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# Leaf Functional Traits of *Amaranthus tricolor* as Affected by Different Types of Acid Deposition

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## Abstract

The progressively diversified in the types of acid deposition may complicate the pronounced effects of acid deposition on the structure, function, and stability of ecosystems. Meanwhile, leaf functional traits are important indices in relation to the relationship between various environmental factors and leaf functioning of plant species. This study aims to assess the effects of five types of acid deposition with different  $SO_4^{2-}$  to  $NO_3^{-}$  ratios (1:0, sulfuric acid; 3:1, sulfuric-rich acid; 1:1, mixed acid; 1:3, nitric-rich acid; 0:1, nitric acid) on the leaf functional traits of one of the common crops *Amaranthus tricolor* L. Sulfuric-rich acid deposition and mixed acid deposition significantly decreased leaf shape index of *A. tricolor*. This may be attributed to the decreased soil pH values mediated by acid deposition which could show negative effects on leaf growth of *A. tricolor*. Sulfuric-rich acid deposition triggered more negative effects on leaf functional traits (especially leaf length, leaf width, leaf shape index, and leaf N concentration) of *A. tricolor* than nitric-rich and/or nitric acid deposition. This may be attributed to nitric deposition possibly exerting a fertilizing effect but not sulfuric deposition. Another reason may be the difference in exchange capacity with hydroxyl groups (OH<sup>-</sup>) between  $SO_4^{2-}$  and  $NO_3^{-}$ . Therefore, the ratio of  $SO_4^{2-}$  to  $NO_3^{-}$  in acid deposition may be a key factor determining the effects of acid deposition on leaf functional traits of *A. tricolor*.

### **Keywords**

Acid Deposition, Leaf Functional Traits, Amaranthus tricolor

# 1. Introduction

At present, acid deposition has become one of the important components of global environmental change. East Asia (mainly China), Western Europe, and North America are currently the major areas of acid deposition [1], [2], [3], [4]. Acid deposition can trigger pronounced effects on the structure, function, and stability of ecosystems, such as the modification of plant species composition [5] and plant litter decomposition [6], [7], [8], [9], the restrained plant growth [10], [11], [12], the engendered soil acidification [6], [7], [9], the reduction in soil microbial function [4], [6], [7], [9], and the enhanced allelopathic effects of invasive plants on native species [8], [12].

Sulfur dioxide (SO<sub>2</sub>) and nitrogen oxides (NO<sub>x</sub>) are the main source of acid deposition [1], [3], [4]. For some decades now, the proportion of sulfate ion (SO<sub>4</sub><sup>2-</sup>) in precipitation tends to decrease due to the emerging policies on the controlling and mitigating SO<sub>2</sub> emissions and the adjustment of energy structure in China, whereas the relative contribution of nitrate ion (NO<sub>3</sub><sup>-</sup>) to acidification increased progressively due to the substantial increase in the amount of nitrogenous fertilizer application and the number of motor vehicles in China [13], [14]. Accordingly, the type of acid rain changes gradually from acid rain dominated by sulfuric acid to acid rain with a mixture of sulfuric and nitric acids (mixed acid rain, MAR), and then to acid rain dominated by nitric acid in China [9], [13], [14], [15]. More important, the progressively diversified in the types of acid deposition may complicate the

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pronounced effects on the structure, function, and stability of ecosystems [9], [12], [15].

Leaf functional traits can facilitate plants to occupy a wide variety of environmental conditions. Thus, leaf functional traits are important indices in relation to the relationship between various environmental factors and leaf functioning [16], [17], [18], [19]. Consequently, determination of the effects of different types of acid deposition (especially MAR) on the leaf functional traits of is important in illuminating the effects of acid deposition on the structure, function, and stability of ecosystems, especially the growth of plant species.

The present study aims to determine the effects of five types of acid deposition with different  $SO_4^{2^-}$  to  $NO_3^-$  ratios (1:0, sulfuric acid; 3:1, sulfuric-rich acid; 1:1, mixed acid; 1:3, nitric-rich acid; 0:1, nitric acid) on the leaf functional traits of one of the common crops *Amaranthus tricolor* L. *A. tricolor* is a herbaceous annual plant of the Amaranthaceae family. This plant is native to China. Since ancient times, the species is widely cultivated as a vegetable in China, where acid deposition is prevalent in recent decades, particularly in the Yangtze River Delta region [1], [2], [3]. The present study addressed the following hypotheses: (1) acid deposition can have adverse effects on the leaf functional traits of *A. tricolor*; and (2) the effects of acid deposition on the leaf functional traits of *A. tricolor* and traits of *A. tricolor* are solved by the types of acid deposition.

### 2. Materials and Methods

### 2.1. Experimental Design

Experiments were performed by using pot cultivation experiment. A. tricolor seeds were bought from one seller in a local vegetable market. The seeds were placed in garden pot. The diameter of the garden pot is 25 cm. Six seedlings of A. tricolor with uniform and strongly growing were incubated per garden pot. The seedlings of A. tricolor were treated with five types of simulated acid deposition. Five types of simulated acid deposition were prepared by mixing  $0.5 \text{ M L}^{-1}$  $H_2SO_4$  and 0.5 M L<sup>-1</sup> HNO<sub>3</sub> at ratios of 1:0 (sulfuric acid, SA), 3:1 (sulfuric-rich acid, SRA), 1:1 (mixed acid, MAR), 1:3 (nitric-rich acid, NRA), and 0:1 (nitric acid, NA). For the five types of simulated acid deposition, the pH values of basic solution were finally buffered to 4.5 by adding the five stock solutions. For CK (control), the pH value of basic solution was finally buffered to 5.6 by adding the stock solutions of H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> at a ratio of 1:1. In this study, pH 5.6 was the natural values of unpolluted rainfall, and pH 4.5 was the approximate annual average pH value of rainfall in the study site. Three replicates were performed per treatment. The samples were incubated at room temperature for approximately three months. The plant sample replicates per treatment were harvested after incubation to determine the indices of leaf functional traits of A. tricolor. In one plant sample, five adult and intact leaves were selected randomly to determine the functional traits.

# 2.2. Determination of Leaf Functional Traits of *A. tricolor*

Petiole length was measured using a ruler [18], [19]. Leaf shape index was calculated as the ratio of leaf length to the corresponding leaf width [16], [18], [19], [20]. The leaf length is the maximum value along the midrib, whereas the leaf width is the maximum value perpendicular to the midrib [16]. Leaf length and leaf width were measured using a ruler [18], [19], [21]. Relative chlorophyll and N concentrations in the leaves were estimated with a hand-held plant nutrient meter (TYS-3N, China). TYS-3N was used to calculate the index in "SPAD units" based on absorbance at 650 nm and 940 nm [21], [22]. SLA was computed using the ratio of the leaf area to the corresponding leaf dry weight  $(cm^2 g^{-1})$  [18], [19], [21], [23], [24]. Leaf moisture was calculated by subtracting the leaf dry weight from the leaf wet weight; the difference was then divided by the leaf wet weight. Single-leaf wet weight was determined using an electronic balance. Single-leaf dry weight was obtained by initially subjecting the samples to oven drying at 60°C for 24 h to achieve a constant weight. The final single-leaf dry weight was then determined using an electronic balance with an accuracy of 0.001 g [18], [19]. Leaf thickness was calculated through the overlap of five leaves using a Vernier caliper with an accuracy of 0.01 mm [18], [19], [21].

#### 2.3. Determination of Soil pH Values

The pH values of soil samples were measured using a soil acidity meter *in situ* [ZD instrument (ZD-06), P. R. China] [18], [21].

### 2.4. Statistical Analysis

Differences among various dependent variables were assessed by using an analysis of variance between groups followed by multiple comparisons with the S-N-K test using SPSS Statistics (version 22.0; IBM, Armonk, NY, USA). Statistically significant differences were set at *P* values equal to or lower than 0.05.

### 3. Results

SRA and MAR exhibited significantly negative effects on leaf shape index of *A. tricolor* comparison with control treatment (Tables 1 and 2, P < 0.05). While, all types of acid deposition did not exert significant effects on petiole length, leaf length, leaf width, leaf chlorophyll and N concentrations, SLA, single-leaf wet and dry weights, leaf moisture, and leaf thickness of *A. tricolor* (Tables 1 and 2, P > 0.05).

Leaf length of *A. tricolor* under SRA treatment was significantly lower than those under SA, MAR, NRA, and NA treatments (Table 1, P < 0.05). Leaf width of *A. tricolor* under NRA treatment was significantly higher than that under SRA treatment (Table 1, P < 0.05). Leaf shape index of *A. tricolor* under NRA and NA treatments were significantly higher than those under SRA and MAR treatments (Table 1, P < 0.05). Leaf N concentration of *A. tricolor* under SRA and NA treatments (Table 1, P < 0.05).

treatment (Table 1, P < 0.05). However, the difference in petiole length, leaf chlorophyll concentration, SLA, single-leaf wet and dry weights, leaf moisture, and leaf thickness of *A. tricolor* among the five types of simulated acid

deposition were not significant (Table 1, P > 0.05).

Acid deposition decreed soil pH values but this change was not significant (P > 0.05, data not shown).

**Table 1.** Differences in leaf functional traits of A. tricolor under different treatments. Data with different letters in a vertical row indicate a significant difference (P < 0.05). "ns" means no significant difference (P > 0.05). Abbreviations: PL, petiole length (cm); LL, leaf length (cm); LW, leaf width (cm); LSI, leaf shape index; LCC, leaf chlorophyll concentration (SPAD); LNC, leaf N concentration (mg g<sup>-1</sup>); SLA, specific leaf area (cm<sup>2</sup> g<sup>-1</sup>); SLWW, single-leaf wet weight (g); SLDW, single-leaf dry weight (g); LM, leaf moisture (%); LT, leaf thickness (mm); CK, control; SA, sulfuric acid; SRA, sulfuric-rich acid; MAR, mixed acid; NRA, nitric-rich acid; NA, nitric acid.

	PL	LL	LW	LSI	LCC	LNC	SLA	SLWW	SLDW	LM	LT
CK	3.578	6.933	4.611	1.500	22.389	2.111	148.366	0.327	0.031	90.229	0.332
	±0.541ns	±0.967ab	±0.584ab	±0.025b	±1.251ns	±0.073ab	±6.896ns	$\pm 0.087$ ns	±0.007ns	±0.527ns	±0.013ns
SA	4.189	8.322	5.722	1.454	25.944	2.378	148.512	0.547	0.053	90.488	0.319
	±0.179ns	±0.863a	±0.580ab	±0.006bc	±1.658ns	±0.118a	±10.855ns	±0.133ns	±0.014ns	±0.322ns	±0.025ns
SRA	3.267	5.678	4.089	1.386	21.778	2.067	167.378	0.280	0.029	89.394	0.296
	±0.252ns	±0.386b	±0.204b	±0.026c	±0.401ns	±0.038b	±4.481ns	±0.023ns	±0.002ns	±1.506ns	$\pm 0.018$ ns
MAR	3.611	8.022	5.733	1.400	23.211	2.189	153.554	0.433	0.045	89.806	0.317
	±0.483ns	±0.344a	±0.250ab	±0.016c	±0.881ns	±0.056ab	$\pm 15.863$ ns	±0.101ns	±0.013ns	±0.710ns	±0.031ns
NRA	3.544	8.889	6.000	1.481	24.956	2.300	149.172	0.413	0.045	89.217	0.315
	±0.059ns	±0.323a	±0.217a	±0.006b	±0.563ns	±0.033ab	$\pm 10.087 ns$	±0.107ns	±0.013ns	±0.563ns	±0.021ns
NA	3.633	8.856	5.544	1.598	26.022	2.378	138.996	0.493	0.052	89.252	0.312
	$\pm 0.168$ ns	±0.422a	±0.289ab	±0.021a	$\pm 0.484$ ns	±0.029a	±7.431ns	±0.121ns	±0.011ns	$\pm 0.459 ns$	$\pm 0.008$ ns

**Table 2.** ANOVA of the effects of different types of acid deposition on leaf functional traits of A. tricolor. Abbreviations have the same meanings as described in Table 1.

		Sum of Squares	df	Mean Square	F	Р
PL	Between Groups	1.363	5	0.273	0.835	0.549
	Within Groups	3.919	12	0.327		
	Total	5.282	17			
LL	Between Groups	23.626	5	4.725	4.237	0.019
	Within Groups	13.381	12	1.115		
	Total	37.007	17			
LW	Between Groups	8.566	5	1.713	3.754	0.028
	Within Groups	5.476	12	0.456		
	Total	14.043	17			
LSI	Between Groups	0.089	5	0.018	17.216	< 0.0001
	Within Groups	0.012	12	0.001		
	Total	0.101	17			
LCC	Between Groups	50.774	5	10.155	3.499	0.035
	Within Groups	34.822	12	2.902		
	Total	85.596	17			
LNC	Between Groups	0.272	5	0.054	4.242	0.019
	Within Groups	0.154	12	0.013		
	Total	0.426	17			
SLA	Between Groups	1305.930	5	261.186	0.879	0.524
	Within Groups	3564.317	12	297.026		
	Total	4870.247	17			
SLWW	Between Groups	0.150	5	0.030	0.963	0.477
	Within Groups	0.373	12	0.031		
	Total	0.522	17			
SLDW	Between Groups	0.002	5	0.000	0.879	0.524
	Within Groups	0.004	12	0.000		
	Total	0.006	17			
LM	Between Groups	4.301	5	0.860	0.467	0.794
	Within Groups	22.098	12	1.842		
	Total	26.399	17			
LT	Between Groups	0.002	5	0.000	0.318	0.892
	Within Groups	0.015	12	0.001		
	Total	0.017	17			

# 4. Discussion

Previous studies founded that acid deposition exhibited significantly negative effects on the growth characters of plants obviously [10], [11], [12]. The results of this study also showed that sulfuric-rich acid deposition and mixed acid deposition significantly decreased leaf shape index of A. tricolor. This may be ascribed to the decreased soil pH values mediated by acid deposition [1], [10], [12] which could produce negative effects on leaf growth of A. tricolor. Another reason may be the increased cation leaching triggered by acid deposition [1], [25]. Balasubramanian et al. [10] also founded that simulated acid deposition exert negative effects on growth characters of plant, such as leaf dry weight, single leaf size, SLA, leaf area index. However, nitric acid deposition significantly increased leaf shape index of A. tricolor compare with control. The result may be ascribed to nitric deposition possibly exerting a fertilizing effect [26], [27]. Meanwhile, all types of acid deposition did not trigger significantly affects on petiole length, leaf length, leaf width, leaf chlorophyll and N concentrations, SLA, single-leaf wet and dry weights, leaf moisture, and leaf thickness of A. tricolor in this study. Meanwhile, the effects of acid deposition on leaf shape index of A. tricolor were higher than those of other indices of *A. tricolor* in this study. The result was less consistent with the first hypothesis. The results of previous study [10] and this study suggest that the effects of acid deposition on plant growth vary with species and leaf functional traits. The reason may be ascribed to the difference in plant types, the acidity and/or type of acid deposition, soil physicochemical properties, and/or the time scale of the studies.

An interesting finding is that the effects of acid deposition on leaf functional traits of A. tricolor varied with the acid deposition types in this study. In particular, sulfuric-rich acid deposition triggered more negative effects on leaf length, leaf width, and leaf shape index of A. tricolor than nitric-rich acid deposition. Meanwhile, the effects of sulfuric-rich acid deposition leaf length, leaf shape index, and leaf N concentration of A. tricolor is higher than those of nitric acid deposition. Thus, sulfuric-rich acid deposition triggered more negative effects on leaf functional traits of A. tricolor than nitric-rich or nitric acid deposition. The result may be attributed to nitric deposition possibly exerting a fertilizing effect [26], [27]. By contrast, sulfuric deposition does not exert a fertilizer effect [26]. The phenomenon may also be a consequence of the difference in the exchange capacity with hydroxyl groups (OH<sup>-</sup>) between  $SO_4^{2-}$  and  $NO_3^{-}$  [9], [28]. The result supports the second hypothesis, suggesting that the ratio of  $SO_4^{2-}$  to  $NO_3^{-}$  in acid deposition was a key factor determining the effects of acid deposition on leaf functional traits of A. tricolor. Some previous studies have also confirmed that the ratio of  $SO_4^{2-}$  to  $NO_3^{-}$  in acid deposition could reveal notable effects on some ecological processes, such as soil microbial biomass [29], plant litter decomposition

[9], plant seedling growth [11], [12], and the allelopathic effects of invasive species on native plant [12].

### 5. Conclusion

The purpose of this study was to determine the effects of five types of acid deposition with different  $SO_4^{2-}$  to  $NO_3^{-}$ ratios (1:0, sulfuric acid; 3:1, sulfuric-rich acid; 1:1, mixed acid; 1:3, nitric-rich acid; 0:1, nitric acid) on the leaf functional traits of Amaranthus tricolor. Results showed that sulfuric-rich acid deposition and mixed acid deposition significantly inhibited leaf shape index of A. tricolor. This may be attributed to the decreased soil pH values mediated by acid deposition which could exert negative effects on negative effects on leaf growth of A. tricolor. Sulfuric-rich acid deposition triggered more negative effects on leaf length, leaf width, and leaf shape index of A. tricolor than nitric-rich acid deposition. Meanwhile, the effects of sulfuric-rich acid deposition leaf length, leaf shape index, and leaf N concentration of A. tricolor is higher than those of nitric acid deposition. Thus, sulfuric-rich acid deposition triggered more negative effects on leaf functional traits of A. tricolor than nitric-rich or nitric acid deposition. This may be attributed to nitric deposition possibly exerting a fertilizing effect but not sulfuric deposition. Another reason may be the difference in exchange capacity with hydroxyl groups (OH<sup>-</sup>) between SO<sub>4</sub><sup>2-</sup> and NO<sub>3</sub>. This showed that the ratio of  $SO_4^{2-}$  to  $NO_3^{-}$  in acid deposition was a key factor that profoundly affected the effects of acid deposition on leaf functional traits of A. tricolor. The diversified in the types of acid deposition mediated by the changes in the ratio of SO<sub>4</sub><sup>2-</sup> to NO<sub>3</sub><sup>-</sup> in acid deposition progressively altered occur in the natural ecosystem in the coming decades. Therefore, the growth of plant species via the variation in leaf functional traits would also change consequently.

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