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RESEARCH ARTICLE

Photosynthetic and growth responses of *Schima superba* seedlings to sulfuric and nitric acid depositions

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Abstract A continuing rise in acid deposition can cause forest degradation. In China, acid deposition has converted gradually from sulfuric acid deposition (SAD) to nitric acid deposition (NAD). However, the differing responses of photosynthesis and growth to depositions of sulfuric vs. nitric acid have not been well studied. In this study, 1-year-old seedlings of *Schima superba*, a dominant species in subtropical forests, were treated with two types of acid deposition SO_4^{2-}/NO_3^- ratios (8:1 and 0.7:1) with two applications (foliar spraying and soil drenching) at two pH levels (pH 3.5 and pH 2.5) over a period of 18 months. The results showed that the intensity, acid deposition type, and spraying method had significant effects on the physiological characteristics and growth performance of seedlings. Acid deposition at pH 2.5 via foliar application reduced photosynthesis and growth of *S. superba*, especially in the first year. Unlike SAD, NAD with high acidity potentially alleviated the negative effects of acidity on physiological properties and growth, probably due to a fertilization effect that improved foliar nitrogen and chlorophyll contents. Our results suggest that trees were damaged mainly by direct acid stress in the short term, whereas in the long term, soil acidification was also likely to be a major risk to forest ecosystems. Our data suggest that the shift in acid deposition type may complicate the ongoing challenge of anthropogenic acid deposition to ecosystem stability.

Keywords Sulfuric and nitric acid depositions · Foliar application · Soil drench · Photosynthetic properties · Growth

Introduction

Acid deposition is one of the most serious environmental issues caused by rapid economic development worldwide (Reis et al. 2012; Vet et al. 2014). It is mainly formed by dissolution of sulfur dioxide (SO_2) and nitrogen oxides (NO_x) and can be generally defined as either sulfuric acid deposition (SAD) or nitric acid deposition (NAD). During the last few decades, there have been large reductions in the amount of sulfur (S) and nitrogen (N) deposited in the USA and Europe, which is in line with the implementation of strict emission reduction policies (Vet et al. 2014). However, in China, S and N depositions have remained at high levels (Larssen et al. 2006). The model-based pattern estimated that most of eastern China had total depositions from 20 to 50.2 kg S ha^{-1} $year^{-1}$ and from 20 to 38.6 kg N ha^{-1} $year^{-1}$ in the 2000s, higher than the values in Europe and North America (Vet et al. 2014). Moreover, Zhao et al. (2009) predicted that oxidized N and reduced N deposition would increase by 30 and 57 %, respectively, in China from 2005 to 2020, although S deposition would decrease by 20 %. If this trend continues, N deposition is expected to make a larger contribution to acid deposition than S deposition in the near future, similar to what happened in Europe and North America. This argument is partially supported by the fact that the ratio of the equivalent concentrations of SO_4^{2-} to NO_3^- in

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precipitation has gradually declined from 4.28 to 1.81, over the last 20 years in Lin'an (Zhejiang Province), eastern China (Li et al. 2010).

The impacts of acid deposition on forest ecosystems have been an increasing ecological concern during the past several decades, since large inputs of acidic S and N compounds into forests via acid deposition may have negative effects on ecosystem function. Empirical studies have reported that acid deposition can cause soil acidification (Stevens et al. 2009), reduce the functioning of microbial communities (Kuperman and Edwards 1997), decrease enzyme activity (Ling et al. 2010; Wang et al. 2010), and even alter forest plant community composition (Schaberg et al. 2001; Liu et al. 2013a). Investigations have also shown that acid deposition affects plants' physiological properties and causes decreases in plant productivity (Vries et al. 2014). However, most empirical studies in China have focused on the effects of a single type of acid deposition, especially SAD (Fan and Wang 2000; Liang et al. 2015). So far, very little is known about whether the effects of different acid deposition types on plant growth performance are similar or not.

A few studies have compared growth and the physiological responses of tree species to different types of acid deposition (Chen et al. 2013; Lee et al. 2006), but the question whether or not SAD and NAD have different effects remains unresolved. For instance, some studies found that relative to NAD, SAD had a stronger negative effect on the growth of *Arabidopsis thaliana* (L.) Heynh. (Lee et al. 2006), *Liquidambar formosana* Hance, and *Schima superba* Gardner & Champ (Chen et al. 2013). In contrast, other studies observed that the detrimental outcome of NAD on plant growth was higher than that of SAD (Mai et al. 2010; Tao et al. 2013). In general, it is expected that plants will respond to SAD and NAD differently because of the differences in toxicity and nutritional importance between S and N. With a high intensity of SO_4^{2-}, SAD can cause visible symptoms such as chlorosis and necrosis, increased lipid peroxidation and cell membrane penetrability (Feng et al. 2002; Liu and Liu 2011; Chen et al. 2013; Liang and Wang 2013) and a changed protein profile (Wang et al. 2013). More importantly, plants' physiological activities can be affected by decreases in the leaves' photosynthesis, respiration rates, and nutritional status (Kuki et al. 2008; Wen et al. 2011; Dolatabadian et al. 2013), resulting in a reduction in tree growth. Similar to the toxicity of S, gases and aerosols of N may change plants' physiological properties (Chen et al. 2013). On the other hand, because N is an essential nutrient for plant growth, NAD and N depositions can have positive effects on plant growth in certain environments where N is the limiting factor (Mo et al. 2008; Liu et al. 2011; Zhang et al. 2013). For example, a nitrogen addition experiment at Dinghushan showed that a lower rate of N addition had a positive effect on plant growth but a higher N addition rate had a negative effect (Mo et al. 2008). However, excess inputs of N may cause nutritional imbalances and restrict plant growth (Liu et al. 2013b; Zhang et al. 2013). Thus, unlike those of SAD, the effects of NAD on plant growth depend on N availability and NAD intensity in forest ecosystems.

Even though chronic S and N depositions may have detrimental effects on forest ecosystems (Vries et al. 2014), the responses of ecosystems to such effects may vary at different time scales. It is well known that trees may be damaged directly by foliar injuries or indirectly by soil chemistry modifications (Aznar et al. 2009). Through canopy interception and foliar uptake, acid deposition can rapidly affect plants' physiological activities, as mentioned above. On the other hand, plant growth can also be triggered by soil chemical modifications. Acid deposition, especially NAD in the short term, has been found to increase soil N availability, consequently elevating foliar N concentration and improving plant production (Li et al. 2011; Lu et al. 2010; Jung and Chang 2012). However, long-term acid deposition can cause depletion of the basic cations and reduce soil fertility (Liu et al. 2007c). Increases in soil acidity can induce a nutrient imbalance in trees, especially for species with high nutrient demands, and may even release some toxic ions such as Al^{3+}, Mn^{2+}, and other heavy metals from the soil, thus having detrimental effects on plant growth (Liu et al. 2007a, b). These contrasting results suggest that the indirect impact of acid deposition on plant growth caused by soil chemistry modification might be either positive due to the fertilization effect of N deposition in the short term or negative because of toxic effects in the long term. Thus, it is reasonable to expect that the direct foliar impact of acid deposition and the indirect impact through soil chemistry modification on plant growth are not synchronized and can be affected by the type of acid deposition.

S. superba is a heliophilous and dominant species in evergreen broadleaf forests that is distributed over large areas in subtropical China. Previous studies have shown that the population size of *S. superba* has decreased during the past decade (Liu et al. 2007a; Chen et al. 2013). However, it is not clear how tree growth is affected by different types of acid deposition or by direct leaf injury and indirect soil chemistry modifications. In this study, we used *S. superba* to assess the impacts of SAD and NAD on its growth performance by using two spraying methods: a direct foliar spray and an indirect soil drench. Specifically, the objectives of this study were to investigate (1) whether the responses of plant growth differed between SAD and NAD treatments, and (2) whether the responses of plants' physiological properties and growth were similar for direct foliar injuries and the indirect effects of soil acidification with respect to both SAD and NAD.

Materials and methods

Research site and plant materials

This study was conducted in a greenhouse in Tiantong National Forest Ecosystem Observation and Research Station (29° 48′ N, 121° 47′ E), Zhejiang Province, in eastern China. Annual precipitation in this area is 1375 mm, and mean monthly temperatures range from 4.2 °C in winter (January) to almost 28.1 °C in summer (July) (Wang et al. 2007). All of the precipitation samples were qualified as acid rain (pH <5.6), and annual volume-weighted mean (VWM) pH value of rainfall is approximately 4.25 in 2007 (Li et al. 2010). The vegetation in this region is subtropical evergreen broadleaf forests, and *Castanopsis fargesii* Franch., *S. superba*, *Castanopsis carlesii* (Hemsl.) Hayata, and *Cyclobalanopsis nubium* (Hand.-Mazz.) Chun are common tree species.

In this study, we selected *S. superba* as a target species to assess the impacts of acid deposition on growth performance. In December 2009, 1-year-old seedlings of *S. superba* propagated from seed were purchased from a nearby seedling breeding center of the Ningbo forestry administration (Zhengjiang, China). Seedlings were individually transplanted into plastic pots (26 cm in diameter, 28 cm in height). Each pot was filled with native yellowish-brown lateritic soil (soil organic carbon, 0.98 %; total nitrogen, 1.36 g kg^{-1}; total phosphorus, 0.27 g kg^{-1}; total potassium, 1.41 %), mixed with forest litter (mainly wood chips) at a ratio of 1:1 (*v*/*v*) to loose soil. The seedlings were pre-incubated in a greenhouse for 3 months. By the time of acid exposure, seedlings had a height of 32 cm and a basal diameter of 4.8 mm on average.

Experimental design

In order to achieve our objectives, we split the study into three parts. The first part was to study changes in plants' physiological properties and growth parameters under increased acid deposition. The second part was to investigate the different responses of plant growth between SAD and NAD. The third part was to assess how plant growth differs between acid deposition via a direct foliar spray and an indirect soil drench for both SAD and NAD. The experiment used a three-way (2 × 2 × 2) factorial design plus a blank control. There were nine treatments in total: a control (CK) without H_2SO_4 and HNO_3 but with the application of water filtered with activated carbon (pH 6.0); four SAD treatments: SF3.5, SS3.5, SF2.5, and SS2.5; and four NAD treatments: NF3.5, NS3.5, NF2.5, and NS2.5, where the first letter indicates the type of acid deposition (S = SAD, N = NAD), the second letter indicates the spraying method (S = soil, F = foliar) and the numbers 3.5 or 2.5 indicate the pH value. The greenhouse was divided into three parts. Each of these treatments was arranged into three random blocks as replicates, each having ten individual plastic pots (i.e., 9 treatments × 3 blocks × 10 pots = 270 pots).

Two different pH levels (pH 3.5 and pH 2.5) were chosen. pH 3.5 is the current lowest pH value of precipitation in this region. Considering that the pH value of precipitation will probably decrease in the future, we added a more intense acid deposition treatment. For this treatment, pH 3.0 was applied in the first 3 months to avoid severe damage from heavy acid deposition during seedling establishment; this was then adjusted to pH 2.5 until the end of the experiment. According to previous acid rain records, as well as the tendency of the S/N mole ratio in the rainfall in this area, the solutions of SAD and NAD were prepared by adding a mixture of H_2SO_4 and HNO_3 in a 8:1 mol ratio for SAD and in a 0.7:1 ratio for NAD. The ratio of 8:1 was approximately the maximum value of the S/N mole ratio of rainfall during the 1990s, and 0.7:1 was the minimum value recorded recently at the study site. The foliar application was sprayed as an acid solution on the plant surface with a manual sprayer, which was covered with a thin film to avoid depositing acid onto the soil. The same volume of filtered water (pH 6.0) was provided to the soil as supplementary irrigation. The soil drench was an irrigated acid solution supplied to the soil surface. In order to keep the same extent of disturbance, filtered water was also sprayed onto the plant surface until water dropping (runoff) was observed.

From 1st April 2010 to 30th October 2011 (except January and February in 2011), each treated seedling received 300 mL of acid solution every 5 days. The acid solution was equivalent to 325 mm precipitation during the experiment. The control was sprayed with the same volume of filtered water on both foliage and soil. Inputs of hydrogen, S, and N during the experiment are detailed in Table 1. For pH 3.5 treatments, the level of S (or N) deposition under SAD (or NAD) was equivalent to 0.4× the atmospheric S (or N) deposition (Li et al. 2010). For high acid depositions, the level of S (or N) deposition almost tripled the levels observed in the studied region. No fertilizer was supplied to the pots. The position of the plants was changed every 15 days to eliminate positional effects. All pots were well irrigated with filtered water to avoid drought stress. From 1st June to 30th September, the greenhouse was covered with shade cloth to avoid high temperature stress. The transmittance of the greenhouse was about 75 %,

Table 1 Inputs of hydrogen (H), sulfur (S), and nitrogen (N) (kg ha^{-1} $year^{-1}$) during the experiment

Acid deposition treatments	H	S	N
SAD at pH 3.5	1.03	15.53	0.85
SAD at pH 2.5	8.11	122.12	6.68
NAD at pH 3.5	1.03	9.63	6.02
NAD at pH 2.5	8.11	75.69	47.31

SAD sulfuric acid deposition, *NAD* nitric acid deposition

and the maximum photosynthetic photon flux density (PPFD) in the greenhouse was less than 1000 μmol m^{-2} s^{-1}.

Measurements of physiological properties and tree growth

Physiological properties

We measured plant photosynthetic rate (A_{sat}) and stomatal conductance (gs) on 20th July 2010, 20th October 2010, and 20th May 2011. Within the greenhouse, the uppermost fully expanded leaves (at the third to fifth leaf position from the apex) from three or four individual plants were randomly selected for each block. Photosynthetic parameters were quantified with a portable photosynthetic system (LI-6400, LI-COR Inc., Lincoln, NE, USA). Measurements were first performed by increasing the PPFD to estimate the saturating light values for mature leaves. We found that the saturation PPDF of *S. superba* was <800 μmol m^{-2} s^{-1}. Considering the relatively low PPFD in the greenhouse, the system controlled the saturating PPFD at 800 μmol m^{-2} s^{-1} for this species. All measurements were conducted from 09:00 to 14:00 hours, avoiding the mid-vein. The relative value of each variable was calculated as a percentage of the control values for each sampling time.

After completion of the photosynthetic measurements, leaves were collected to test their physiological characteristics. Four leaf discs (1 cm in diameter each) per plant were taken from the leaves and plunged immediately into liquid N. The sampled leaf discs were extracted with 4 cm^3 95 % ethanol in the dark for 48 h at 4 °C, and the absorbance of leaf pigment extracts was measured at 470, 646, and 663 nm. Total chlorophyll (Chl) and carotenoid (Car) were calculated according to specific absorption coefficients according to Lichtenthaler (1987). The remaining leaves were oven-dried at 80 °C until the weight was constant. Finally, the samples were used to determine the leaf nitrogen concentration (LN) using a flow-injection auto-analyzer (Skalar, San^{++} System, The Netherlands) in July 2010 and May 2011.

Growth parameters

Plant height and stem basal diameter of each experimental seedling were measured in April 2010, November 2010 and October 2011. Stem basal diameter was measured in two directions at a fixed position at 5 cm of stem height from soil surface. The relative growth rates (RGRs) of plant height (H), stem basal diameter (D) and D^2H were determined as: $RGR_H = \frac{LnH_2 - LnH_1}{t_2 - t_1}$, $RGR_D = \frac{LnH_2 - LnH_1}{t_2 - t_1}$ and $RGR_{D^2H} = \frac{LnD_2{}^2H_2 - LnD_1{}^2H_1}{t_2 - t_1}$, where H_1 and D_1 were the initial height and diameter, H_2 and D_2 were the final height and diameter, and $(t_2 - t_1)$ was the exposure time. Plants were harvested and separated into leaves, stems and roots on October 2011. Dry mass was determined after oven-drying at 80 °C until a constant weight was reached.

Soil pH value

Soil sampling was conducted in July 2010 and October 2011. Within each plot, three soil cores (1.0 cm in internal diameter) were collected randomly from the upper soil layer (0–10 cm) to form one mixed sample. Soil samples were air-dried and passed through a 2-mm sieve before analysis. The soil pH values were measured with a pH meter (PB-10, Sartorius, Shanghai, China) at a soil/water ratio of 1:2.5 after shaking equilibration for ~30 min.

Data analysis

In this study, each block was treated as an independent experimental unit, which means that we obtained the average of all plants within each block and used that as one replicate for the data analysis. Three-way analyses of variance (ANOVAs) were used to evaluate the effects of the acid deposition intensity, the type of acid deposition and the spraying method, and their interactions on growth and physiological traits. We grouped the treatments to test the different effects of acid deposition for each concentration, each acid deposition type and each spraying method. The treatments were first divided into four groups to test the different effects of acid deposition for each concentration via the same spraying method for both SAD and NAD: (1) CK vs. SF3.5 vs. SF2.5, (2) CK vs. SS3.5 vs. SS2.5, (3) CK vs. NF3.5 vs.NF2.5, and (4) CK vs. NS3.5 vs. NS2.5. Similarly, the treatments were then divided into four groups to test the different effects of acid type on seedlings under the same pH level and via the same spraying method: (a) CK vs. SF3.5 vs. NF3.5, (b) CK vs. SS3.5 vs. NS3.5, (c) CK vs. SF2.5 vs. NF2.5, and (d) CK vs. SS2.5 vs. NS2.5. Finally, the treatments were divided into four groups to test the effects of different spraying methods at the same pH level for both SAD and NAD: (i) CK vs. SF3.5 vs. SS3.5, (ii) CK vs. NF3.5 vs. NS3.5, (iii) CK vs. SF2.5 vs. SS2.5, and (iv) CK vs. NF2.5 vs. NS2.5. A repeated-measure ANOVA was performed to detect significant differences among treatments, sampling times and their interaction. One-way ANOVA with Least-significant difference (LSD) comparison was used for each instance when the interaction of treatments and time was significant. The correlations among physiological parameters were examined using the Pearson product–moment correlation coefficient. All data were analyzed using the SPSS statistical package (ver. 17, SPSS, Chicago, IL, USA) and checked for deviations from normality and for homogeneity of variance before analysis. Statistical significant differences were set with p values less than 0.05 unless otherwise stated.

Results

Changes in plants' physiological properties, growth parameters and soil pH value with increased acid deposition

After 3 months of exposure, acid deposition via a foliar spray at high acidity induced yellow-brown spots on the upper surface of young leaves. These symptoms appeared to be more intense under SAD than under NAD in the first year. In contrast, no obvious symptoms of acid deposition injury were found under foliar pH 3.5 treatments or for all treatments using the soil drench.

A three-way ANOVA showed that the physiological traits of A_{sat}, gs, Chl, Car, and LN were not affected by the intensity of acid deposition in the first growing season (Table 2). One-way ANOVA revealed that A_{sat} under treatment with foliar SAD at pH 2.5 was significantly lower than that under pH 3.5 and in CK in the seventh month (Table 3). In addition, gs decreased significantly with increased acid intensity under foliar applications of SAD and NAD in the third month. However, in the second growing season, Chl and Car were significantly higher under the pH 2.5 treatments (mean ± SE, 0.84 ± 0.12 mg g^{-1} FW and 0.23 ± 0.02 mg g^{-1} FW, respectively) than under the pH 3.5 treatments (mean ± SE, 0.55 ± 0.04 mg g^{-1} FW and 0.18 ± 0.01 mg g^{-1} FW, respectively) (Table 4). Moreover, for Chl and Car, significant interactions between intensity and spraying type were detected (Table 2). One-way ANOVA showed that the contents of Chl and Car were the highest under NAD at pH 2.5 via soil drenching and were significantly greater than those under NAD at pH 3.5 via soil drenching and in CK (Table 3). In repeated-measure ANOVA, no interaction between acid treatment and sampling time was observed in LN and RGR. SAD via soil drenching at pH 2.5 significantly increased LN relative to CK, and NAD via soil drenching at pH 2.5 significantly increased LN relative to the pH 3.5 treatment and CK.

With respect to growth parameters, the three-way ANOVA showed that RGR_H and $RGR_D{}^2{}_H$ were higher under the pH 2.5 treatments (0.60 ± 0.06 mm m^{-1} day^{-1} and 1.65 ± 0.07 m m^3 m^{-3} day^{-1}) than under the pH 3.5 treatments (0.40 ± 0.03 mm m^{-1} day^{-1} and 1.47 ± 0.07 m m^3 m^{-3} day^{-1}), but only in the second growing season (Fig. 1; Table 5). There was no significant change in RGR_D or biomass accumulation in response to acid intensity. Moreover, no significant interaction among intensity, type and spraying method was detected for all growth parameters. The RGR_H decreased significantly under foliar NAD at pH 3.5 and pH 2.5. Leaf biomass decreased significantly under foliar SAD at pH 2.5 compared with foliar SAD at pH 3.5 and in CK (Fig. 2; Table 3). Acid deposition also decreased stem and root biomass, although this decrease was not statistically significant. The relative value of total biomass decreased significantly under foliar NAD in the pH 3.5 treatment (25.1 %) but not under foliar NAD in the pH 2.5 treatment.

Soil pH value was significantly influenced by sampling time and the interaction between acid treatment and sampling time (Fig. 3). In the third month, soil pH value was not influenced by acid application. In contrast, in the 18th month, soils receiving high-acidity treatments had a significantly lower pH value than the control soil. The value of soil pH decreased

Table 2 Results of three-way ANOVA for variability of plant physiological properties induced by the intensity (I), acid deposition type (T), and spraying method (S)

Time	Variables	I	T	S	I × T	I × S	T × S	I × T × S
Month 3	A_{sat} (μmol m^{-2} s^{-1})	0.123	*0.001*	*(0.008)*	0.097	0.670	0.299	0.891
	gs (mmol mol^{-1})	0.260	*0.006*	0.302	0.238	*0.014*	0.703	0.074
	Chl (mg g^{-1} FW)	0.604	0.904	(*0.029*)	0.732	0.601	0.448	0.997
	Car (mg g^{-1} FW)	0.844	0.634	(*0.041*)	0.994	0.762	0.484	0.874
	LN (mg g^{-1} DW)	0.709	0.053	(*0.021*)	0.448	0.479	0.216	0.676
Month 7	A_{sat} (μmol m^{-2} s^{-1})	0.305	0.896	(*0.050*)	*0.003*	0.671	0.405	0.106
	gs (mmol mol^{-1})	0.421	0.608	0.774	0.787	0.071	0.747	0.896
	Chl (mg g^{-1} FW)	0.962	0.322	(*0.006*)	0.316	0.956	0.422	0.671
	Car (mg g^{-1} FW)	0.734	0.404	(*0.021*)	0.301	0.839	0.381	0.391
Month 15	A_{sat} (μmol m^{-2} s^{-1})	0.695	0.187	0.995	0.756	0.362	0.580	0.597
	gs (mmol mol^{-1})	0.098	0.330	*0.021*	0.909	0.251	0.766	0.664
	Chl (mg g^{-1} FW)	(*<0.001*)	(*<0.001*)	(*0.016*)	0.052	*0.004*	*0.036*	*0.044*
	Car (mg g^{-1} FW)	(*0.001*)	(*<0.001*)	(*0.006*)	0.103	*0.001*	*0.031*	0.209
	LN (mg g^{-1} DW)	(*<0.001*)	(*0.002*)	(*<0.001*)	0.377	*0.036*	0.554	0.119

p values are presented in the table. If the mean values were greater in pH 2.5/NAD/soil drenching compared with that in pH 3.5/SAD/foliar spraying, *p* values are shown in parentheses. All the significant effects are set in italics

A_{sat} light-saturated photosynthetic rate, *gs* stomatal conductance, *Chl* leaf chlorophyll content, *Car* leaf carotenoid content, *LN* leaf nitrogen content

Table 3 Post hoc multiple comparisons for physiological properties and growth parameters of seedlings with different intensities

Time	Variables	CK vs. SF3.5 vs. SF2.5		CK vs. SS3.5 vs. SS2.5		CK vs. NF3.5 vs.NF2.5		CK vs. NS3.5 vs. NS2.5	
		ANOVA (p)	Multiple comparisons CK/SF3.5/SF2.5	ANOVA (p)	Multiple comparisons CK/SS3.5/SS2.5	ANOVA (p)	Multiple comparisons CK/NF3.5/NF2.5	ANOVA (p)	Multiple comparisons CK/NS3.5/NS2.5
Month 3	A_{sat}	0.397	ns	0.500	ns	0.497	ns	0.126	ns
	gs	*0.046*	a a b	0.234	ns	*0.004*	a b c	0.112	ns
	Chl	0.602	ns	0.955	ns	0.209	ns	0.840	ns
	Car	0.363	ns	0.961	ns	0.202	ns	0.930	ns
Month 7	A_{sat}	*0.023*	a a b	0.289	ns	0.080	ns	0.804	ns
	gs	*0.041*	a ab b	*0.001*	a b b	*0.016*	a b b	*0.013*	a b b
	Chl	0.124	ns	0.782	ns	0.608	ns	0.521	ns
	Car	0.112	ns	0.666	ns	0.391	ns	0.530	ns
Month 15	A_{sat}	0.075	ns	0.170	ns	0.159	ns	0.087	ns
	gs	0.651	ns	0.211	ns	0.252	ns	0.244	ns
	Chl	*0.035*	b b a	0.066	ns	0.051	ns	*0.007*	b b a
	Car	0.521	ns	*0.023*	ab b a	0.402	ns	*0.005*	b b a
	LN*	0.228	ns	*0.044*	b ab a	0.113	ns	*0.009*	b b a
	RGR_H*	0.102	ns	0.406	ns	*0.024*	a b b	0.587	ns
	RGR_D*	0.648	ns	0.181	ns	0.870	ns	0.646	ns
	$RGR_D{}^2{}_H$*	0.283	ns	0.129	ns	0.173	ns	0.479	ns
Month 18	W_{Root}	0.587	ns	0.333	ns	0.105	ns	0.331	ns
	W_{Stem}	0.159	ns	0.450	ns	0.096	ns	0.623	ns
	W_{Leaf}	*0.026*	a a b	0.611	ns	0.094	ns	0.121	ns
	RW_{Total}	0.409	ns	0.423	ns	*0.037*	a b ab	0.574	ns

Different letters on the same row indicated significant difference among CK, pH 3.5 and pH 2.5 via the same spraying method for both SAD and NAD ($p < 0.05$; a > b > c). The numbers 3.5 or 2.5 indicate the pH value

SAD sulfuric acid deposition, *NAD* nitric acid deposition, *SF* SAD with foliar spraying, *SS* SAD with soil drenching, *NF* NAD with foliar spraying, *NS* NAD with soil drenching

*no significant interaction between acid treatment and sampling time

significantly under SAD and NAD at pH 2.5 in month 18 than that in month 3, which indicates that the cumulative effect of acid deposition on soils was present. In addition, no significant difference in soil pH was observed between SAD and NAD.

Differences in plants' physiological properties and growth between SAD and NAD

The three-way ANOVA showed that NAD had stronger negative effect on A_{sat} than SAD in the early part of experiment (i.e., in the third month), but it had a positive effect on Chl and LN after 15 months of acid treatment (Table 2). NAD increased Chl and LN by 76.3 and 43.1 %, respectively, compared with only 15.2 and 15.1 % under SAD, respectively. In addition, the interaction between acid type and spraying method affected Chl and Car, and the interactions among type, spraying method and intensity affected Chl significantly (Table 2). Furthermore, we found that the reduction in RGR_D and $RGR_D{}^2{}_H$ was greater under NAD than under SAD in the second growing season (Table 5).

For moderate acidity (i.e., pH 3.5), we observed that the reduction in A_{sat} was greater under foliar application of NAD than under foliar SAD in the 7th month (Table 6). In the 15th month, the contents of Chl and Car were significantly higher under NAD via soil application than under SAD. The repeated-measure ANOVA showed that LN was increased in the NAD soil application treatment, but this effect was not significant for SAD. In addition, leaf biomass and the relative value of total biomass decreased significantly under foliar NAD compared with foliar SAD and in CK. All these results suggested that seedlings were more sensitive to NAD than SAD at moderate acidity.

For high acidity (i.e., pH 2.5), the reduction in A_{sat} under NAD was greater than that under SAD after 3 months of the experiment (Table 6). However, in the 7th month, the reductions in A_{sat} and Chl were greater under foliar application of SAD than under foliar NAD. In the second season, the increase in A_{sat} was greater under NAD than under SAD, though

Table 4 Means and standard error (SE) of plant physiological properties in different acid deposition treatments

Time	Variables	CK	Sulfuric acid deposition				Nitric acid deposition			
			SF3.5	SS3.5	SF2.5	SS2.5	NF3.5	NS3.5	NF2.5	NS2.5
Month 3	A_{sat} (μmol m^{-2} s^{-1})	9.58 ± 0.32	9.07 ± 0.28	10.55 ± 0.71	9.00 ± 0.31	10.7 ± 0.91	8.70 ± 0.09	9.23 ± 0.86	7.15 ± 0.65	9.13 ± 0.02
	gs (mmol mol^{-1})	0.14 ± 0.01	0.15 ± 0.02	0.13 ± 0.01	0.11 ± 0.00	0.16 ± 0.02	0.12 ± 0.00	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
	Chl (mg g^{-1} FW)	0.82 ± 0.10	0.78 ± 0.13	0.88 ± 0.21	0.64 ± 0.15	0.85 ± 0.09	0.66 ± 0.10	0.91 ± 0.19	0.58 ± 0.04	0.95 ± 0.16
	Car (mg g^{-1} FW)	0.20 ± 0.01	0.17 ± 0.02	0.19 ± 0.05	0.15 ± 0.03	0.19 ± 0.02	0.16 ± 0.03	0.22 ± 0.05	0.15 ± 0.01	0.22 ± 0.03
	LN (mg g^{-1} DW)	13.82 ± 1.32	13.43 ± 1.52	14.93 ± 0.58	14.88 ± 0.65	14.93 ± 0.17	14.67 ± 0.50	17.24 ± 0.47	14.61 ± 0.17	16.81 ± 0.89
Month 7	A_{sat} (μmol m^{-2} s^{-1})	6.81 ± 0.48	6.74 ± 0.25	6.54 ± 0.14	5.25 ± 0.13	6.01 ± 0.26	5.42 ± 0.16	6.38 ± 0.26	6.24 ± 0.34	6.62 ± 0.56
	gs (mmol mol^{-1})	0.20 ± 0.01	0.15 ± 0.02	0.13 ± 0.00	0.12 ± 0.01	0.14 ± 0.01	0.16 ± 0.02	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01
	Chl (mg g^{-1} FW)	1.88 ± 0.11	1.38 ± 0.28	2.10 ± 0.19	1.30 ± 0.10	1.86 ± 0.41	1.58 ± 0.31	1.90 ± 0.25	1.70 ± 0.13	2.14 ± 0.11
	Car (mg g^{-1} FW)	0.47 ± 0.03	0.35 ± 0.07	0.49 ± 0.04	0.32 ± 0.02	0.42 ± 0.09	0.40 ± 0.06	0.42 ± 0.05	0.39 ± 0.03	0.48 ± 0.04
Month 15	A_{sat} (μmol m^{-2} s^{-1})	5.67 ± 0.34	6.53 ± 0.10	6.78 ± 0.48	6.74 ± 0.34	6.19 ± 0.22	6.81 ± 0.37	7.07 ± 0.49	6.90 ± 0.56	6.94 ± 0.36
	gs (mmol mol^{-1})	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
	Chl (mg g^{-1} FW)	0.47 ± 0.01	0.48 ± 0.03	0.44 ± 0.01	0.57 ± 0.01	0.67 ± 0.07	0.65 ± 0.08	0.62 ± 0.05	0.72 ± 0.06	1.39 ± 0.24
	Car (mg g^{-1} FW)	0.18 ± 0.00	0.16 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.01	0.21 ± 0.02	0.33 ± 0.04
	LN (mg g^{-1} DW)	7.59 ± 0.18	7.14 ± 0.30	8.52 ± 0.61	9.38 ± 0.78	11.3 ± 0.25	9.37 ± 0.30	9.91 ± 0.45	9.56 ± 0.06	13.2 ± 1.20

$n = 3$ for all samples. The numbers 3.5 or 2.5 indicate the pH value

SAD sulfuric acid deposition, *NAD* nitric acid deposition, *SF SAD* with foliar spraying, *SS SAD* with soil drenching, *NF NAD* with foliar spraying, *NS NAD* with soil drenching

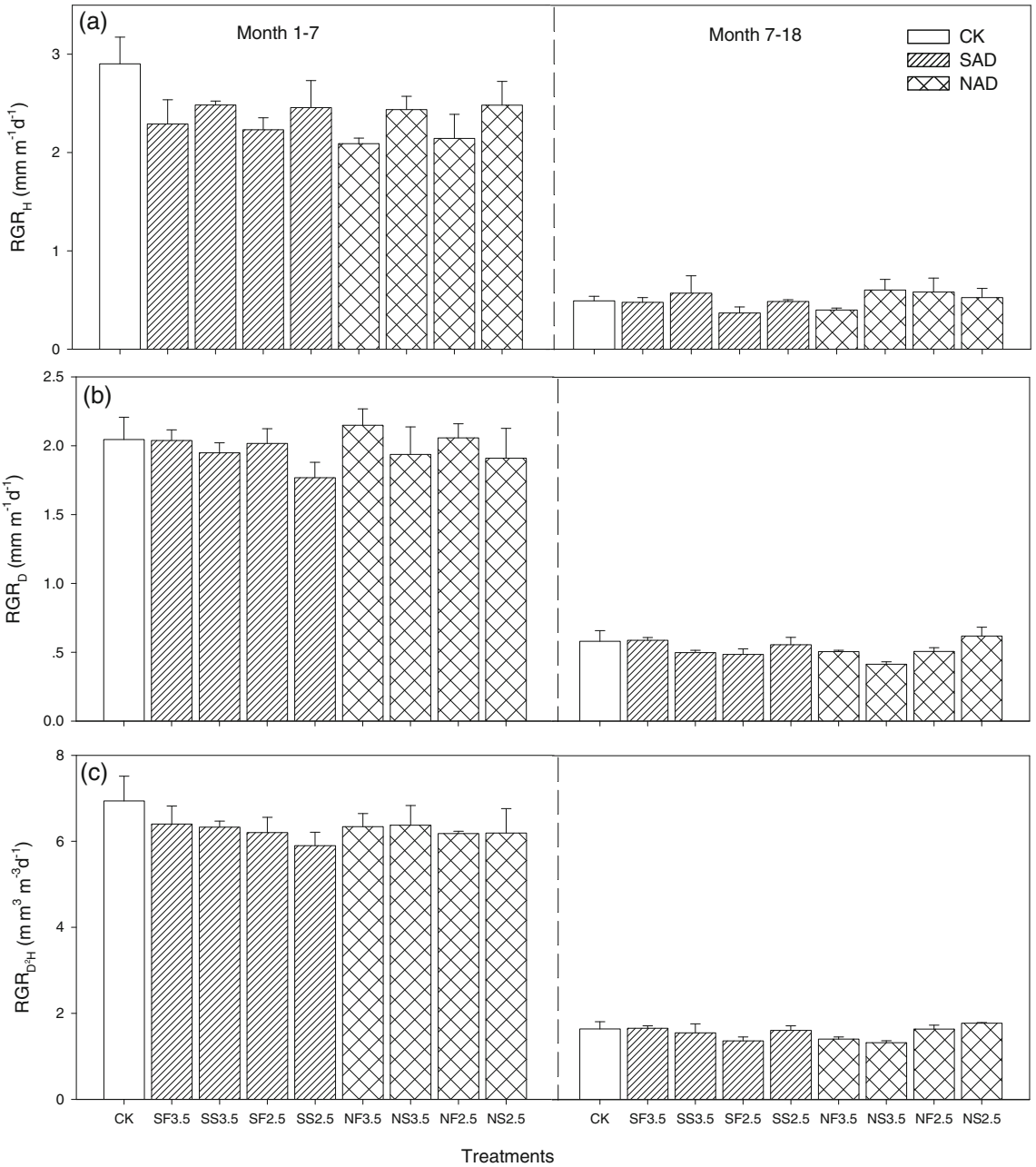

Fig. 1 Effects of acid deposition on relative growth rates (*RGRs*) of stem height (**b**), basal diameter (**b**), and D^2H (**c**). *Vertical bars* represent the standard error (*SE*) of the mean ($n = 3$). *SAD* sulfuric acid deposition, *NAD* nitric acid deposition, *SF* SAD with foliar spraying, *SS* SAD with soil drenching, *NF* NAD with foliar spraying, *NS* NAD with soil drenching; the numbers 3.5 or 2.5 indicate the pH value

not significantly. Chlorophyll was significantly higher under foliar application of NAD than under SAD and in CK, and Car was significantly higher under NAD via soil application than under SAD and in CK. Moreover, we found that LN increased significantly under NAD via soil drenching compared with the SAD treatment and in CK, whereas RGR and biomass did not differ significantly between SAD and NAD. These results suggested that NAD increased LN and Chl due to a fertilization effect in the second year but did not significantly alleviate the negative effects of acid stress on plants' physiological properties and growth.

Differences in plants' physiological properties and growth between foliar spray and soil drench

A three-way ANOVA showed that the reduction in A_{sat} was significantly greater under foliar application than under soil application in the third and seventh months. The contents of

Chl, Car, and LN under soil application were significantly higher than those under foliar application (Table 2). Similar trends were also found for the responses of RGR_D and $RGR_D{}^2{}_H$ in the second growing season (Table 5).

For SAD, we found that A_{sat} in month 7 and leaf biomass under foliar applications at pH 2.5 were significantly lower than those in CK. SAD via soil application at pH 2.5 induced a significant increase in LN and Chl in month 15, but no significant increase was seen under foliar application (Table 7).

For NAD, the increases in LN, Chl, and Car in the second growing season were significantly greater under soil application at pH 2.5 than under foliar application. However, growth parameters and biomass accumulation did not differ between the foliar spray and the soil drench.

Relationships among the physiological properties of seedlings

There was a significant positive relationship between LN and A_{sat} under foliar application ($r=0.834$; $p=0.003$) and under soil application ($r=0.743$; $p=0.014$) (Appendix 1). However, the relative value of A_{sat} was not correlated to the relative value of LN under foliar spraying ($r=0.550$; $p=0.099$) or under soil drenching ($r=0.148$; $p=0.704$). It is suggested that the relationship between LN and A_{sat} was not caused by the acid deposition treatment. The relationships between the relative value of Chl and LN ($r=0.737$; $p=0.001$) and between Chl and A_{sat} ($r=0.685$; $p<0.001$) were significant positive correlations, but these varied depending on the spraying method. As shown in Fig. 4, significant correlations were found between the relative value of Chl and LN under foliar application ($r=0.701$; $p=0.024$) and under soil application ($r=0.900$; $p=0.001$). The relative value of Chl was strongly and positively correlated with A_{sat} under foliar application ($r=0.864$; $p<0.001$) but not under soil application ($r=0.218$; $p=0.453$). It is therefore suggested that acid deposition via foliar spraying and soil drenching had different effects on N availability and the photosynthetic characteristics of seedlings.

Discussion

Effects of acid deposition intensity on plant photosynthesis and growth

Acid deposition, as a stress factor, can impact a wide array of plants' physiological and growth processes (Vries et al. 2014). This is the first study to investigate the composite effects of acid deposition (considering acid intensity, acid type, and spraying method) on a native broadleaf tree species in subtropical regions. Our results showed that compared with the foliar treatments at pH 3.5, the foliar treatments at pH 2.5 induced more severe foliar symptoms for current-year leaves of *S. superba* during this experiment. The injury symptoms were similar to those seen in 3-month-old *L. formosana* and *S. superba* seedlings exposed to simulated acid rain at pH 3.0 by Chen et al. (2013). This supported the earlier results that visible foliar damage symptoms would intensify with increasing acid deposition (Neufeld et al. 1985; Leith et al. 1989; Dixon and Kuja 1995).

Growth parameters and biomass accumulation are the major criteria under consideration when critical values of acid deposition affecting plants are discussed, as a change in these can reflect damage or disruption to metabolic and physiological processes (Feng et al. 2002). In this study, biomass accumulation was less affected after two growing

Table 5 Results of three-way ANOVA for variability of relative growth rates and biomass accumulation induced by the intensity (I), acid deposition type (T), and spraying method (S)

Time	Variables	I	T	S	I × T	I × S	T × S	I × T × S
Months 1–7	RGR_H (mm m^{-1} day^{-1})	0.449	0.403	0.426	0.342	0.356	0.133	0.654
	RGR_D (mm m^{-1} day^{-1})	0.067	0.225	0.586	0.159	0.753	0.673	0.702
	$RGR_D{}^2{}_H$ ($\times 10^{-3}$ m^3 m^{-3} day^{-1})	0.169	0.811	0.490	0.177	0.453	0.313	0.515
Months 7–18	RGR_H (mm m^{-1} day^{-1})	(*0.002*)	0.721	(*0.013*)	0.783	0.065	0.725	0.293
	RGR_D (mm m^{-1} day^{-1})	0.818	*0.011*	0.698	0.369	0.746	0.238	0.312
	$RGR_D{}^2{}_H$ ($\times 10^{-3}$ m^3 m^{-3} day^{-1})	(*0.022*)	*0.026*	(*0.013*)	0.468	0.070	0.443	0.702
Month 18	W_{Root} (g)	0.235	0.441	0.416	0.216	0.783	0.224	0.741
	W_{Stem} (g)	0.346	0.210	0.465	0.120	0.780	0.584	0.992
	W_{Leaf} (g)	0.571	0.344	0.681	0.059	0.822	0.315	0.743
	RW_{Total} (g)	0.289	0.814	0.445	0.059	0.943	0.241	0.988

p values are presented. If the mean values were greater in pH 2.5/NAD/soil drenching compared with that in pH 3.5/SAD/foliar spraying, *p* values were shown in parentheses. All the significant effects are set in italics

RGR_H relative growth rate of stem height, RGR_D relative growth rate of basal diameter, $RGR_D{}^2{}_H$ relative growth rate of D^2H, W_{Root} root dry weight, W_{Stem} stem dry weight, W_{Leaf} leaf dry weight, RW_{Total} relative value of total dry weight

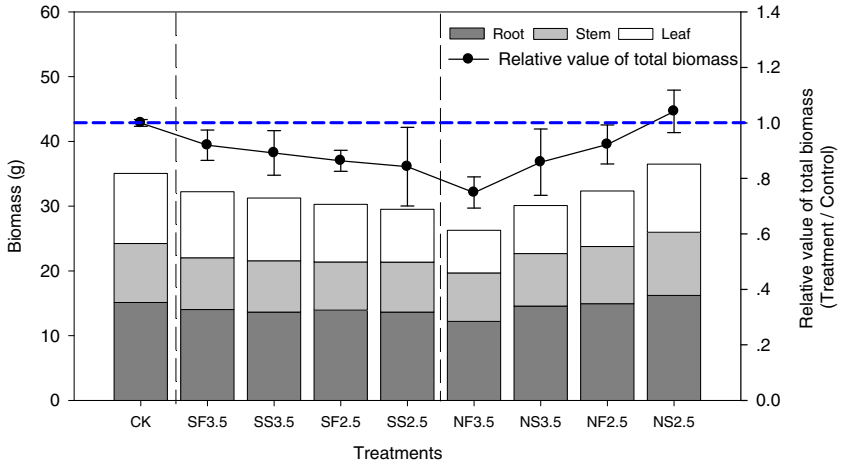

Fig. 2 Effects of acid deposition on biomass accumulation and the relative value of total biomass. *Vertical bars* represent the standard error of the mean ($n = 3$). Data were measured at 18 months after acid addition

seasons. Similar results were also found in Shan (1994), in which the total biomass of *S. superba* increased by 32.6 % after exposure to acid rain at pH 3.0 and decreased by 55.5 % at pH 2.0. This indicated that *S. superba* is not an acid-sensitive tree species and the threshold may be lower than pH 2.5 for this species (Shan 1994; Hogan 1998). Nevertheless, in the real world, most pH values of precipitation vary from 3.5 to 5.6 in this region (Li et al. 2010). Thus, direct visible foliar injuries and reductions in biomass to this species are limited in the short term.

In previous studies, Chen et al. (2013) and Liu et al. (2007a) found that *L. formosana* and *Pinus massoniana* Lamb. were more susceptible to acid deposition than *S. superba* in south China. However, some evergreen broadleaf trees, such as *Michelia macclurei* Dandy, *Cyclobalanopsis glauca* (Thunb.) Oerst., and *Cinnamomum camphora* (L.) J. Presl., showed more resistance to acid than *S. superba* (Shan 1994; Zhang et al. 2012). This suggested that conifers and deciduous forests exposed to acid deposition are more vulnerable than evergreen broadleaf forests. The lower susceptibility of evergreen broadleaf trees to acid deposition can be related to anatomical characteristics such as a thicker cuticle and mesophyll. Cuticle thickness can interfere with rain water retention on the leaf blade (Silva et al. 2005). The thick and compact mesophyll, with a larger quantity of Chl per unit of leaf surface, would enhance photosynthetic capacity, which is capable of feeding detoxification processes more intensely (Zhang et al. 2012). Evidence for this includes the fact that evergreen broadleaf forests continue to grow relatively well under acid deposition conditions, though widespread decline and dieback of *P. massoniana* in southwestern China, red spruce (*Picea rubens* Sarg.) in the northeastern USA (Leith et al. 1989), and sugar maple (*Acer saccharum* Marsh.) in Canada (Dixon and Kuja 1995) have been reported.

Despite no significant impacts on the biomass accumulation of seedlings being found, acid deposition affected plants' photosynthetic processes significantly, indicating that the responses of foliar physiology were more sensitive than growth. In the first growing season, the effects of acid deposition were a decrease in A_{sat}, along with a decline in gs and Chl. This demonstrated that acid deposition could induce stomatal closure and damage cell structures, which is consistent with other observational studies (Neufeld et al. 1985; Chen et al. 2013). Surprisingly, A_{sat} and Chl increased in the second year, unlike what we expected. The discrepancy in the changes in A_{sat} and Chl may result from a species' physiological acclimation and

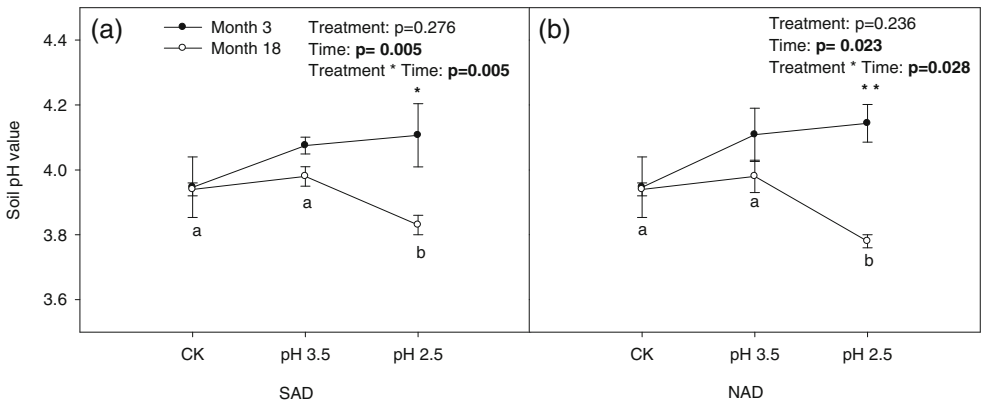

Fig. 3 Response of soil pH value to SAD (**a**) and NAD (**b**). *Vertical bars* represent the standard error of the mean ($n = 3$). *Different low letters* indicated significant difference among acid treatments for each sampling time ($p < 0.05$; a > b). *p less than 0.05; **p less than 0.01—between months 3 and 18 for each treatment, respectively. *SAD* sulfuric acid deposition, *NAD* nitric acid deposition

Table 6 Post hoc multiple comparisons for physiological properties and growth parameters of seedlings with different acid deposition types

Times	Variables	CK vs. SF3.5 vs. NF3.5		CK vs. SS3.5 vs. NS3.5		CK vs. SF2.5 vs. NF2.5		CK vs. SS2.5 vs. NS2.5	
		ANOVA (p)	Multiple comparisons CK/SF3.5/NF3.5	ANOVA (p)	Multiple comparisons CK/SS3.5/NS3.5	ANOVA (p)	Multiple comparisons CK/SF2.5/NF2.5	ANOVA (p)	Multiple comparisons CK/SS2.5/NS2.5
Month 3	A_{sat}	0.120	ns	0.410	ns	*0.022*	a a b	*0.046*	ab a b
	gs	0.202	ns	0.323	ns	*0.003*	a b b	*0.044*	ab a b
	Chl	0.581	ns	0.931	ns	0.322	ns	0.747	ns
	Car	0.370	ns	0.875	ns	0.231	ns	0.798	ns
Month 7	A_{sat}	*0.040*	a a b	0.660	ns	*0.049*	a b ab	0.478	ns
	gs	0.162	ns	*0.001*	a b b	*0.002*	a b b	*0.013*	a b b
	Chl	0.412	ns	0.664	ns	*0.027*	a b a	0.707	ns
	Car	0.344	ns	0.499	ns	*0.017*	a b ab	0.696	ns
Month 15	A_{sat}	0.073	ns	0.133	ns	0.162	ns	0.071	ns
	gs	0.455	ns	0.532	ns	0.482	ns	0.272	ns
	Chl	0.058	ns	*<0.001*	b b a	*0.006*	b b a	*0.009*	b a a
	Car	0.316	ns	*0.014*	a b a	0.066	ns	*0.007*	b b a
	LN*	0.248	ns	*0.017*	b ab a	0.122	ns	*0.011*	b b a
	RGR_H*	0.060	ns	0.496	ns	*0.049*	a b b	0.514	ns
	RGR_D*	0.979	ns	0.490	ns	0.727	ns	0.464	ns
	$RGR_D{}^2{}_H$*	0.392	ns	0.333	ns	0.123	ns	0.281	ns
Month 18	W_{Root}	0.083	ns	0.355	ns	0.677	ns	0.275	ns
	W_{Stem}	0.151	ns	0.624	ns	0.103	ns	0.457	ns
	W_{Leaf}	*0.012*	a a b	0.076	ns	0.261	ns	0.515	ns
	RW_{Total}	*0.021*	a a b	0.410	ns	0.197	ns	0.599	ns

Different letters on the same row indicated significant difference among CK, SAD, and NAD under the same pH level and spraying method ($p < 0.05$; a > b > c)

*no significant interaction between acid treatment and sampling time

repairing process by increasing antioxidative enzyme activity and the synthesis of specific metabolites under acid deposition conditions after some time (Liang et al. 2015). In addition, the impacts of acid deposition on plants can be positive due to the availability of nutrients (S and N) or negative due to a weakening of the forest condition (Vries et al. 2014). As there was no fertilization supplied during the experiment, nutrients gradually became a limiting factor. In the second year, the fertilization effect of acid deposition may have played an important part in improving plants' physiological activities and increasing the tolerance to acid deposition.

SAD and NAD affected tree photosynthesis and growth differently

In general, S and N are two main components of acid deposition. Consistent with our expectations, SAD and NAD affected tree growth and photosynthesis differently in many cases. For example, the reductions in A_{sat} in the first growing season and total biomass at the end of experiment were greater under NAD than under SAD at pH 3.5 via foliar application, indicating that the negative effects of NAD on seedlings were more significant than those of SAD. This may contribute to the greater oxidation stress of HNO_3 on plants' physicochemical activities. This strong oxidation is more likely to destroy the cell membrane due to the peroxidation of polyunsaturated fatty acids, and to reduce the photosynthetic pigment, A_{sat} and other physiological activities of seedlings (Mai et al. 2010). Meanwhile, strong oxidation of NO_3^- may further inhibit the activity of nitrate reductase and glutamate synthase and decrease the absorption and assimilation of N nutrients (Mai et al. 2010).

However, in the second year, we found that NAD, especially at pH 2.5, significantly elevated LN and Chl more than SAD did. Moreover, NAD at pH 2.5 via the soil drench treatment increased biomass accumulation, though not significantly. This result implied that adding N via deposition had a positive fertilization effect in the studied forest ecosystem (Liu et al. 2013b, 2015), which is consistent with the N-limited ecosystem hypothesis of Aber et al. (1998) and the results of short-term fertilization studies in southern China (e.g., Mo et al. 2008). In this study, a significant positive relationship existed between LN and Chl, and between Chl and A_{sat}, suggesting that N deposition increased A_{sat} to a

Table 7 Post hoc multiple comparisons for physiological properties and growth parameters of seedlings with different spraying methods

Time	Variables	CK vs. SF3.5 vs. SS3.5		CK vs. SF2.5 vs. SS2.5		CK vs. NF3.5 vs. NS3.5		CK vs. NF2.5 vs. NS2.5	
		ANOVA (p)	Multiple comparisons CK/SF3.5/SS3.5	ANOVA (p)	Multiple comparisons CK/SF2.5/SS2.5	ANOVA (p)	Multiple comparisons CK/NF3.5/NS3.5	ANOVA (p)	Multiple comparisons CK/NF2.5/NS2.5
Month 3	A_{sat}	0.166	ns	0.192	ns	0.298	ns	*0.018*	a b b
	gs	0.318	ns	0.083	ns	0.217	ns	*0.009*	a b b
	Chl	0.890	ns	0.424	ns	0.448	ns	0.145	ns
	Car	0.711	ns	0.368	ns	0.489	ns	0.126	ns
Month 7	A_{sat}	0.829	ns	*0.040*	a b ab	0.061	ns	0.695	ns
	gs	*0.030*	a ab b	*0.004*	a b b	*0.033*	a b b	*0.006*	a b b
	Chl	0.106	ns	0.254	ns	0.602	ns	0.093	ns
	Car	0.149	ns	0.221	ns	0.627	ns	0.133	ns
Month 15	A_{sat}	0.129	ns	0.116	ns	0.100	ns	0.136	ns
	gs	0.439	ns	0.273	ns	0.491	ns	*0.031*	b a ab
	Chl	0.276	ns	*0.032*	b ab a	0.082	ns	*0.009*	b b a
	Car	0.095	ns	0.100	ns	0.492	ns	*0.006*	b b a
	LN*	0.407	ns	*0.033*	b ab a	*0.010*	b b a	*0.009*	b b a
	RGR_H*	0.241	ns	0.114	ns	0.054	ns	0.254	ns
	RGR_D*	0.408	ns	0.188	ns	0.407	ns	0.923	ns
	$RGR_D{}^2{}_H$*	0.477	ns	0.105	ns	0.303	ns	0.431	ns
Month 18	W_{Root}	0.382	ns	0.706	ns	0.104	ns	0.521	ns
	W_{Stem}	0.313	ns	0.326	ns	0.456	ns	0.778	ns
	W_{Leaf}	0.622	ns	*0.015*	a b ab	*0.019*	a b b	0.475	ns
	RW_{Total}	0.425	ns	0.108	ns	0.067	ns	0.420	ns

Different letters on the same row indicated significant difference among CK, foliar spraying, and soil drenching at the same pH level for each of SAD and NAD ($p < 0.05$; a > b > c)

*no significant interaction between acid treatment and sampling time

certain extent. These results are consistent with many other studies. For example, Jacobson et al. (1990) showed that simulated SAD decreased aboveground biomass but NAD increased aboveground biomass for red spruce. Lee et al. (2006) reported that, compared with the control, shoot and root growth in *A. thaliana* treated with NAD decreased by 14 and 17 %, respectively, but specimens treated with SAD decreased by 24 and 25 % for shoot and root growth, respectively. Chen et al. (2013) found that seedlings of *L. formosana* and *S. superba* suffered more in terms of photosynthesis and the antioxidant system under SAD than under NAD at pH 3.0.

Direct and indirect effects of acid deposition on plant photosynthesis and growth

Comparing the direct (via foliage) and indirect (via soil) effects of acid deposition on plants is vital for distinguishing the confounding effects of two processes that often happen simultaneously. In our experiment, we found that the responses of plant physiological properties and growth were different for direct foliar injuries and indirect effects through soil chemistry modifications. Negative effects are more serious under direct foliar contact, especially in the first year, consistent with the work of Aznar et al. (2009). This indicated that soil, as a buffer substance, can accumulate atmospheric pollutants and reduce direct damage on plants. Meanwhile, plants could benefit from the nutrients in soils supplied by acid deposition (Jung and Chang 2012).

However, when N deposition exceeds the nutritional demands of plants, it would reduce net photosynthesis by decreasing the activity of Rubisco in the leaves. The N saturation hypothesis predicts that high accumulation of N in the foliage will dilute the pool of other nutrients, causing a nutritional imbalance and increasing the risk of climatic factors, and finally leading to tree decline (Aber et al. 1998). In this study, the correlation between LN and A_{sat} was stronger under foliar spraying than under soil drenching. Moreover, increased Chl resulting from elevated LN improved A_{sat} under foliar spraying; however, increased Chl did not affect A_{sat} under soil drenching (Fig. 4). This implies that A_{sat} under the soil drench treatment was probably limited by other factors. Phosphorus, rather than N, may be the limiting factor in this type of soil and hence the effects of NAD on a forest

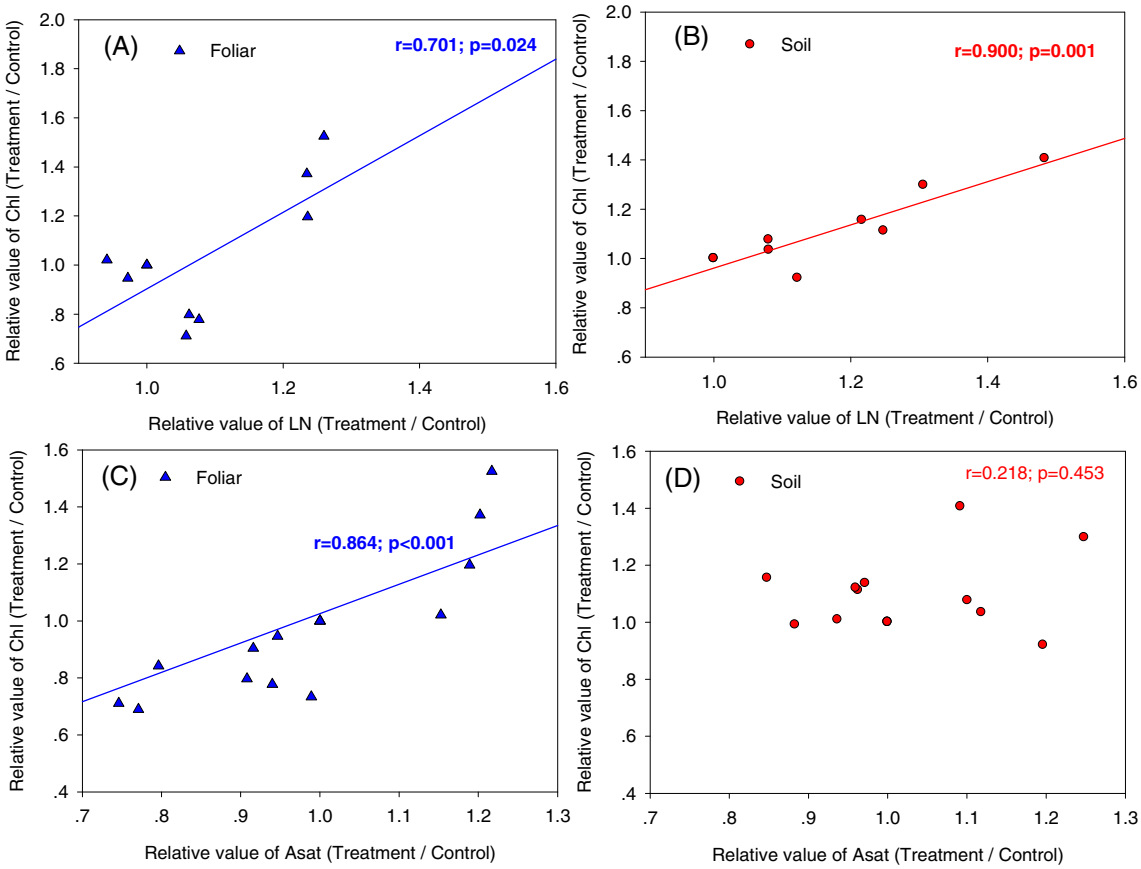

Fig. 4 The relationships between the relative values of LN and Chl and between the relative values of Chl and A_{sat} in the leaves of *S. superba* seedlings. The relative value of each variable was calculated as a percentage of the control values for each sampling time. *Foliar*, acid deposition via foliar spraying; soil, acid deposition via soil drenching

ecosystem are more complex than those of SAD. In the near future, changes in the energy structure in China may aggravate the influence of acid deposition on forest ecosystems, which may cause further forest degradation.

Moreover, subtropical forest soils, which are poorly buffered against low pH, are sensitive to such acid depositions. In this study, acid addition significantly decreased the soil pH value under the pH 2.5 treatments. Additionally, multi-year experiments have shown that soil acidification further increased the concentration of free-moving metallic ions (e.g., Al^{3+}, Mn^{2+}), which can be highly toxic to plants (Liu et al. 2007b). Several studies have indicated that soil acidification resulting from acid deposition, especially NAD, is the main cause of long-term forest decline (Ulrich et al. 1980; Zhao et al. 2009; Vries et al. 2014).

Conclusions

Measurements of plants' physiological properties, growth, and biomass under eight different acid deposition treatments over 18 months revealed that acid intensity, acid deposition type (SAD vs. NAD), and spraying method had significant effects on the physiological properties of *S. superba* in the study region. Foliar acid deposition at pH 2.5 inhibited photosynthesis in the first year and also caused a slight reduction in RGR and biomass accumulation. Compared with SAD, NAD at moderate acidity had a stronger negative effect on A_{sat} and biomass. For high-intensity acid deposition, NAD potentially alleviated the negative effects of acidity on photosynthesis and growth, which increased LN and Chl due to a fertilization effect. Trees were damaged mainly by direct acid stress rather than by soil acidification in this short-term experiment. Our data indicate that the effects of NAD on the forest ecosystem are more complex than those of SAD and the shift in acid deposition type (from SAD to NAD) further complicates the ongoing challenge of anthropogenic acid deposition to ecosystem stability and increases the risks of ecosystem degradation.

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