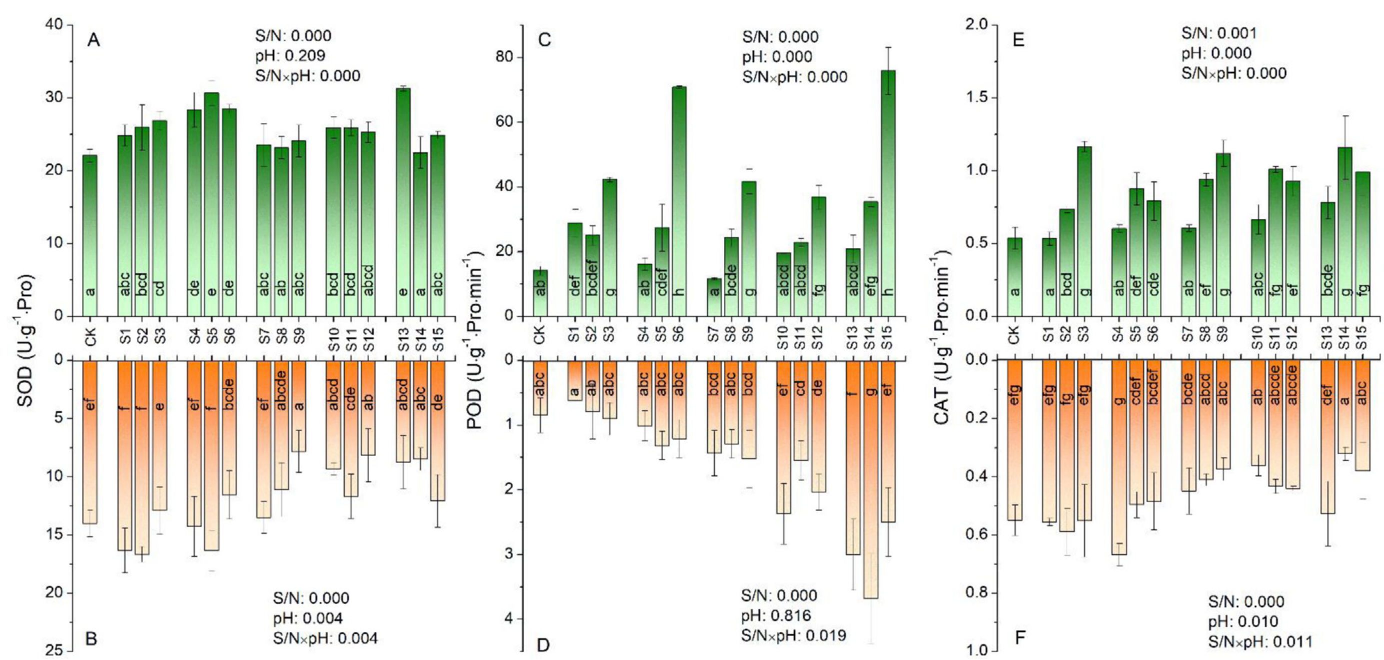

Fig. 4. Changes of the foliar (green, A, C, E) and root (orange, B, D, F) enzymatic antioxidants under different simulated acid rain treatments. S/N, acid rain S/N ratio; pH, acid rain pH; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase. The experimental treatments are: CK = control check; S1 = pH 4.5, S:N 1:0; S2 = pH 3.5, S:N 1:0; S3 = pH 2.5, S:N 1:0; S4 = pH 4.5, S:N 5:1; S5 = pH 3.5, S:N 5:1; S6 = pH 2.5, S:N 5:1; S7 = pH 4.5, S:N 1:1; S8 = pH 3.5, S:N 1:1; S9 = pH 2.5, S:N 1:1; S10 = pH 4.5, S:N 1:5; S11 = pH 3.5, S:N 1:5; S12 = pH 2.5, S:N 1:5; S13 = pH 4.5, S:N 1:0; S14 = pH 3.5, S:N 1:0; S15 = pH 2.5, S:N 1:0. Different letters indicate significant difference ($p < 0.05$) among different acid rain acidity with the same acid rain S/N ratio and same season based on one-way ANOVA, followed by a Duncan test. Two-way ANOVA was applied to indicate significant difference among variances. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

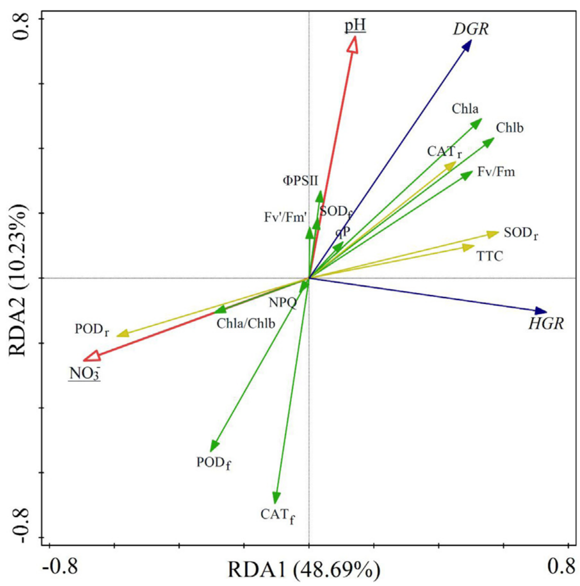

Fig. 5. Redundancy analysis (RDA) of sapling growth rates and acid rain, leaf and root properties. The angle and length of the arrows indicate the direction and strength of the relationship of sapling growth rates and acid rain, leaf and root properties. HGR, sapling growth rate of height; DGR, sapling growth rate of basal diameter; NO_3^-, the percentage of NO_3^- in total amount of SO_4^{2-} and NO_3^- in acid rain; pH; acid rain pH; Chla, the content of chlorophyll a; Chlb, the content of chlorophyll b; Chla/Chlb, the ratio of Chla to Chlb; Fv/Fm, the maximal efficiency of Photosystem II (PSII) photochemistry; Fv'/Fm', actual PSII efficiency; ΦPSII, the effective efficiency of PSII photochemistry; qP, photochemical quenching coefficient; NPQ, non-photochemical quenching coefficient; SOD_f, foliar superoxide dismutase activity; POD_f, foliar peroxidase activity; CAT_f, foliar catalase activity; TTC, triphenyl tetrazolium chloride, root activity; SOD_r, root superoxide dismutase activity; POD_r, root peroxidase activity; CAT_r, root catalase activity.

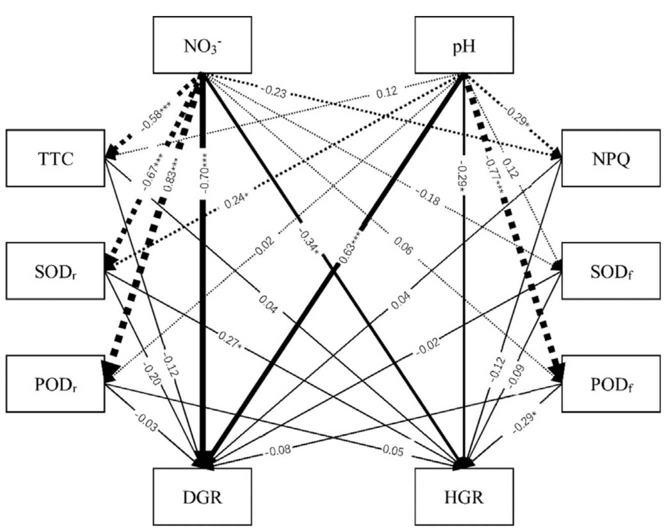

Fig. 6. Structural equation models of acid rain S/N ratio (the ratio of SO_4^{2-} to NO_3^-) and intensity (pH) effects on tree aboveground biomass (AB). (χ^2 = 13.713; df = 9, p = 0.133 > 0.05; GFI = 0.930 > 0.900; RMSEA = 0.072). Numbers on arrows are standardized path coefficients. The width of arrows indicates the strength of the causal influence. Solid arrows mean a direct effect on growth rate; dashes represent an indirect path to growth rate. HGR, sapling growth rate of height; DGR, sapling growth rate of basal diameter; NO_3^-, the percentage of NO_3^- in total amount of SO_4^{2-} and NO_3^- in acid rain; pH; acid rain pH; Chla/Chlb, the ratio of chlorophyll a to chlorophyll b; NPQ, non-photochemical quenching coefficient; CAT_f, foliar catalase activity; SOD_r, root superoxide dismutase activity; POD_r, root peroxidase activity; CAT_r, root catalase activity.

large amounts of nitrogen oxide pollutant to be emitted into the ambient atmosphere (Hao et al., 2002; Liu et al., 2017). NO_3^- in acid rain would stimulate seedling growth as a nitrogenous fertilizer in the short term (Lee and Weber, 1979; Mofunanya and Soonen, 2017). However, the ability of exchange with hydroxyl groups (OH^-) of NO_3^- is lower than that of SO_4^{2-}, which would result in more severe soil acidification (Lindberg et al., 1990). In our study, we found that the height growth rates of Chinese fir sapling with acid rain treatments at S/N 0:1 and pH 4.5 and 2.5 were significantly lower than that with CK treatments, and growth rates of basal diameter significantly decreased as NO_3^- concentration increased in acid rain. It may be because acid rain directly influenced soil properties and then indirectly affected plant growth (Tamm et al., 1977). Earlier studies found that nitric acid rain had more inhibitory effects on soil pH and microbial activity than sulfuric acid rain (Lv et al., 2014; Liu et al., 2017), and nitric acid rain slowed down soil carbon, nitrogen, and phosphorus mineralization (Lv et al., 2014).

Plants absorb energy by leaf chlorophyll from light to support photosynthetic production (Krause and Weis, 1991; Du et al., 2017; Ren et al., 2018). Therefore, leaf chlorophyll content shows a strong correlation with plant growth. In our study, Chla and Chlb contents of Chinese fir sapling significantly decreased as acid rain pH decreased. This was in accordance with the findings that leaf chlorophyll content showed a reduction of 6.71% per pH unit across 67 terrestrial plant species in China (Du et al., 2017). In addition, we also found that the ratios of Chla to Chlb were significantly affected by acid rain pH. Prior studies pointed out that chlorophyll contents decreased by acid rain may be due to foliar leaching of magnesium, which is one of the major components of chlorophyll (Morrison et al., 1984; Liu et al., 2011). However, acid rain S/N ratio only significantly affected the Chlb contents in our study. What's more, under the stronger acid rain (pH = 2.5), Chla and Chlb contents with acid rain S/N 1:5 were significantly lower than those with S/N 1:0. This suggests that decreasing acid rain S/N ratio would increase the inhibitory effects of acid rain pH on plant growth. The induced Chl fluorescence as a kinetic parameter was an ideal method to research and explore the effect of biotic or abiotic stress on plants (Guo et al., 2016). Yu et al. (2002) reported that acid rain treatments induced a significant decrease in the Fv/Fm ratio and ΦPSII. In this study, we found that Fv/Fm ratio and NPQ with most acid rain treatments were significantly lower than those with CK treatments. Plants would produce enzymatic antioxidants, such as SOD, POD and CAT, to cope with acid rain stress (Kazemi et al., 2010; Liu et al., 2018). In our study, stronger acid rain (pH = 3.5, 2.5) significantly increased POD and CAT activities in leaves compared to CK treatments, which is consistent with Velikova et al. (2000). In addition, under the strongest acid rain treatments (pH = 2.5), POD activities in leaf with acid rain S/N 5:1 and 0:1 were significantly higher than those with CK treatments, which also indicated that increasing acid rain NO_3^- concentration would increase the stress of acid rain pH on plant growth.

Acid rain also would inhibit plant growth by leaching of soil nutrient cations and increasing the availability of toxic metals, such as aluminum toxicity (Vanguelova et al., 2007; Du et al., 2017). Plant roots would suffer from the toxicity of aluminum, as an effect of acid rain. Liu et al. (2018) reported that fine root biomass significantly decreased with acid rain pH and S/N ratio decreased. In this study, we found that root activity decreased as acid rain pH decreased when SO_4^{2-} concentration was higher than NO_3^- concentration in acid rain. Root activities first increased and then decreased with acid rain pH decreased when acid rain S/N ratios were, respectively, 1:1, 1:5 and 0:1. This may be because the promoting effect of N fertilizers in nitric acid rain on root activity is higher than the inhibitory impacts of acid rain pH at acid rain pH 3.5. The RDA analysis showed that SOD and CAT activities in roots had positive correlations with sapling growth rate, whereas, POD activity in leaf had negative correlation with growth rates and increased as NO_3^- concentration increased in acid rain. This suggested that sapling growth of Chinese fir could resist the stress of acid rain by scavenging and detoxification of active oxygen species in roots (Velikova et al., 2000). In addition, the SEM model indicated that both the NO_3^- concentration and pH in acid rain had stronger inhibitory effects on growth rates of Chinese fir sapling. It suggested that the change of acid rain types further complicates the ongoing challenge of Chinese fir plantation development in southern China.

It should be noted that we simulated acid rain only over one year in greenhouse. In field, Chinese fir would experience a more complicated environment. In the future, we should design a *meso*cosm experiment for long-term study of the effects of acid rain S/N ratio on Chinese fir growth.

5. Conclusion

Using a one-year greenhouse experiment, acid rain treatments with different pH and S/N ratios changed Chinese fir sapling growth. In the period of simulated acid rain, Chinese fir sapling height and basal diameter growth rate decreased as acid rain pH and S/N ratios decreased. Both the contents of Chla and Chlb with stronger acid rain treatments (pH= 3.5, 2.5) were significantly lower than those with CK treatments. The chlorophyll fluorescence parameters, Fv/Fm ratio and NPQ with most acid rain treatments were significantly lower than those with CK treatments. Root activities first increased and then decreased as acid rain pH decreased when acid rain S/N ratios were 1:1, 1:5 and 0:1. The Chinese fir DGR and HGR had positive correlations with Chla, Chlb, Fv/Fm ratio, root activity, CAT and SOD activities in roots under the stress of acid rain with different pH and S/N ratios. In summary, acid rain with high NO_3^- concentration would change foliar and root properties and inhibit Chinese fir sapling growth. This inhibitory effect of high NO_3^- concentration in acid rain may seriously alter the sustainable development of Chinese fir plantation in southern China.

Acknowledgements

Financial support for this study by the National Natural Science Foundation of China (Grant no. 31470709, 31200534), National Special Fund for Forestry Scientific Research in the Public Interest (Grant no. 201504406), Agricultural Science and Technology Innovation Fund of Jiangsu Province (Grant no. CX(17)1004), Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Doctorate Fellowship Foundation of Nanjing Forestry University (2149113) is gratefully acknowledged. USGS's. We would thank Prof. Donald L. DeAngelis, from the Wetland and Aquatic Research Center, U.S. Geological Survey, for his valuable comments andsuggestions that have greatly improved the quality of this manuscript.

References

Blanco, J.A., Wei, X., Jiang, H., Jie, C.Y., Xin, Z.H., 2012. Enhanced nitrogen deposition in south-east china could partially offset negative effects of soil acidification on biomass production of Chinese fir plantations. Can. J. For. Res. 42 (42), 437–450.

Cape, J.N., 1993. Direct damage to vegetation caused by acid rain and polluted cloud: definition of critical levels for forest trees. Environ. Pollut. 82 (2), 167–180.

Chan, C.K., Yao, X., 2008. Air pollution in mega cities in China. Atmos. Environ. 42, 1–42.

Du, E., Dong, D., Zeng, X., Sun, Z., Jiang, X., Vries, W.D., 2017. Direct effect of acid rain on leaf chlorophyll content of terrestrial plants in china. Sci. Total Environ. 605–606, 764–769.

Duan, H., Cao, S., Zheng, H., Hu, D., Lin, J., Lin, H., Hu, R., Sun, Y., Li, Y., 2016. Variation in the growth traits and wood properties of Chinese fir from six provinces of southern China. Forests 7 (9), 192.

Fan, H.B., Wang, Y.H., 2000. Effects of simulated acid rain on germination, foliar damage, chlorophyll contents and seedling growth of five hardwood species growing in China. For. Ecol. Manag. 126 (3), 321–329.

Gassama, U.M., Puteh, A.B., Abd-Halim, M.R., Kargbo, B., 2015. Influence of municipal wastewater on rice seed germination, seedling performance, nutrient uptake, and chlorophyll content. J. Crop Sci. Biotechnol. 18 (1), 9–19.

Guo, Y.Y., Yu, H.Y., Kong, D.S., Yan, F., Zhang, Y.J., 2016. Effects of drought stress on growth and chlorophyll fluorescence of *Lycium ruthenicum* Murr. seedlings. Photosynthetica 54 (4), 524–531.

Hao, J., Tian, H., Lu, Y., 2002. Emission inventories of NOx from commercial energy consumption in china, 1995–1998. Environ. Sci. Technol. 36 (4), 552.

Kazemi, N., Khavari-Nejad, R.A., Fahimi, H., Saadatmand, S., Nejad-Sattari, T., 2010. Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* L. under nickel stress. Sci. Hortic. 126, 402–407.

Khan, M.N., Mobin, M., Abbas, Z.K., Siddiqui, M.H., 2017. Nitric oxide-induced synthesis of hydrogen sulfide alleviates osmotic stress in wheat seedlings through sustaining antioxidant enzymes, osmolyte accumulation and cysteine homeostasis. Nitric Oxide 68, 91–102.

Krause, G.H., Weis, E., 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu. Rev. Plant Biol. 42 (1), 313–349.

Lee, J.J., Weber, D.E., 1979. The effects of simulated acid rain on seedling emergence and growth of eleven woody species. For. Sci. 25, 393–398.

Li, S., Huang, P., Ding, G., Zhou, L., Tang, P., Sun, M., Zheng, Y., Lin, S., 2017. Optimization of hormone combinations for root growth and bud germination in Chinese fir (*Cunninghamia lanceolata*) clone leaf cuttings. Sci. Rep 7 (1), 5046.

Liang, G., Hui, D., Wu, X., Wu, J., Liu, J., Zhou, G., Zhang, D., 2016. Effects of simulated acid rain on soil respiration and its components in a subtropical mixed conifer and broadleaf forest in southern China. Environ. Sci. 18, 246–255.

Liao, L., Chen, C., 1992. Relationships between simulated acid rain, soil acidification and root growth of Chinese fir and *Schima superba*. Chin. J. Ecol. 11 (1), 23–28.

Lindberg, S.E., Bredemeier, M., Schaefer, D.A., Qi, L., Okita, T., 1990. Atmospheric concentrations and deposition of nitrogen and major ions in conifer forests in the United States and Federal Republic of Germany. Atmos. Environ. 24 (8), 2207–2220.

Liu, T.W., Wu, F.H., Wang, W.H., Chen, J., Li, Z.J., Dong, X.J., Patton, J., Pei, Z.M., Zheng, H.L., 2011. Effects of calcium on seed germination, seedling growth and photosynthesis of six forest tree species under simulated acid rain. Tree Physiol. 31 (4), 402.

Liu, X., Zhang, B., Zhao, W., Wang, L., Xie, D., Huo, W., Zhang, J., 2017. Comparative effects of sulfuric and nitric acid rain on litter decomposition and soil microbial community in subtropical plantation of Yangtze River Delta region. Sci. Total Environ. 601, 669–678.

Liu, X., Zhao, W.R., Meng, M.J., Fu, Z.Y., Xu, L.H., Zha, Y., Yue, J.M., Zhang, S.F., Zhang, J.C., 2018. Comparative effects of simulated acid rain of different ratios of SO_4^{2-} to NO_3^- on fine root in subtropical plantation of China. Sci. Total Environ. 618, 336–346.

Lv, Y., Wang, C., Jia, Y., Wang, W., Ma, X., Du, J., Tian, X., 2014. Effects of sulfuric, nitric, and mixed acid rain on litter decomposition, soil microbial biomass, and enzyme activities in subtropical forests of China. Appl. Soil Ecol. 79, 1–9.

Ma, Z., Zhang, X., Zhang, C., Wang, H., Chen, F., Fu, X., Fang, X., Sun, X., Lei, Q., 2017. Accumulation of residual soil microbial carbon in Chinese fir plantation soils after nitrogen and phosphorus additions. J. For. Res. 1–10.

Mofunanya, A., Soonen, L., 2017. Physiological and morphological responses of *Amaranthus hybridus* L. (green) to simulated nitric and sulphuric acid rain. Br. J. Appl. Sci. Technol. 21 (4), 1–12.

Morrison, I.K., 1984. Acid rain, forests and forestry. In: Stone, E.L. (Ed.). In: Proceedings of the 6th North American Forest Soils Conference, University of Tennessee, Knoxville, pp. 209–219.

Niu, H., He, Y., Xi, X.L., Jie, S., Du, J., Zhang, T., Pu, T., Xin, H., Chang, L., 2014. Chemical composition of rainwater in the Yulong Snow Mountain region, Southwestern China. Atmos. Res. 144, 195–206.

Osório, M.L., Gonçalves, S., Coelho, N., Osório, J., Romano, A., 2013. Morphological, physiological and oxidative stress markers during acclimatization and field transfer of micropropagated Tuberaria major, plants. Plant Cell Tiss. Org. 115 (1), 85–97.

Ramlall, C., Varghese, B., Ramdhani, S., Pammenter, N.W., Bhatt, A., Berjak, P., Sershen, 2015. Effects of simulated acid rain on germination, seedling growth and oxidative metabolism of recalcitrant-seeded *Trichilia dregeana* grown in its natural seed bank. Physio. Plant 153 (1), 149.

Ren, X., Zhu, J., Liu, H., Xu, X., Liang, C., 2018. Response of antioxidative system in rice (*Oryza sativa*) leaves to simulated acid rain stress. Ecotox. Environ. Saf. 148, 851–856.

Singh, A., Agrawal, M., 2008. Acid rain and its ecological consequences. J. Environ. Biol. 29 (1), 15–24.

Sun, X., Wang, Y., Li, H., Yang, X., Sun, L., Wang, X., Wang, T., Wang, W., 2016. Organic acids in cloud water and rainwater at a mountain site in acid rain areas of south china. Environ. Sci. Pollut. Res. 23 (10), 9529.

Tamm, C.O., Wiklander, G., Popović, B., 1977. Effects of application of sulphuric acid to poor pine forests. Water Air Soil Poll. 8 (1), 75–87.

Tang, X., Lu, Y., Fehrmann, L., Forrester, D.I., Guisasola-Rodríguez, R., Pérez-Cruzado, C., Kleinnet, C., 2016. Estimation of stand-level aboveground biomass dynamics using tree ring analysis in a Chinese fir plantation in Shitai county, Anhui province, China. New For. 47 (2), 319–332.

Teisseire, H., Guy, V., 2000. Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (*Lemna minor*). Plant Sci. 153, 65–72.

Tu, J., Wang, H., Zhang, Z., Jin, X., Li, W., 2005. Trends in chemical composition of precipitation in Nanjing, China, during 1992–2003. Atmos. Res. 73 (3), 283–298.

Vanguelova, E.I., Hirano, Y., Eldhuset, T.D., Sas-Paszt, L., Bakker, M.R., Püttsepp, Ü., Godbold, D., 2007. Tree fine root Ca/Al molar ratio-indicator of Al and acidity stress. Plant Biosyst. 141, 460–480.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, 59–66.

Wang, T., Yang, W., Xie, Y., Shi, D., Ma, Y., Sun, X., 2017. Effects of exogenous nitric oxide on the photosynthetic characteristics of bamboo (*Indocalamus barbatus*, McClure) seedlings under acid rain stress. Plant Growth Regul. 82 (1), 1–10.

Ying, C., Lin, F.Z., Hua, Y., Li, Y., Fei, H., Wang, J.L., Luo, Y.Y., Cao, F.L., 2014. Effect of varying NaCl doses on flavonoid production in suspension cells of ginkgo biloba:

relationship to chlorophyll fluorescence, ion homeostasis, antioxidant system and ultrastructure. Acta Physiol. Plant. 36 (12), 3173–3187.
Yu, J.Q., Ye, S.F., Huang, L.F., 2002. Effects of simulated acid precipitation on photosynthesis, chlorophyll fluorescence, and antioxidative enzymes in *Cucumis sativus* L. Photosynthetica 40 (3), 331–335.
Zhang, G., Liu, D., He, X., Yu, D., Pu, M., 2017. Acid rain in Jiangsu province, eastern China: tempo-spatial variations features and analysis. Atmos. Pollut. Res. 8 (6), 1031–1043.
Zhang, Y., Tao, Y., Zhang, H., Wang, L., Sun, G.Q., Sun, X., Erinle, K.O., Feng, C.C., Song, Q.X., Li, M., 2015. Effect of di-n-butyl phthalate on root physiology and rhizosphere microbial community of cucumber seedlings. J. Hazard. Mater. 289, 9–17.
Zhao, Y., Duan, L., Xing, J., Larssen, T., Nielsen, C.P., Hao, J., 2009. Soil acidification in China: is controlling SO_2 emissions enough? Environ. Sci. Technol. 43 (21), 8021.