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Comparative effects of simulated acid rain of different ratios of SO_4^{2-} to NO_3^- on fine root in subtropical plantation of China

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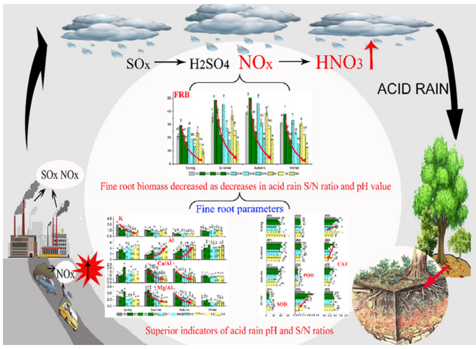
HIGHLIGHTS

- Fine root biomass (FRB) significantly decreased as acid rain S/N ratio and pH decreased.
- Acid rain S/N ratio and pH had stronger direct effects on FRB than indirect effects.
- Fine-root Al, Ca/Al and Mg/Al ratio were useful indicators of acid rain stress.

GRAPHICAL ABSTRACT



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ABSTRACT

The influence of acid rain on forest trees includes direct effects on foliage as well as indirect soil-mediated effects that cause a reduction in fine-root growth. In addition, the concentration of NO_3^- in acid rain increases with the rapidly growing 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 5:1 (S), 1:1 (SN) and 1:5 (N), on fine-root growth from March 2015 to February 2016. Results showed that fine roots were more sensitive to the effects of acid rain than soils in the short-term. Both soil pH and fine root biomass (FRB) significantly decreased as acid rain pH decreased, and also decreased with the percentage of NO_3^- increased in acid rain. Acid rain pH significantly influenced soil total carbon and available potassium in summer. Higher acidity level ($pH = 2.5$), especially of the N treatments, had the strongest inhibitory impact on soil microbial activity after summer. The structural equation modelling results showed that acid rain S/N ratio and pH had stronger direct effects on FRB than indirect effects via changed soil and fine root properties. Fine-root element contents and antioxidant enzymes activities were significantly affected by acid rain S/N ratio and pH during most seasons. Fine-root Al ion content, Ca/Al, Mg/Al ratios and catalase activity were used as better indicators than soil parameters for evaluating the effects of different acid rain S/N ratios and pH on forests. Our results suggest that the ratio of SO_4^{2-} to NO_3^- in acid rain is an important factor which could affect fine-root growth in subtropical forests of China.

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1. Introduction

Fine root, which is usually defined as roots ≤2 mm in diameter, is an ephemeral part of the root system and has fast turnover (Lai et al., 2016). Fine roots play a crucial role as the primary path for water and nutrient uptake by plants (Miyamoto et al., 2016; Ohashi et al., 2016) and for movement of carbon and energy from plant canopy to soil (Matamala et al., 2003; Santos et al., 2016). Thus, to understand forest ecosystem processes, it is essential to assess the fine root dynamics (Xu et al., 2013). Fine roots are sensitive to the change of environment, such as by fertilization (Majdi, 1994; Mo et al., 2008; Wurzburger and Wright, 2015; Fahey et al., 2016), air pollution (Nadelhoffer, 2000) and changes in climatic conditions (Majdi, 2005; Yuan and Chen, 2010). Bakker (1999) used the fine root parameters as indicators of sustainability of forest ecosystems, such as fine-root Ca, Al, Mg and Ca/Al. Freschet et al. (2017) and Matamala and Schlesinger (2000) found that fine-root growth was sensitive to the changes of atmospheric condition.

Acid rain, which is one of the most important global environmental problems, caused soil acidification in forest soil and indirectly affected fine roots (Esher et al., 1992; Persson et al., 1995; Fan and Wang, 2000). Following Northeast America and Central Europe, China has become the third region in the world seriously affected by acid rain during the past decades (Solberg et al., 2006; Singh and Agrawal, 2007; Liang et al., 2016). In southern China, the fine root biomass (FRB) in the mixed conifer and broadleaf forest and the broadleaved forest revealed a negative response to the increasing level of acid rain (Liang et al., 2013). In a secondary forest in subtropical China, acid rain treatments resulted in a decrease in topsoil root density in comparison with control treatments (Chen et al., 2012). Moreover, acid rain has occurred in about 40% of the entire territory of China (Tu et al., 2005; Wang et al., 2007). However, few researches conducted a comprehensive study on the effects of acid rain on FRB, the activities of fine-root antioxidant enzymes, the changes of fine-root elements, and the indirect effect of soil acidification on fine roots.

The sulfate ions (SO_4^{2-}) in precipitation have decreased significantly (Lv et al., 2014) due to the implementation of a series of SO_2 control measures in China (Chan and Yao, 2008). Meanwhile, however, the amount of motor vehicles has increased rapidly in China, and NO_x is emitted into atmosphere through tailpipe (Zhao et al., 2009). Therefore, the relative content of nitrate ions (NO^{3-}) in acid rain has increased significantly as well. The ratio of SO_4^{2-} to NO^{3-} in precipitation has decreased from 6 in 2003 to 5 in 2014 in Nanjing, China (Tu et al., 2005; Lv et al., 2014). So, the ratio of SO_4^{2-}/NO^{3-} in acid rain may continue to gradually decrease in the future, and the fine-root growth then would face a different profound challenge.

To explore the effects of acid rain with different ratios of SO_4^{2-} to NO_3^- on fine roots in forest ecosystem, we have established a series of mesocosm experiments in a subtropical plantation of China. 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 soil and fine root parameters that are sensitive indicators of forest productivity, focusing mainly on the potential use of fine-root parameters as indicators of acid rain. Based on the previous studies and reports (Vanguelova et al., 2007; Lv et al., 2014; Liu et al., 2017), we hypothesized that: (1) acid rain would depress the fine-root growth, and the inhibitory effects would increase as acid rain S/N ratio decreases; (2) fine root parameters such as fine-root Al, Ca, Mg, Ca/Al and Mg/Al would be more sensitive to acid rain than soil parameters.

2. Materials and methods

2.1. Study site

The study area was located in the Tong Shan forestry farm (31°37′N, 118°51′E), Nanjing, China. The altitude of the Tong Shan forestry farm ranges from 38 m to 388 m above mean sea level. It is currently dominated by mature monoculture plantations consisting of three major species: *Quercus acutissima*, *Cunninghamia lanceolata* and *Phyllostachys edulis*. It has a subtropical monsoon climate with an annual mean temperature of 15.1 °C, annual cumulative hours of sunshine of 2199.5 h, and annual precipitation of 1117.29 mm (2002–2013). The rainy season is mostly from June to August. The annual average pH value of rainfall is approximately 5.15 (Tu et al., 2005), with an annual acid rain frequency ($pH < 5.6$, total events of acid rain/total events of rainfall) of approximately 55.8% (Lv et al., 2014). The experimental plots were located in a *Q. acutissima* plantation. At the time of the study (2015–2016), the trees were about 48 years old; the average tree height of *Q. acutissima* was 13.8 m; the stand density was 425 $trees \cdot ha^{-1}$; the average diameter at breast height (DBH) was 25.8 cm; and the leaf area indices (LAI), estimated by a LAI-2200 canopy analysis system (LI-COR, Lincoln, USA), ranged over 0.1–3.87, and the existing density of litter was 18.04 $t \cdot hm^{-2}$. The soil type of *Q. acutissima* plantation was yellow brown soil. The soil properties of pH, total carbon (TC), total nitrogen(TN), total sulfur (TS), available phosphorus (AP), available potassium (AK) were 4.11 ± 0.06, 33.51 ± 6.04 $mg \cdot g^{-1}$, 3.21 ± 0.50 $mg \cdot g^{-1}$, 0.86 ± 0.21 $mg \cdot g^{-1}$, 2.50 ± 0.43 $mg \cdot kg^{-1}$ and 38.10 ± 9.59 $mg \cdot kg^{-1}$, respectively.

2.2. Experimental design

One hundred and twenty discrete plots (0.6 m × 2.0 m), separated from each other by approximately 5 m, were chosen in *Q. acutissima* plantation (Fig. S1). In November 2015, we dug a ditch in the middle of each plot, 2.0 m long × 20 cm wide × 20 cm deep, and cleaned the roots from the ditch. The ditches were filled with 15 cm deep mixture of river sand and soil (2:1, v/v) in order to identify and collect fine roots of *Q. acutissima* quickly. Then we covered the sand with 5 cm soil and covered the soil surface with fallen leaves. The stock solution of sulfate acid rain (S) was prepared by mixing 0.5 $mol \cdot L^{-1}$ H_2SO_4 and 0.5 $mol \cdot L^{-1}$ HNO_3 at molar ratio of 5:1 that corresponded to the general anion composition of rainfall in Nanjing city (Wang et al., 2007; Lv et al., 2014). The stock solution of two other acid rain mixtures (SN and N) was prepared by mixing 0.5 $mol \cdot L^{-1}$ H_2SO_4 and 0.5 $mol \cdot L^{-1}$ HNO_3 at molar ratio (S/N ratio) of 1:1 and 1:5, respectively. A completely randomized design with 12 replicates (three replicates per season) was used with ten simulated acid rain (SAR) treatments (Fig. S1): CK (pH 6.6, the local stream water as the control), S1 (S/N 5:1, pH 4.5), SN1 (S/N 1:1, pH 4.5), N1 (S/N 1:5, pH 4.5), S2 (S/N 5:1, pH 3.5), SN2 (S/N 1:1, pH 3.5), N2 (S/N 1:5, pH 3.5), S3 (S/N 5:1, pH 2.5), SN3 (S/N 1:1, pH 2.5) and N3 (S/N 1:5, pH 2.5). The S3, SN3 and N3 treatments respectively supplied 0.25 $g \cdot m^{-2}$, 0.92 $g \cdot m^{-2}$ and 2.29 $g \cdot m^{-2}$ nitrogen to plots compared to CK treatments. The nitrogen contents added to soil by pH 2.5 treatments were 10 times that by pH 3.5 SAR treatments and 100 times that by pH 4.5 SAR treatments. The acid rain solution was applied to each plot twice a month using a sprinkler from March 2015 to February 2016. The total amount of simulated acid rain was 62.07 mm based the monthly precipitation from 2002 to 2013. This total amount was 5.55% of the mean annual precipitation of the study area. The amount of simulated acid rain during period (spring, summer, autumn and winter) was 12.79 mm (20.62% of total amounts), 32.35 mm (52.11%), 9.35 mm (15.08%) and 7.57 mm (12.19%).

Fine root samples from each ditch were collected after spring (May 30–31, 2015), summer (August 30–31, 2015), autumn (November 29–30, 2015) and winter (February 28–29, 2016) acid rain applied (Fig. S1). Fine root samples were kept in sealed bags in a small refrigerator with 4 °C and taken back to the laboratory for further study. Fine root biomass was determined by collecting the fine roots from the ditches. Soil adhering to fine root samples was carefully removed, and the roots were then manually rinsed with distilled water. Then we weighed the biomass of fresh fine roots and measured the water content of fine root and antioxidant enzymes activities as soon as possible. Meanwhile, soil samples were collected from the top layers (0–5 cm) of each ditch. All soil samples were also kept in sealed bags in small refrigerator with 4 °C. Soil samples were passed through a 2-mm sieve to remove leaves, plant roots, gravel, and stones. Half of these soil samples were air-dried for subsequent chemical analysis. The other half of these soil samples were then kept in a refrigerator at −20 °C for further phospholipid fatty acid (PLFA) analysis.

2.3. Soil properties

The collected soil samples were air-dried and the ants and other invertebrates, stones, roots, seeds, coarse organic matter, and other impurities removed by sieving through a 2-mm mesh. The assay methods of soil pH, total carbon (TC), total nitrogen (TN), available phosphorus (AP) and available potassium (AK) were described by Liu et al. (2017). Soil pH was determined at a 1:2.5 soil: solution ratio (in deionized water) by using a PB-10 pH meter (Sartorius GmbH, Göttingen, Germany) after shaking for 1 h. Soil total carbon (TC) and total nitrogen (TN) were determined using an elemental analyzer (Vario EL III, ELementar, Germany) after the soil samples were further ground. Available phosphorus (AP) of soil samples was extracted with ammonium fluoride (NH_4F, 0.03 $mol \cdot L^{-1}$) and hydrochloric acid (HCl, 0.025 $mol \cdot L^{-1}$), and measured by UV–Vis spectrophotometer. Available potassium (AK) of soil samples was determined by the extraction with CH_3COONH_4 (1 $mol \cdot L^{-1}$), and measured by flame photometer.

The soil microbial biomass was estimated by phospholipid fatty acid (PLFA) analysis using the procedure described by Guo et al. (2016). The PLFAs were extracted from 3 g fresh soils by adding 15.2 mL Bligh-Dyer solvent [chloroform, methanol, and citrate buffer (0.15 M, pH 4.0); 1:2:0.8, v/v/v]. Following the process of soil lipid extraction, silicic acid chromatography and the methylation of polar lipids with methyl nondecanoate (19:0), the lipids were evaporated using a nitrogen evaporator. The separated fatty acids were identified using a gas chromatography (Agilent 6890 N, USA) fitted with a MIDI peak identification system. Total PLFAs (tPLFAs) concentration (nanomole PLFA per gram soil) was used as an index of the total microbial biomass.

2.4. Fine-root element content analysis

Fine root samples, oven-dried at 60 °C until constant weight was reached, were finely ground using a 0.5-mm sieving mill. A 0.25 g sample was extracted with concentrated nitric acid and concentrated perchloric acid (5:1). The digestion solution, including K, Ca, Mg, Al ions, was quantitatively analyzed by atomic absorption spectrometer (AA900T, Perkin Elmer, MA, USA). The fine-root total nitrogen (TN-r) was determined using an elemental analyzer (Vario EL III, ELementar, Germany).

2.5. Fine-root antioxidant enzymes activities analysis

Prior to determination of antioxidant enzyme activities, a crude enzyme extract was prepared by homogenizing 2–3 g 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 $mg \cdot protein^{-1} \cdot min^{-1}$ (Khan et al., 2017). Protein content (Pro) was determined according to Bradford (1976) and Teisseire and Guy (2000) using bovine albumin for calibration.

2.6. Statistical analyses

The Duncan test was used when one-way ANOVA (SPSS Inc., Chicago, Ill., USA) showed that acid rain treatment effects on soil properties and fine root properties were significant. Two-way ANOVA was used to test the main effects and interactions of acid rain pH, S/N ratios and season on soil and fine roots properties by SPSS 19.0. Redundancy discriminant analysis (RDA) was performed to reveal the relationships between the acid rain pH, S/N ratios, fine root traits and soil properties 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 fine root and soil properties in the short-term (one year). The model was used to test whether acid rain S/N ratio and pH influenced the fine root biomass directly or indirectly through modifying soil characteristics and/or fine root elements and antioxidant enzymes activities. SEM analyses were performed using AMOS 24.0 (SPSS Inc., Chicago, Ill., USA).

3. Results

3.1. Soil properties

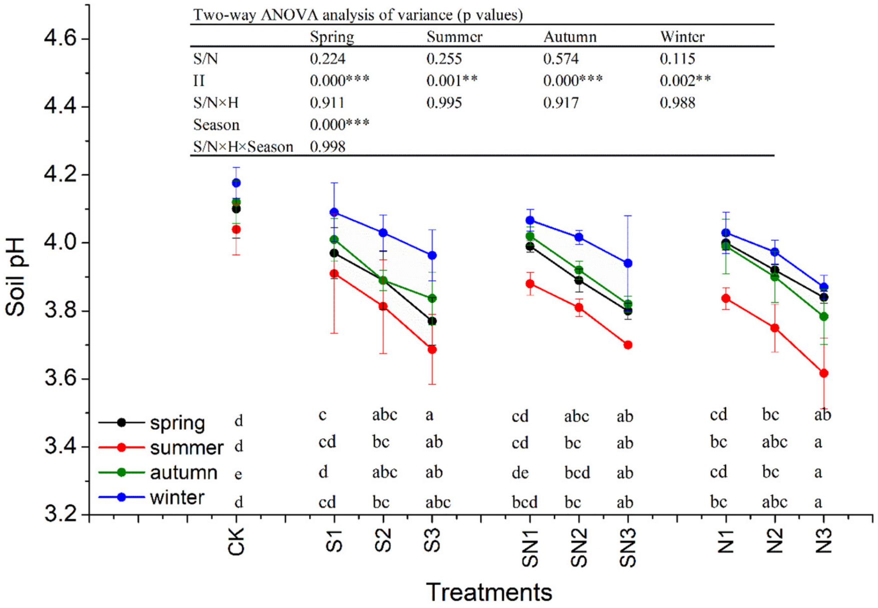
In acid rain treatment groups, we found a clear declining trend of soil pH with higher acidity input, and acid rain pH significantly influenced the soil pH (Fig. 1). However, there was no significant difference of acid rain S/N ratios ($p > 0.05$). Soil pH was significantly higher without acid rain input than with stronger acid rain (pH = 3.5, 2.5) ($p < 0.05$) over four experimental seasons (Fig. 1). In spring, summer and autumn, soil pH with pH 2.5 treatments was significantly higher than with pH 4.5 treatments ($p < 0.05$).

Soil nutrients (TC, TN and AP) showed significant differences among seasons ($p < 0.001$). After spring, there were significant differences for soil TC, TN and AP among acid rain S/N ratios ($p < 0.05$) (Table 1). TC with both S1 and S2 was significantly lower than that with N2, and TN with S1 was significantly lower than that with N3 ($p < 0.05$). After summer, TN and AK with acid rain treatments were lower than those with CK treatments, but stronger acid rain led to higher TN and AK than weaker acid rain (pH = 4.5). After winter, there were significant differences for AK among acid rain S/N ratios ($p < 0.01$). In addition, statistically significant interaction of acid rain S/N ratio and pH influencing soil AK was found in our study after winter (Table 1).

The microbial biomass values (tPLFAs) obtained from the 0–5 cm soil layer at the end of each season with different experimental treatments were shown in Fig. 2, and there were significant differences among four seasons. After spring, there was strongly significant difference among acid rain S/N ratios ($p < 0.001$), and the soil microbial biomass increased as the acid rain S/N ratio decreased. In addition, the soil microbial biomass in spring and autumn displayed significant differences among acid rain pH values ($p < 0.05$), which contrasted. Acid rain pH increased microbial biomass after spring and decreased microbial biomass after autumn, respectively. After summer and winter, S, SN and N had negative impacts on soil microbial biomass, but they were not significant ($p > 0.05$).

3.2. Fine root biomass

FRB significantly decreased with acid rain pH decreased over four seasons and had significant differences among acid rain S/N ratios ($p < 0.001$) (Fig. 3). All of the stronger acid rain treatments (pH = 2.5) significantly decreased FRB ($p < 0.05$). However, weaker acid rain treatments (pH = 4.5) significantly accelerated FRB ($p < 0.05$), except for SN1 and N1 in winter. Statistically significant interactions of acid rain S/N ratios and pH influences on FRB were not found over four seasons ($p > 0.05$).



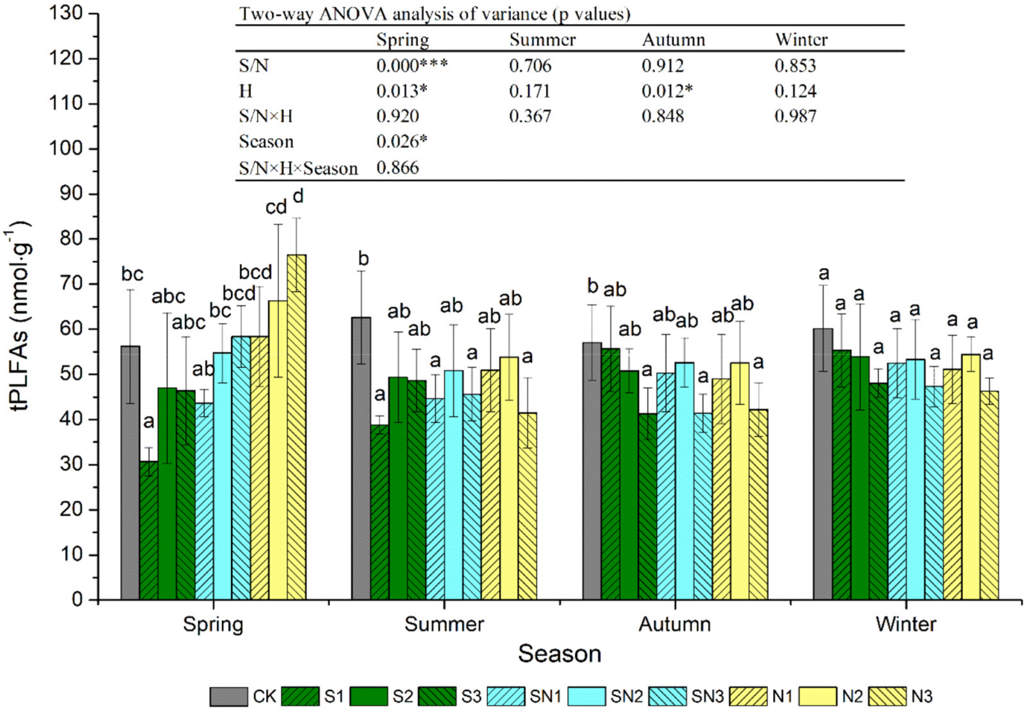
	Spring	Summer	Autumn	Winter
S/N	0.224	0.255	0.574	0.115
H	0.000***	0.001**	0.000***	0.002**
S/N×H	0.911	0.995	0.917	0.988
Season	0.000***			
S/N×H×Season	0.998			

Fig. 1. Changes of the soil pH value at 0–10 cm soil layer under different simulated acid rain treatments. S/N, the ratio of SO_4^{2-} to NO_3^-; H, acid rain pH. The experimental treatments are: CK = control check; S1 = pH 4.5, S/N 5:1; S2 = pH 3.5, S/N 5:1; S3 = pH 2.5, S/N 5:1; SN1 = pH 4.5, S/N 1:1; SN2 = pH 3.5, S/N 1:1; SN3 = pH 2.5, S/N 1:1; N1 = pH 4.5, S/N 1:5; N2 = pH 3.5, S/N 1:5; N3 = pH 2.5, S/N 1:5. Different letters indicate significant difference ($p < 0.05$) among different treatments in same season based on one-way ANOVA, followed by a Duncan test. Two-way ANOVA was applied to indicate significant difference among variances (no CK treatments). *** indicates significant difference at $p < 0.001$; ** indicates significant difference at $p < 0.01$; * indicates significant difference at $p < 0.05$.

Table 1
Effects of acid rain with different S/N ratios (S/N) and pH (H) addition on abiotic soil variables (mean ± SD) over four seasons.

	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
	TC (g·kg^{-1})				TN (g·kg^{-1})			
CK	30.39 ± 1.85ab	41.74 ± 5.02a	28.01 ± 2.81a	32.75 ± 5.74ab	3.07 ± 0.45ab	3.80 ± 0.29c	2.66 ± 0.22a	3.30 ± 0.33a
S1	23.49 ± 5.06a	34.19 ± 2.08a	34.07 ± 1.27a	27.18 ± 10.15a	2.36 ± 0.35a	3.11 ± 0.09ab	3.00 ± 0.12a	2.81 ± 0.66a
S2	25.40 ± 5.03a	33.78 ± 9.22a	31.11 ± 7.42a	33.15 ± 3.88a	2.62 ± 0.39ab	3.03 ± 0.46a	3.14 ± 0.39a	3.23 ± 0.30a
S3	27.85 ± 7.92ab	38.87 ± 7.17a	35.76 ± 4.97a	31.50 ± 2.71a	2.77 ± 0.53ab	3.51 ± 0.15abc	3.35 ± 0.39a	2.89 ± 0.50a
SN1	27.79 ± 5.24ab	38.04 ± 2.89a	34.50 ± 3.95a	26.90 ± 4.00a	2.77 ± 0.23ab	3.36 ± 0.06abc	3.05 ± 0.29a	2.70 ± 0.23a
SN2	29.17 ± 1.58ab	34.83 ± 1.88a	33.85 ± 4.99a	31.23 ± 3.09a	2.84 ± 0.37ab	3.33 ± 0.39abc	3.27 ± 0.42a	3.13 ± 0.55a
SN3	31.59 ± 1.60ab	33.84 ± 0.98a	31.66 ± 3.34a	30.16 ± 3.21a	3.02 ± 0.44ab	3.61 ± 0.36abc	3.32 ± 0.26a	3.19 ± 0.31a
N1	30.05 ± 6.01ab	41.36 ± 8.93a	28.71 ± 3.39a	27.56 ± 4.67a	3.08 ± 0.16ab	3.72 ± 0.27bc	2.84 ± 0.46a	2.63 ± 0.21a
N2	34.87 ± 2.25b	33.49 ± 2.98a	34.95 ± 4.13a	30.44 ± 7.18a	2.96 ± 0.13ab	3.12 ± 0.42ab	3.15 ± 0.16a	2.90 ± 0.62a
N3	29.61 ± 2.33ab	37.10 ± 4.65a	27.54 ± 2.74a	31.83 ± 2.61a	3.28 ± 0.60b	3.71 ± 0.48bc	3.11 ± 0.64a	3.07 ± 0.18a
Analysis of variance (p values)								
S/N	0.041*	0.729	0.242	0.890	0.032*	0.172	0.587	0.758
H	0.396	0.316	0.726	0.169	0.287	0.033*	0.246	0.159
S/N × H	0.601	0.479	0.189	0.981	0.942	0.562	0.984	0.843
Season	0.000***				0.000***			
S/N × H × Season	0.696				0.983			
	AP (mg·kg^{-1})				AK (mg·kg^{-1})			
CK	2.50 ± 0.37c	3.11 ± 0.66c	2.19 ± 0.56ab	2.22 ± 0.61ab	35.63 ± 8.53a	52.24 ± 16.01c	33.18 ± 4.88abc	31.36 ± 10.38abc
S1	1.90 ± 0.50abc	2.58 ± 0.74abc	2.39 ± 0.33ab	1.47 ± 0.51a	26.41 ± 5.39a	24.94 ± 12.02a	30.08 ± 4.82abc	24.24 ± 3.12a
S2	2.48 ± 0.39c	2.89 ± 0.79bc	2.95 ± 1.00b	2.18 ± 0.67ab	34.41 ± 9.16a	44.51 ± 8.20bc	35.34 ± 5.47bc	37.39 ± 6.57bc
S3	2.07 ± 0.40abc	1.90 ± 0.54a	2.41 ± 0.66ab	2.41 ± 0.33b	31.92 ± 12.49a	34.56 ± 8.10ab	25.41 ± 2.25a	32.22 ± 2.22abc
SN1	2.11 ± 0.09bc	2.57 ± 0.28abc	2.30 ± 0.10ab	1.58 ± 0.35a	29.36 ± 4.53a	25.70 ± 6.97a	30.56 ± 3.88abc	28.67 ± 1.51ab
SN2	2.18 ± 0.25bc	2.60 ± 0.37abc	2.73 ± 0.58b	2.12 ± 0.27ab	36.67 ± 4.01a	39.08 ± 1.06abc	34.75 ± 4.12abc	31.67 ± 4.12abc
SN3	1.74 ± 0.33ab	2.27 ± 0.21abc	2.08 ± 0.19ab	1.67 ± 0.18ab	34.40 ± 4.05a	33.51 ± 5.57ab	27.36 ± 2.59ab	27.36 ± 2.59ab
N1	2.05 ± 0.28abc	1.85 ± 0.31a	2.13 ± 0.27ab	1.65 ± 0.25ab	31.89 ± 6.74a	25.66 ± 5.87a	30.89 ± 4.60abc	39.30 ± 5.68c
N2	1.72 ± 0.29ab	2.05 ± 0.14ab	2.09 ± 0.23ab	1.86 ± 0.12ab	38.40 ± 4.16a	36.17 ± 6.11abc	34.32 ± 7.84abc	33.87 ± 5.20abc
N3	1.49 ± 0.14a	2.62 ± 0.39abc	1.73 ± 0.28a	1.48 ± 0.50a	32.92 ± 7.14a	47.95 ± 12.45bc	37.10 ± 6.34c	36.64 ± 5.21bc
Analysis of variance (p values)								
S/N	0.048*	0.325	0.050	0.170	0.556	0.612	0.237	0.006**
H	0.075	0.513	0.105	0.052	0.113	0.002**	0.103	0.239
S/N × H	0.179	0.064	0.840	0.180	0.980	0.206	0.204	0.024*
Season	0.000***				0.362			
S/N × H × Season	0.109				0.388			

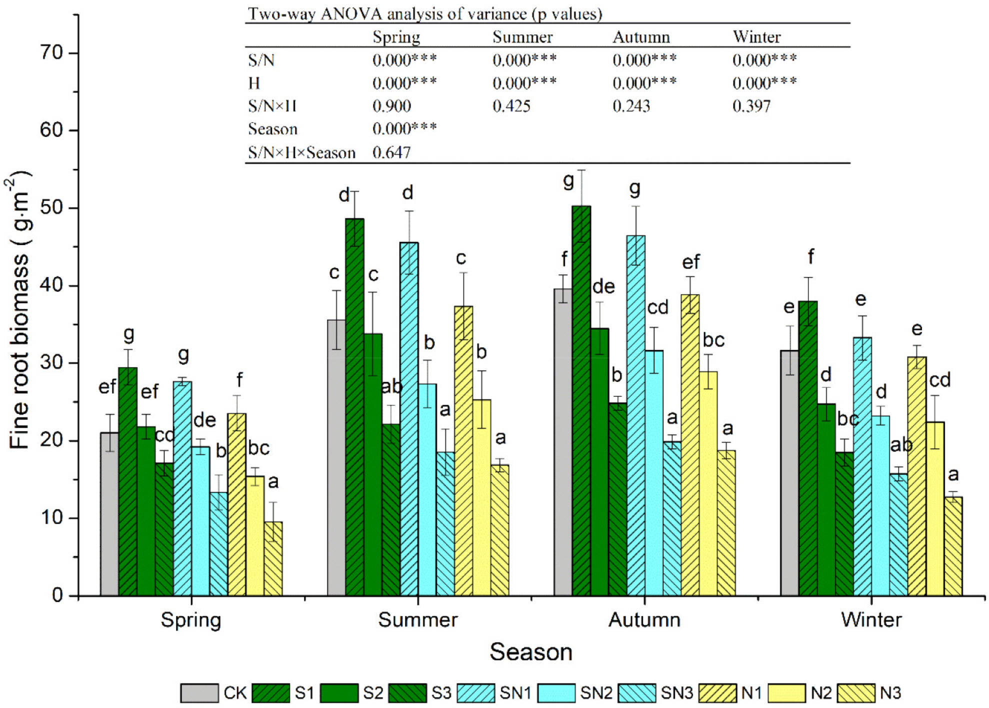
Note: The values in bracket are standard deviation (n = 3). Capital letters for a given variable indicate significant difference ($p < 0.05$) among different treatments of one season based on one-way ANOVA, followed by a Duncan test. Two-way ANOVA was applied to indicate significant difference among variances (no CK treatments). *** indicates significant difference at $p < 0.001$; ** indicates significant difference at $p < 0.01$; * indicates significant difference at $p < 0.05$. S/N, the ratio of SO_4^{2-} to NO_3^-; TC, total carbon; TN, total nitrogen; AP, available phosphorus; AK, available potassium. The experimental treatments are: CK = control check; S1 = pH 4.5, S/N 5:1; S2 = pH 3.5, S/N 5:1; S3 = pH 2.5, S/N 5:1; SN1 = pH 4.5, S/N 1:1; SN2 = pH 3.5, S/N 1:1; SN3 = pH 2.5, S/N 1:1; N1 = pH 4.5, S/N 1:5; N2 = pH 3.5, S/N 1:5; N3 = pH 2.5, S/N 1:5.



Two-way ANOVA analysis of variance (p values)

	Spring	Summer	Autumn	Winter
S/N	0.000***	0.706	0.912	0.853
H	0.013*	0.171	0.012*	0.124
S/N×H	0.920	0.367	0.848	0.987
Season	0.026*			
S/N×H×Season	0.866			

Fig. 2. Changes of the soil microbial biomass (tPLFAs) at 0-10 cm soil layer under different simulated acid rain treatments. S/N, the ratio of SO_4^{2-} to NO_3^- in acid rain; H, acid rain pH. The experimental treatments are: CK = control check; S1 = pH 4.5, S/N 5:1; S2 = pH 3.5, S/N 5:1; S3 = pH 2.5, S/N 5:1; SN1 = pH 4.5, S/N 1:1; SN2 = pH 3.5, S/N 1:1; SN3 = pH 2.5, S/N 1:1; N1 = pH 4.5, S/N 1:5; N2 = pH 3.5, S/N 1:5; N3 = pH 2.5, S/N 1:5. 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 (no CK treatments). *** indicates significant difference at $p < 0.001$; ** indicates significant difference at $p < 0.01$; * indicates significant difference at $p < 0.05$.



Two-way ANOVA analysis of variance (p values)

	Spring	Summer	Autumn	Winter
S/N	0.000***	0.000***	0.000***	0.000***
H	0.000***	0.000***	0.000***	0.000***
S/N×H	0.900	0.425	0.243	0.397
Season	0.000***			
S/N×H×Season	0.647			

Fig. 3. Changes of the fine root biomass under different simulated acid rain treatments. S/N, the ratio of SO_4^{2-} to NO_3^- in acid rain; H, acid rain pH. The experimental treatments are: CK = control check; S1 = pH 4.5, S/N 5:1; S2 = pH 3.5, S/N 5:1; S3 = pH 2.5, S/N 5:1; SN1 = pH 4.5, S/N 1:1; SN2 = pH 3.5, S/N 1:1; SN3 = pH 2.5, S/N 1:1; N1 = pH 4.5, S/N 1:5; N2 = pH 3.5, S/N 1:5; N3 = pH 2.5, S/N 1:5. 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 (no CK treatments). *** indicates significant difference at $p < 0.001$.

Table 2
Analysis of variance (p values) of effects of acid rain with different S/N ratios (S/N) and pH (H) addition on fine root antioxidant enzymes activities.

		Spring	Summer	Autumn	Winter		Spring	Summer	Autumn	Winter
S/N	SOD	0.022*	0.101	0.382	0.001**	POD	0.813	0.471	0.042*	0.000***
H		0.000***	0.706	0.003**	0.002**		0.001**	0.382	0.017*	0.000***
S/N × H		0.474	0.129	0.254	0.452		0.795	0.996	0.226	0.000***
Season		0.000***					0.000***			
S/N × H × season		0.070					0.000***			
S/N	CAT	0.543	0.046*	0.194	0.005**					
H		0.000***	0.000***	0.000***	0.541					
S/N × H		0.792	0.000***	0.088	0.849					
Season		0.000***								
S/N × H × season		0.019*								

Note: Two-way ANOVA was applied to indicate significant difference among variances (no CK treatments). *** indicates significant difference at $p < 0.001$; ** indicates significant difference at $p < 0.01$; * indicates significant difference at $p < 0.05$. S/N, the ratio of SO_4^{2-} to NO_3^-; SOD, superoxide dismutase activity; POD, peroxidase activity; CAT, catalase activity.

3.3. Fine-root antioxidant enzymes activities

Significant seasonal differences of fine-root antioxidant enzymes activities were found in our study (Table 2, $p < 0.001$). After spring, N3 treatment significantly increased fine-root SOD activity compared to CK, N1 and N2 treatments ($p < 0.05$) (Fig. 4 A1). Under SN treatment, SOD activity with high acid rain acidity (pH = 2.5) was significantly higher than that with weaker acidity treatments (pH = 4.5, 3.5) ($p < 0.05$). In summer and autumn, there were no significant differences between CK and acid rain treatments, and we only found that the SN3 significantly increased SOD compared to SN2 in summer ($p < 0.05$). SOD activity in winter was lower than that in other seasons. POD activity first increased (pH = 3.5) and then decreased (pH = 2.5) with acid rain pH decreased in spring (Fig. 4 A2). In autumn, we only found N3 treatment significantly increased POD activity ($p < 0.05$). In spring and autumn, we found significant differences among acid rain pH for SOD and POD activity (Table 2). In winter, fine-root POD activities were the highest among the four seasons, and significant differences were found among both acid rain S/N ratios and pH ($p < 0.001$). Meanwhile, statistically significant interaction of acid rain S/N ratio and pH

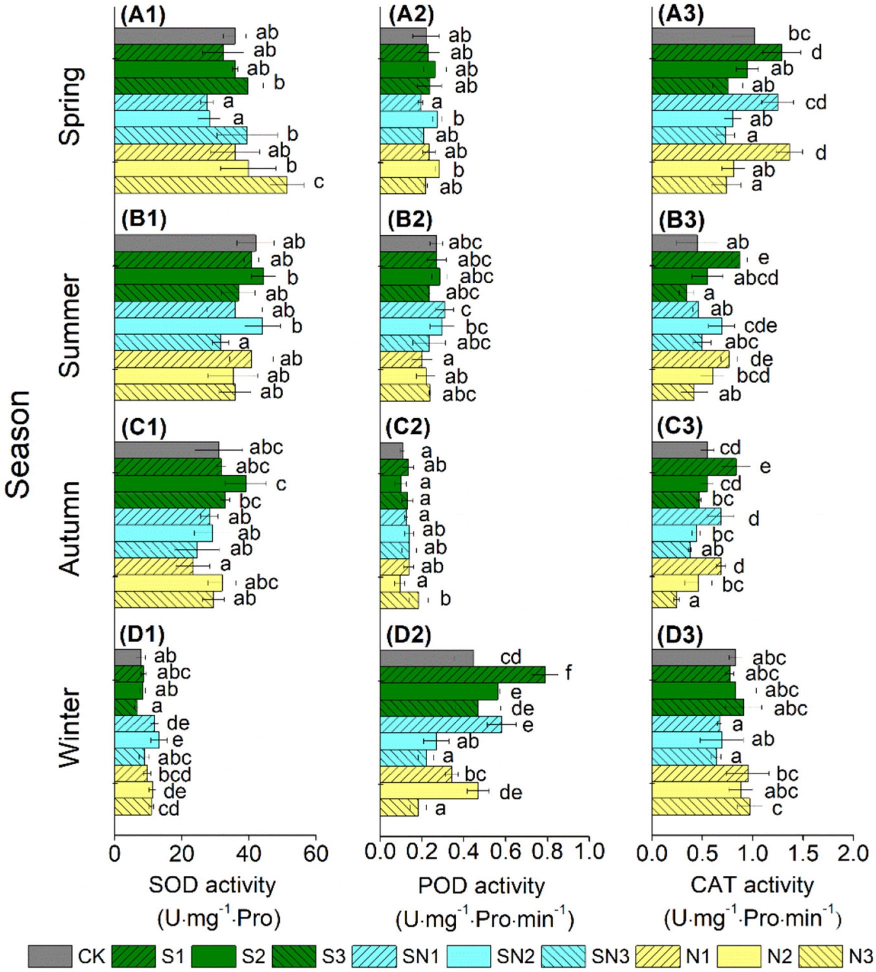


Fig. 4. Changes of the fine-root enzymatic antioxidants under different simulated acid rain treatments. SOD, superoxide dismutase; POD, peroxidase; CAT, catalase. The experimental treatments are: CK = control check; S1 = pH 4.5, S/N 5:1; S2 = pH 3.5, S/N 5:1; S3 = pH 2.5, S/N 5:1; SN1 = pH 4.5, S/N 1:1; SN2 = pH 3.5, S/N 1:1; SN3 = pH 2.5, S/N 1:1; N1 = pH 4.5, S/N 1:5; N2 = pH 3.5, S/N 1:5; N3 = pH 2.5, S/N 1:5. S/N, the ratio of SO_4^{2-} to NO_3^- in acid rain. 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.

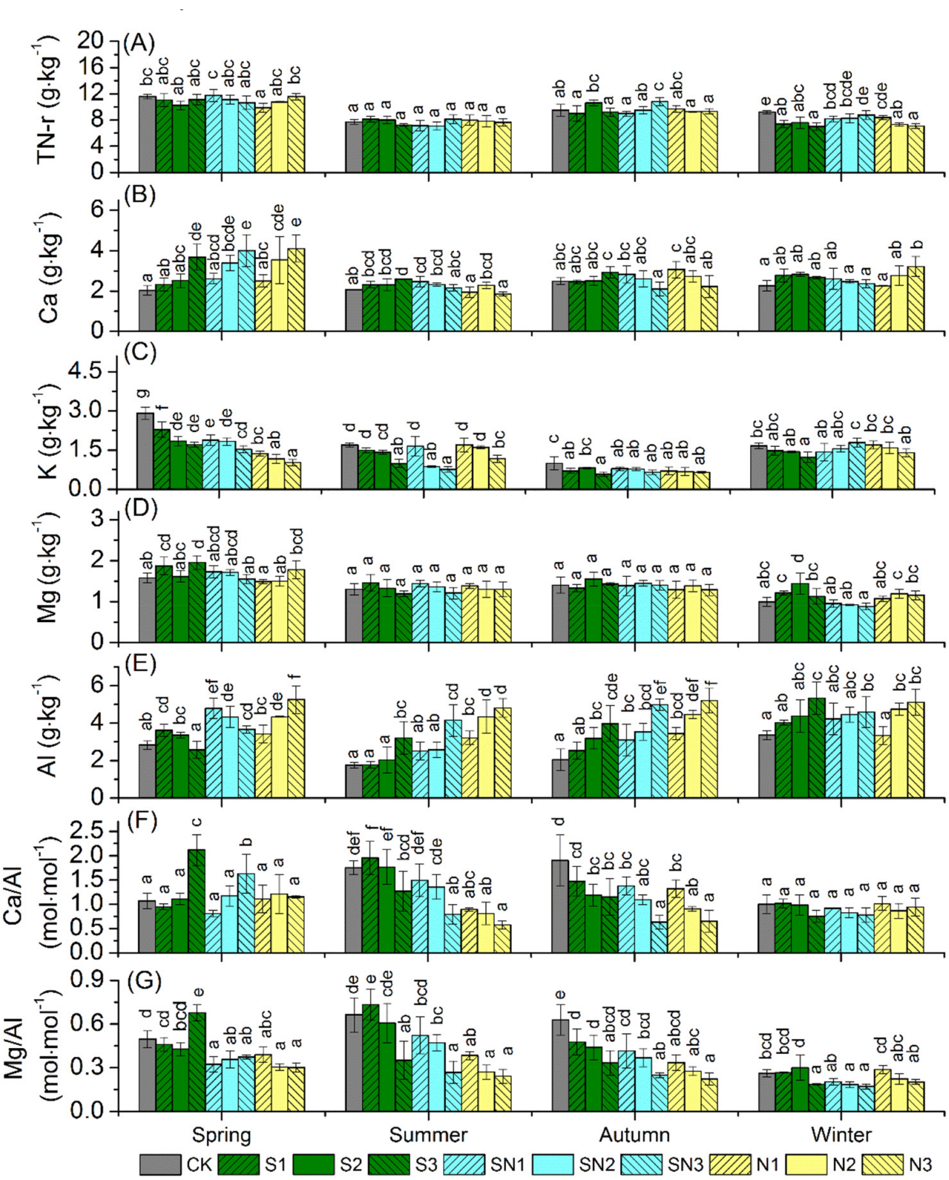


Fig. 5. Changes of the fine-root element contents under different simulated acid rain treatments. TN-r, total nitrogen in fine roots; Ca/Al, the ratio of Ca ion to Al ion in fine roots; Mg/Al, the ratio of Mg ion to Al ion in fine roots. The experimental treatments are: CK = control check; S1 = pH 4.5, S/N 5:1; S2 = pH 3.5, S/N 5:1; S3 = pH 2.5, S/N 5:1; SN1 = pH 4.5, S/N 1:1; SN2 = pH 3.5, S/N 1:1; SN3 = pH 2.5, S/N 1:1; N1 = pH 4.5, S/N 1:5; N2 = pH 3.5, S/N 1:5; N3 = pH 2.5, S/N 1:5. S/N, the ratio of SO_4^{2-} to NO_3^- in acid rain. 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.

influencing POD activity was found in winter (Table 2). CAT activities decreased with acid rain pH decreased in spring, summer and autumn (Fig. 4 A3, B3, C3), and there were significant differences among acid rain pH (Table 2). In spring and summer, S1 and N1 significantly increased CAT activity compared to CK ($p < 0.05$). SN3 and N3 led to significantly lower CAT activity compared to CK ($p < 0.05$) in spring and autumn. In winter, we found significant differences among acid rain S/N ratios ($p < 0.01$) (Table 2). In addition, statistically significant interactions of acid rain S/N ratio, pH and season influencing POD ($p < 0.001$) and CAT ($p < 0.05$) activity were found in our study (Table 2).

3.4. Fine-root elements

Acid rain caused changes in the concentration of fine-root ions and TN examined (Fig. 5). Fine-root elements concentrations showed significant differences among four seasons (Table 3, $p < 0.001$). The concentration of fine-root K decreased and Al and Ca increased with the effect of acid rain (Fig. 5B, C, E). However, acid rain had weak effects on TN and Mg (Fig. 5A, D), especially in summer and autumn. The significant differences for K among acid rain S/N ratios were found in spring and summer. In spring, summer and autumn, we also found that the K content significantly decreased with acid rain pH decreased (Fig. 5C, Table 3). The concentrations of Al showed significant differences among acid rain S/N ratios in spring, summer and autumn, and significantly increased with acid rain pH decreased in summer, autumn and winter (Fig. 5E, Table 3). Statistically significant interactions of acid rain S/N ratios and pH influencing Ca were found in summer, autumn and winter in our study ($p < 0.05$) (Table 3). The fine-root Ca/Al ratios and Mg/Al ratios significantly increased with acid rain pH decreased after summer, except for Ca/Al ratio in winter (Table 3). In addition, Ca/Al ratios and Mg/Al ratios decreased with the acid rain S/N ratio decreased (Fig. 5F, G), and there were significant differences for Ca/Al ratios and Mg/Al ratios among acid rain S/N ratios both in summer and autumn (Table 3). In addition, statistically significant interactions of acid rain S/N ratio, pH and season influencing fine-root TN, K, Ca/Al and Mg/Al were found in our study.

Table 3
Analysis of variance (p values) of effects of acid rain with different S/N ratios (S/N) and pH (A) addition on the contents of fine root elements.

Season		Spring	Summer	Autumn	Winter		Spring	Summer	Autumn	Winter
S/N	TN-r	0.403	0.415	0.539	0.002**	K	0.000***	0.001**	0.368	0.057
H		0.504	0.941	0.101	0.337		0.000***	0.000***	0.018*	0.722
S/N × H		0.046*	0.108	0.004**	0.056		0.351	0.017	0.412	0.043*
Season		0.000***					0.000***			
S/N × H × season		0.000***					0.001**			
S/N	Ca	0.140	0.001**	0.620	0.156	Mg	0.015*	0.990	0.246	0.000***
H		0.000***	0.464	0.133	0.395		0.126	0.051	0.218	0.090
S/N × H		0.792	0.018*	0.036*	0.040*		0.033*	0.820	0.887	0.229
Season		0.000***					0.000***			
S/N × H × season		0.146					0.204			
S/N	Al	0.000***	0.000***	0.002**	0.813	Ca/Al	0.146	0.000***	0.018*	0.313
H		0.700	0.000***	0.000***	0.006**		0.000***	0.001**	0.000***	0.078
S/N × H		0.000***	0.539	0.775	0.311		0.007**	0.717	0.405	0.508
Season		0.000***					0.000***			
S/N × H × season		0.060					0.013*			
S/N	Mg/Al	0.000***	0.000***	0.003**	0.003**					
H		0.002**	0.000***	0.002**	0.002**					
S/N × H		0.000***	0.186	0.943	0.062					
Season		0.000***								
S/N × H × season		0.002**								

Note: Two-way ANOVA was applied to indicate significant difference among variances (no CK treatments). *** indicates significant difference at $p < 0.001$; ** indicates significant difference at $p < 0.01$; * indicates significant difference at $p < 0.05$. S/N, the ratio of SO_4^{2-} to NO_3^-; TN-r, total nitrogen in fine roots; Ca/Al, the ratio of Ca ion to Al ion in fine roots; Mg/Al, the ratio of Mg ion to Al ion in fine roots.

3.5. Linking fine root traits and soil properties under acid rain treatments

The effects of acid rain on fine root traits and soil properties were obvious in summer and autumn due to the large amount of simulated acid rain was applied in summer. In the RDA of acid rain and soil properties with fine root traits as the explanatory variables Axis 1 accounted for 43.40% of the variation in the dataset during summer and autumn, with 22.37% of the variation accounted for by Axis 2 (Fig. 6). High FRB with high acid rain pH, soil pH, fine-root Ca/Al, Mg/Al ratios, CAT activity and acid rain S/N ratio were found at the right-hand end of the ordination plots and were associated with lower fine-root Al content, soil AK, TN and fine-root POD activity. Fine-root TN, Ca ion content and SOD activity increased along the y-axis, whereas the fine-root K ion content and soil TC decreased.

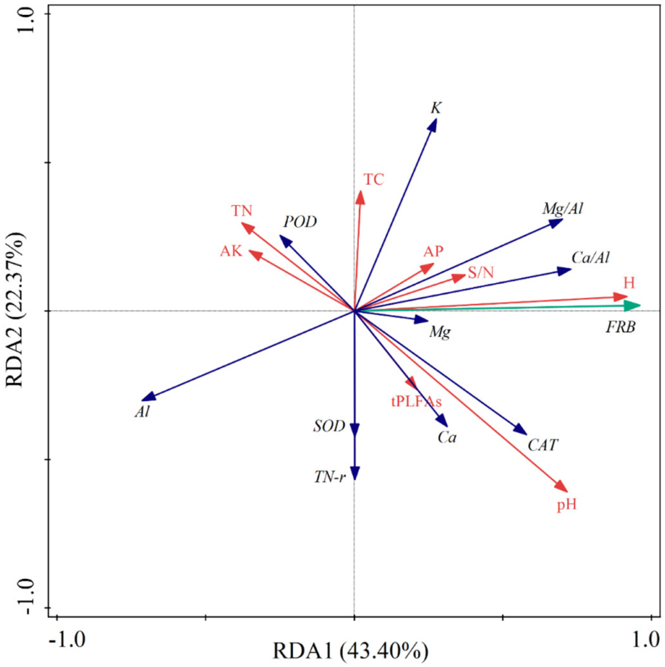


Fig. 6. Redundancy analysis (RDA) analysis of fine root traits and soil properties during summer and autumn. The angle and length of the arrows indicate the direction and strength of the relationship of each fine root and soil index. A, acid rain pH; S/N, acid rain S/N ratio (the ratio of SO_4^{2-} to NO_3^-); FRB, fine root biomass; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; TN-r, total nitrogen in fine roots; K, Ca, Mg, Al, the fine-root ions contents; Ca/Al, the ratio of Ca ion to Al ion in fine roots; Mg/Al, the ratio of Mg ion to Al ion in fine roots. pH, soil pH; TC, total carbon; TN, total nitrogen, AP, available phosphorus; AK, available potassium; tPLFAs, soil microbial biomass.

3.6. SEM results

Fig. 7 showed the structural equation modelling (SEM) as estimated by AMOS. Each of the observed variables was displayed in a rectangle, and each of the latent constructs was displayed in an oval (Xiong et al., 2016). The χ^2 test showed that the model generated $\chi^2 = 35.035$, $df = 29$, and $p = 0.203$ (>0.050). The goodness-of-fit index (GFI) was 0.901 (>0.900), the root mean square error of approximation (RMSEA) was 0.063 (<0.080). There was an inhibitory effect of acid rain S/N ratio on FRB. However, the significant direct effects of acid rain S/N ratio (0.31, $p < 0.001$) on FRB were less obvious than those of acid rain pH (0.89, $p < 0.001$). FRB was less influenced by acid rain S/N ratio (-0.032) and pH (0.011) indirectly through changes in soil properties (pH, TN and AK) and fine root elements (Al, Ca/Al and Mg/Al) than directly. Therefore, the total effects of acid rain S/N ratio and pH on FRB were 0.280 and 0.906, respectively.

We found an inhibitory effect of acid rain S/N ratio on FRB from RDA and SEM analyses. Hence, we used the stepwise multiple regression analysis to investigated the relations between FRB and fine root traits and soil variables under different acid rain S/N ratios (Table 4). Both fine-root CAT activities were selected to model and calculate the FRB under S and N treatments. However, fine-root K ion content and Mg/Al ratio were, respectively, selected in the models of S and SN treatments. In addition, soil pH and fine-root K ion content were sufficient to describe the changes of FRB under SN treatments (Fig. 7).

4. Discussion

Fine roots play important roles in carbon and nitrogen cycling by absorbing nutrients and water from soil and releasing exudates to soil (Li et al., 2015). This study focused on the effects of simulated acid rain on fine root traits, especially when the differences of impacts on fine root among different acid rain S/N ratios were compared. After one-year of simulated acid rain, soil pH significantly decreased with acid rain pH

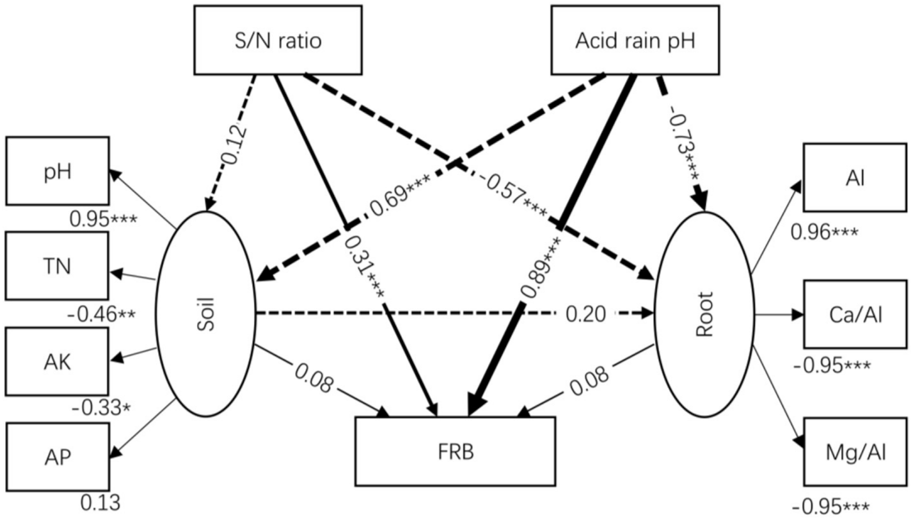


Fig. 7. Structural equation models of acid rain S/N ratio (the ratio of SO_4^{2-} to NO_3^-) and pH effects on fine root biomass. ($\chi^2 = 35.035$; df = 29, $p = 0.203 > 0.05$; GFI = 0.901 > 0.900; CFI = 0.986 > 0.900; RMSEA = 0.063). Numbers on arrows are standardized path coefficients. The width of arrows indicates the strength of the causal influence. Solid arrows mean a direct effect on the fine root biomass; dashes represent an indirect path to fine root biomass. Soil, soil properties; Root, fine root properties; pH, Soil pH; TN, soil total nitrogen, AK, soil available potassium; AP, soil available phosphorus; Al, fine-root aluminium ion content; Ca/Al, the ratio of Ca ion to Al ion in fine roots; Mg/Al, the ratio of Mg ion to Al ion in fine roots.

decreased, and decreased with increasing percentage of NO_3^- in acid rain. This may be because the ability of exchange with hydroxyl groups (OH^-) of NO_3^- was weaker than with those of SO_4^{2-}, which was more easily absorbed by soil particles (Lv et al., 2014; Liu et al., 2017). In this study, fine roots mainly grew in ditches with mixture of river sand and soil (2:1, v/v). It is well known that cutting the roots during the process of digging ditches stimulates them to grow several new ones. In order to identify the effects of ditching on FRB although we had set aside four months for plots to restore stability, we also collected the fine roots from undisturbed soil close to ditch using 5 cm-diameter soil-drilling method (Fig. S2). We found that fine root biomasses collected from undisturbed soil were higher than those collected from ditches, and the rates of fine root decomposition in undisturbed soil were higher than those in ditches in autumn. It perhaps the case that microbial activity or biomass accelerating fine root decomposition in undisturbed soil was stronger than that in ditches. What's more, the same trends of FRB with acid rain S/N ratio and pH were found from Figs. 3 and S2, respectively. So, in this study, we used the fine roots from ditches to analyze the effects of acid rain on biomass.

Prior studies had not found a clear relationship between soil acidity and FRB, as influenced by soil properties, tree species and climates (Miyatani et al., 2016). In our study, FRB significantly decreased with acid rain pH decreased (Persson et al., 1995; Chen et al., 2012; Liang et al., 2013; Hirano et al., 2017). In addition, some studies found that excess nitrogen from atmospheric deposition had a significant impact on fine roots by changing plant carbon allocation patterns, storage of carbohydrates and production of secondary defenses chemicals (Vogt et al., 1993; Li et al., 2015). N fertilizers could increase microbial activity and stimulate plant growth (Li et al., 2016). However, the growth and quality of roots would be inhibited by increasing atmospheric nitrogen deposition (5–30 $g \cdot m^{-2}$ $year^{-1}$) (Chen et al., 2017). In our study, we found that there were significant differences of FRB among acid rain S/N ratios ($p < 0.001$). The FRB with S1 (0.003 $g \cdot m^{-2} \cdot year^{-1}$ N addition) and SN1 (0.009 $g \cdot m^{-2} \cdot year^{-1}$ N addition) treatments were significantly higher than those with CK ($p < 0.05$). This may be because fine roots had adapted to the acidic soil in our study area. They would concentrate in the surface soils to compensate for nutrient deficiencies under weaker acidity treatments (Leuschner et al., 2004), and the nitrogen and sulfur in acid rain would act as a fertilizer, increasing soil fertility (Liu et al., 2017). In addition, N2 and N3 treatments respectively supplied 0.23 $g \cdot m^{-2} \cdot year^{-1}$ and 2.3 $g \cdot m^{-2} \cdot year^{-1}$ nitrogen to soil compared to CK. However, FRB decreased with the ratio of SO_4^{2-} to NO_3^- decreased, and FRB with N2 and N3 treatments were significantly lower than those with CK treatments. It suggested that the promoting effect of N fertilizers in nitric acid rain on fine-root growth was lower than the inhibitory impacts of acid rain.

Table 4
Multiple stepwise linear regression models between FRB and fine root traits and soil variables.

Treatments	Multiple linear stepwise regression equation	p	R^2
S	FRB = − 10.890 + 4.978CAT + 17.677K	0.000	0.725
SN	FRB = − 300.037 + 88.499pH + 10.538K	0.000	0.903
N	FRB = − 1.759 + 2.501CAT + 46.778Mg/Al	0.000	0.848

Note: S, SO_4^{2-}/NO_3^-, 5:1; SN, SO_4^{2-}/NO_3^-, 1:1; N, SO_4^{2-}/NO_3^-, 1:5; FRB, fine root biomass; CAT, fine-root catalase activity; K, fine-root K ion content; pH, soil pH; Mg/Al, the ratio of Mg ion to Al ion in fine roots.

Fine roots would input a substantial nutrient into soil after death (Joslin et al., 1988), but acid rain would inhibit microbial activity influencing fine root decomposition, and then decrease soil fertility (Liu et al., 2017). In this study, the PLFA method was effective in estimating soil microbial biomass (Liu et al., 2017). Soil microbial biomasses were significant negatively impacted by the increased acidity level (pH = 2.5) since summer. In addition, Lv et al. (2014) found that soil microbial biomass exhibited a decreased trend with a decrease in the SO_4^{2-}/NO_3^- ratio in acid rain. Consistent with this trend, we found that higher acidity level of S3 (pH = 2.5) had the strongest inhibitory impact on soil microbial activity in summer. However, in our study, no significant differences under mostly SAR treatments were seen in soil TC, TN, AP and AK (Tanikawa et al., 2014; Miyatani et al., 2016). We found only that soil TN and AK with SAR treatments were lower than those with CK, and significantly increased with acid rain acidity increased after rainy season. This suggested that soil TC, TN, AP and AK cannot be used as good indicators to evaluate the stress on plantations under acid rain. Elemental concentrations in fine roots may be regarded as good indicators of the nutritional conditions in the forest soil (Persson et al., 1995). Bakker (1999), discussed the potential of fine roots as indicators of forest sustainability, and found that fine-root Ca, Mg, Al, Ca/Al and Mg/Al gave a good insight into forests. Vanguelova et al. (2007) concluded that the ratio Ca/Al in roots was widely used as bioindicator in acid soils. In our study, the concentrations of K and Al cation in fine roots were significantly affected by acid rain pH. Nitrogen was the most important macro-nutrient affecting plant growth (Iversen et al., 2017). Fine-root nitrogen content for S3 treatments was lower than that for CK treatment in each season. On the contrary, SN3 treatment increased the fine-root nitrogen concentration in summer and autumn. However, with the NO^{3-} increased in N3 treatments, the fine-root nitrogen content decreased compared to that with SN3 treatments in summer, autumn and winter. This may be because SN3 treatments provided more nitrogen fertilizers to fine roots compared to S3 treatments. However, N3 treatments provided the maximum nitrogen fertilizers, but at the same time N3 treatments had the greatest negative influence on fine-root growth that inhibited the ability of fine root absorb nitrogen from soil. This was consistent with the above results of the effect of acid rain on fine root biomass.

Acid rain demonstrably caused soil acidification, nutrient imbalances, and increased concentration of Al in soil solution (Vanguelova et al., 2007). Meanwhile, fine roots strongly adsorbed Al ions either by exchange processes or formation of insoluble organo-Al complexes that would result in toxicity to fine roots. Consistently, in our study, fine-root Al ion contents with SAR treatments were higher than those with CK, and significantly increased with acid rain acidity increased after summer. Acid rain S/N ratios significantly influenced Al ion contents in fine roots. However, Al toxicity could be alleviated by Ca. Thus, Ca/Al molar ratios in plant tissues had been proposed as good indicators than Al ion concentration itself for evaluating the Al toxicity stress to trees (Cronan and Grigal, 1995; Mao et al., 2017). Šrámek et al. (2014) showed that fine-root Ca/Al < 0.1 strongly indicated Al stress and fine-root Ca/Al < 0.2 demonstrated the adverse effects of Al. The critical thresholds for the fine-root Ca/Al ratio of 0.2 was estimated to represent 90% risk of adverse impact on root growth (Cronan and Grigal, 1995). However, fine-root Ca/Al ratios of mature trees have only rarely been determined below the critical 0.2 (Vanguelova et al., 2007). In our study, Ca/Al ratios decreased significantly with increased acid rain acidity in summer and autumn, and decreased with the S/N ratios decreased in acid rain. That was mostly because the amount of simulated acid rain in summer obviously increased the input of a mass of H^+ into soil. In addition, fine-root Ca/Al ratios were 0.58–1.27 with stronger acid rain ($pH = 2.5$) in this study, which was higher than 0.2. It may be because of the differences of experiment site and tree species and because the amount of our simulated acid rain was only about 5.55% of the annual precipitation. Mg cations could also alleviate Al stress (Bakker, 1999). In this study, significant differences of fine-root Mg/Al ratio among both acid rain pH and S/N ratios were found in four seasons. It suggested that fine-root Ca/Al ratio and Mg/Al ratio could be useful indicators of the stresses of acid rain acidity and S/N ratio.

Plants would suffer oxidative damage with environmental stress (Agarwal and Pandey, 2004). For protection against oxidative damage, plant cells would produce enzymatic antioxidants such as SOD, POD and CAT (Sofo et al., 2005; Kazemi et al., 2010). Acid rain would significantly increase the activity of SOD and guaiacol peroxidase, but decrease the activity of CAT in leaves (Yu et al., 2002). Velikova et al. (2000) found that POD and CAT activities in leaves were enhanced in order to scavenge and detoxicate the active oxygen species. In our study, we found that SOD activity in fine roots significantly increased with acid rain acidity increased in spring, and stronger acid rain ($pH = 2.5$) increased SOD activity compared to CK treatments, which is consistent with Koricheva et al. (1997). However, with the amount of simulated acid rain increased in summer, the fine-root SOD activities with stronger SAR treatments decreased compared to other treatments. It may be because the stronger acid rain profoundly influenced the fine-root growth, and then inhibited the process of SOD production. In addition, weaker acid rain ($pH = 4.5$) increased the fine-root CAT activities compared to CK treatment in spring, summer and autumn, but acid rain acidity significantly decreased the fine-root CAT activities. This suggested that fine roots of *Q. acutissima* could resist the stress of weaker acid rain by scavenging and detoxification of active oxygen species, whereas, stronger acid rain would destroy the enzymatic antioxidants system.

Fine roots were mainly distributed in the top layer of soil that was the main provider of soil nutrients, water, and heat for plant roots (Verma et al., 2014). Chen et al. (2016) found that FRB displayed a significant positive correlation with soil C and N, and that soil nutrients were important to stimulate fine-root growth. However, we only found soil properties such as pH, AP and microbial biomass had a positive correlation with FRB under acid rain. It was because soil nutrients such as TC and TN showed less change with the one-year stress of acid rain. In addition, with the effect of acid rain in our study, FRB also displayed obvious positive correlations with acid rain pH, S/N ratio, fine-root Ca/Al, Mg/Al ratio and CAT activity, while fine-root Al ion content had a significant negative correlation with FRB. Hence, fine-root growth, based on the nutrient supply from soil, was primarily influenced by acid rain pH, S/N ratio and fine root elements during the short-term stress of acid rain.

It should be noted that we only simulated acid rain over four seasons of one year. Meanwhile, the minirhizotron technique is a nondestructive in situ method for studying the dynamics of fine root, which allows the simultaneous measurement of fine-root growth and mortality (Johnson et al., 2016). Our future mesocosm experiment work should use minirhizotron technique for long-term studying the effect of acid rain S/N ratio on fine-root growth.

5. Conclusion

Using a one-year mesocosm experiment, S, SN and N treatments changed fine root traits and soil properties of a plantation ecosystem. In the period of simulated acid rain, soil pH and fine root biomass significantly decreased as acid rain S/N ratio and pH decreased. Acid rain pH significantly influenced soil TC and AK in summer, and S/N ratios significantly affected soil AK in winter. Soil microbial biomasses were negatively impacted by the increased acidity level resulting from all acid rain treatments. Fine root parameters were more sensitive to short-term acid rain stress than soil properties. Fine-root antioxidant enzyme activities with weak acid rain were higher than those with CK treatments, whereas, stronger acid rain inhibited the process of antioxidant enzymes production. In addition, fine-root Al ion content, Ca/Al and Mg/Al ratios were significantly influenced by acid rain S/N ratio and pH. In summary, while SO_4^{2-} concentration is decreasing, NO_3^- concentration is increasing in the future acid rain as a result of rapid economic growth (Lv et al., 2014; Liu et al., 2017). Acid rain with high NO_3^- concentration would change soil nutrients and inhibit fine-root growth. This inhibitory effect may seriously alter the balance of ecosystem C flux, nutrient cycling, and humus formation, which may, in turn, have multiple effects on plantation ecosystem.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.11.073.

References

Agarwal, S., Pandey, V., 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol. Plant. 48, 555–560.

Bakker, M.R., 1999. Fine-root parameters as indicators of sustainability of forest ecosystems. For. Ecol. Manag. 122, 7–16.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

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

Chen, S., Shen, X., Hu, Z., Chen, H., Shi, Y., Liu, Y., 2012. Effects of simulated acid rain on soil CO_2 emission in a secondary forest in subtropical China. Geoderma 189, 65–71.

Chen, L., Mu, X., Yuan, Z., Deng, Q., Chen, Y., Yuan, L.Y., Ryan, L.T., Kallenbach, R.L., 2016. Soil nutrients and water affect the age-related fine root biomass but not production in two plantation forests on the Loess Plateau, China. J. Arid Environ. 135, 173–180.

Chen, G.T., Tu, L.H., Peng, Y., Hu, H.L., Hu, T.X., Xu, Z.F., Liu, L., Tang, Y., 2017. Effect of nitrogen additions on root morphology and chemistry in a subtropical bamboo forest. Plant Soil 412, 441–451.

Cronan, C.S., Grigal, D.F., 1995. Use of calcium/aluminum ratios as indicators of stress in forest ecosystems. J. Environ. Qual. 24, 209–226.

Esher, R.J., Marx, D.H., Ursic, S.J., Baker, R.L., Brown, L.R., Coleman, D.C., 1992. Simulated acid rain effects on fine roots, ectomycorrhizae, microorganisms, and invertebrates in pine forests of the southern United States. Water Air Soil Pollut. 61, 269–278.

Fahey, T.J., Heinz, A.K., Battles, J.J., Fisk, M.C., Driscoll, C.T., Blum, J.D., Johnson, C.E., 2016. Fine root biomass declined in response to restoration of soil calcium in a northern hardwood forest. Can. J. For. Res. 46, 738–744.

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, 321–329.

Freschet, G.T., Valverde-Barrantes, O.J., Tucker, C.M., Craine, J.M., McCormack, L.M., Violle, C., Bonis, A., 2017. Climate, soil and plant functional types as drivers of global fine-root trait variation. J. Ecol. 105, 1182–1196.

Guo, X., Chen, H.Y., Meng, M., Biswas, S.R., Ye, L., Zhang, J., 2016. Effects of land use change on the composition of soil microbial communities in a managed subtropical forest. For. Ecol. Manag. 373, 93–99.

Hirano, Y., Tanikawa, T., Makita, N., 2017. Biomass and morphology of fine roots in eight *Cryptomeria japonica* stands in soils with different acid-buffering capacities. For. Ecol. Manag. 384, 122–131.

Iversen, C.M., McCormack, M.L., Powell, A.S., Blackwood, C.B., Freschet, G.T., Kattge, J., Bodegom, P.M., 2017. A global Fine-Root Ecology Database to address below-ground challenges in plant ecology. New Phytol. 215, 15–26.

Johnson, M.G., Andersen, C.P., Phillips, D.L., Kaldy, J.E., 2016. *Zostera marina* root demography in an intertidal estuarine environment measured using minirhizotron technology. Mar. Ecol. Pro. 557, 123–132.

Joslin, J.D., Kelly, J.M., Wolfe, M.H., Rustad, L.E., 1988. Elemental patterns in roots and foliage of mature spruce across a gradient of soil aluminium. Water Air Soil Pollut. 40, 375–390.

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.

Koricheva, J., Roy, S., Vranjic, J.A., Haukioja, E., Hughes, P.R., Hänninen, O., 1997. Antioxidant responses to simulated acid rain and heavy metal deposition in birch seedlings. Environ. Pollut. 95, 249–258.

Lai, Z., Zhang, Y., Liu, J., Wu, B., Qin, S., Fa, K., 2016. Fine-root distribution, production, decomposition, and effect on soil organic carbon of three revegetation shrub species in northwest China. For. Ecol. Manag. 359, 381–388.

Leuschner, C., Hertel, D., Schmid, I., Koch, O., Muhs, A., Hölscher, D., 2004. Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant Soil 258, 43–56.

Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F., Wu, J., 2015. The effects of simulated nitrogen deposition on plant root traits: a meta-analysis. Soil Biol. Biochem. 82, 112–118.

Li, J.H., Zhang, J., Li, W.J., Xu, D.H., Knops, J.M., Du, G.Z., 2016. Plant functional groups, grasses versus forbs, differ in their impact on soil carbon dynamics with nitrogen fertilization. Eur. J. Soil Biol. 75, 79–87.

Liang, G., Liu, X., Chen, X., Qiu, Q., Zhang, D., Chu, G., Zhou, G., 2013. Response of soil respiration to acid rain in forests of different maturity in southern China. PLoS One 8, e62207.

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.

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.

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.

Majdi, H., 1994. Effects of nutrient applications on fine-root dynamics and root/rhizosphere chemistry in a Norway spruce stand. Rapport-Sveriges Lantbruksuniversitet. Miljoevaard (Sweden), Institutionen foer Ekologi och.

Majdi, H., 2005. Fine root turnover in forest ecosystems. Plant Soil 276, vii–viii.

Mao, Q., Lu, X., Zhou, K., Chen, H., Zhu, X., Mori, T., Mo, J., 2017. Effects of long-term nitrogen and phosphorus additions on soil acidification in an N-rich tropical forest. Geoderma 285, 57–63.

Matamala, R., Schlesinger, W.H., 2000. Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. Glob. Chang. Biol. 6, 967–979.

Matamala, R., Gonzalez-Meler, M.A., Jastrow, J.D., Norby, R.J., Schlesinger, W.H., 2003. Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science 302, 1385–1387.

Miyamoto, K., Wagai, R., Aiba, S.I., Nilus, R., 2016. Variation in the aboveground stand structure and fine-root biomass of Bornean heath (kerangas) forests in relation to altitude and soil nitrogen availability. Trees 30, 385–394.

Miyatani, K., Mizusawa, Y., Okada, K., Tanikawa, T., Makita, N., Hirano, Y., 2016. Fine root traits in Chamaecyparis obtusa forest soils with different acid buffering capacities. Trees 30, 415–429.

Mo, J.M., Zhang, W., Zhu, W.X., Gundersen, P., Fang, Y.T., Li, D.J., Wang, H., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Glob. Chang. Biol. 14, 403–412.

Nadelhoffer, K.J., 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytol. 147, 131–139.

Ohashi, M., Nakano, A., Hirano, Y., Noguchi, K., Ikeno, H., Fukae, R., Finér, L., 2016. Applicability of the net sheet method for estimating fine root production in forest ecosystems. Trees 30, 571–578.

Persson, H., Majdi, H., Clemensson-Lindell, A., 1995. Effects of acid deposition on tree roots. Ecol. Bull. 158–167.

Santos, F., Nadelhoffer, K., Bird, J.A., 2016. Rapid fine root C and N mineralization in a northern temperate forest soil. Biogeochemistry 128, 187–200.

Singh, A., Agrawal, M., 2007. Acid rain and its ecological consequences. J. Environ. Biol. 29, 15.

Sofo, A., Dichio, B., Xiloyannis, C., Masia, A., 2005. Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Funct. Plant Biol. 32, 45–53.

Solberg, S., Aas, W., Økland, T., Eilertsen, O., Angell, V., Liu, Q.R., Zhao, D.W., Xiang, R., Xiao, J., Luo, J.H., 2006. Acid rain in China. Environ. Sci. Technol. 40, 418–425.

Šrámek, V., Fadrhonsová, V., Jurkovská, L., 2014. Ca/Al ratio in Norway spruce fine roots on monitoring plots in the Czech Republic. J. For. Sci. 60, 121–131.

Tanikawa, T., Sobue, A., Hirano, Y., 2014. Acidification processes in soils with different acid buffering capacity in *Cryptomeria japonica* and *Chamaecyparis obtusa* forests over two decades. For. Ecol. Manag. 334, 284–292.

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, 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.

Verma, K.S., Kohli, S., Kaushal, R., Chaturvedi, O.P., 2014. Root structure, distribution and biomass in five multipurpose tree species of Western Himalayas. J. Mt. Sci. 11, 519–525.

Vogt, K.A., Publicover, D.A., Bloomfield, J., Perez, J.M., Vogt, D.J., Silver, W.L., 1993. Belowground responses as indicators of environmental change. Environ. Exp. Bot. 33, 189–205.

Wang, T., Jiang, F., Li, S., Liu, Q., 2007. Trends in air pollution during 1996–2003 and cross-border transport in city clusters over the Yangtze River Delta region of China. Terr. Atmos. Ocean. Sci. 18, 995.

Wurzburger, N., Wright, S.J., 2015. Fine-root responses to fertilization reveal multiple nutrient limitation in a lowland tropical forest. Ecology 96, 2137–2146.

Xiong, Q., Pan, K., Zhang, L., Wang, Y., Li, W., He, X., Luo, H.Y., 2016. Warming and nitrogen deposition are interactive in shaping surface soil microbial communities near the alpine timberline zone on the eastern Qinghai-Tibet Plateau, southwestern China. Appl. Soil Ecol. 101, 72–83.

Xu, W., Liu, J., Liu, X., Li, K., Zhang, D., Yan, J., 2013. Fine root production, turnover, and decomposition in a fast-growth *Eucalyptus urophylla* plantation in southern China. J. Soils Sediments 13, 1150–1160.

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, 331–335.

Yuan, Z.Y., Chen, H.Y., 2010. Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility, and stand age: literature review and meta-analyses. Crit. Rev. Plant Sci. 29, 204–221.

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, 8021–8026.