Insights into the Effects of Simulated Nitrogen Deposition on Leaf Functional Traits of *Rhus Typhina*

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Abstract

The effects of anthropogenic nitrogen (N) deposition on successful plant invaders, particularly potential effects on their leaf functional traits, have stimulated considerable research interest. This study aims to gain insights into the leaf functional traits of the controversial invader *Rhus typhina* in the presence of a gradient of simulated N deposition (control, 0 g L⁻¹; low N, 5 g L⁻¹; medium N, 10 g L⁻¹; and high N, 25 g L⁻¹). Soil pH is decreased under the growth of *R. typhina*. The soil acidification mediated by *R. typhina* may be due to the positive effects of *R. typhina* on soil ammonium concentration and negative effects on soil nitrate concentration. Soil pH decreased under N fertilization due to the release of free H⁺ via the nitrification process. Leaf width, leaf chlorophyll and N concentrations, SLA, and single leaf wet weight of *R. typhina* increased in the presence of all N fertilizers; medium N and high N fertilization also increased leaf length and leaf thickness of *R. typhina* due to the fertilizing effects of the addition of exogenous N on *R. typhina* growth. Thus, *R. typhina* leaves may possess higher resource capture ability as well as higher relative growth rate by reducing material investment per unit area under exogenous N fertilization. Meanwhile, medium N fertilization exerts stronger fertilizing effects on leaf length, leaf width, leaf chlorophyll and N concentrations, single leaf wet weight, and leaf thickness of *R. typhina* than those of high N fertilization. This is possibly because excess N fertilization could drive some unexpected reverse phenomena on leaf growth of *R. typhina*. Thus, leaf growth of *R. typhina* may be presumably attenuated with increasing amounts of anthropogenic N deposited into ecosystems in the future, and thereby pose pronounced effects on its subsequent further invasion.

Keywords: leaf functional traits, SLA, nitrogen deposition, *Rhus typhina*
Introduction

Anthropogenic activities, such as the combustion of fossil fuels, the production and consumption of chemical nitrogen (N) fertilizers, and the rapid development of animal husbandry, have triggered a global process that increases atmospheric N deposited into terrestrial ecosystems [1-3]. East Asia (mainly China), Western Europe, and North America are currently the major areas of N deposition [1, 3-4]. N deposition can incur changes in ecosystem functions, such as changes in soil pH values, soil microbial community and functioning, plant litter decomposition [5-8], and plant growth and physiological performance [9-10]. Meanwhile, invasive plants have also triggered serious impacts on native ecosystems on a global scale, especially affecting the structure and function of the ecosystems in which these invasions occur [11-12].

Many studies have confirmed that certain plants successfully invade certain ecosystems because leaf functional traits [such as higher specific leaf area (SLA)] of those invaders can enable them to acquire an advantage in resource capture and growth rate [13-14]. Generally, leaves with high SLA values were typically associated with high resource acquisition and use efficiency with low investment in leaf construction and protective tissues than those leaves with low SLA values [15-17]. Moreover, leaf size (indicated by leaf length and leaf width), leaf chlorophyll and N concentrations, single leaf wet weight, and leaf thickness are also pivotal indices of leaf functional traits because those indices are also known to be good indicators of resource-use strategy of plant species [15, 18-20]. Based on this, the effects of anthropogenic N deposition on successful invaders have raised considerable interest, particularly with respect to its potential effects on the leaf functional traits of invasive species to encapsulate the mechanism underlying their successful ecological strategy.

This study aims to gain insights into the changes in leaf functional traits of the controversial invader Rhus typhina in the presence of a gradient of simulated N deposition (control, 0 g L\(^{-1}\); low N, 5 g L\(^{-1}\); medium N, 10 g L\(^{-1}\); and high N, 25 g L\(^{-1}\)). R. typhina is a deciduous tree native to North America and introduced to China as a main forestation species by the Botanical Garden, Institute of Botany, the Chinese Academy of Sciences in 1959 [21-22]. This species has some ornamental values because of its fruit clusters (which look like burning torches in late summer and early autumn) and its brilliant red leaves in mid-autumn [21]. Unfortunately, this species can display somewhat invasive characteristics, such as sturdily growth and quick reproduction [21, 23]. Furthermore, this species has been scattered on almost all habitats from downtown to mountains, including roadsides, farmlands, and protected areas in many regions of China [21]. Therefore, R. typhina has been identified as a destructive invader by many botanists [21, 24]. In this present study, leaf functional traits (i.e., leaf size, leaf chlorophyll and N concentrations, SLA, single leaf wet weight, and leaf thickness) of R. typhina under a gradient of N fertilization are assessed to gain insight into its ecological strategy.

The results of this present study could provide a platform to better understand the successful strategy mechanisms of R. typhina and then lay an essential theoretical foundation and practical significance for effective invasion prevention and control, especially under conditions with increasing amounts of anthropogenic N deposited into ecosystems in the future. This study presents the following hypotheses. First, R. typhina can increase soil pH [25-26] because of the preferences of plant N required [27-28] and/or the alkaline substances in the litters and/or root exudations of invasive species [28-29]. Second, soil pH decreased under N fertilization [6-8] due to the release of free H\(^+\) via the nitrification process under N fertilization [30-31]. Third, N fertilization could increase the values of leaf functional traits of R. typhina because N supply could enhance the growth and physiological performance of plant species [9-10, 15, 32].

Materials and Methods

Experimental Design

Root samples of R. typhina were collected mid-August 2015 in Jinan, P. R. China (36.63°N, 117.03°E), which has a warm temperate climate. The annual mean temperature of the area is approximately 13.8°C, annual precipitation is approximately 614 mm, and the rainy season comes in June and July. All root samples were transported into planting pots as soon as possible using nutritious soils.

A mixed N solution was prepared with a 1:1:1 ratio of KNO\(_3\)-N:NH\(_4\)Cl-N:urea-N. The ratio of different N forms in the mixed N was similar to the global average ratio of natural atmospheric N deposition [33-34]. The concentrations were made with distilled water prior to use to create a series with gradient contents, namely CK (control, 0 g L\(^{-1}\)), low N (5 g L\(^{-1}\)), medium N (10 g L\(^{-1}\)), and high N (25 g L\(^{-1}\)). Three replicates were performed per treatment. The samples were incubated in a greenhouse at 25°C for approximately 70 d. During the incubation, deionized water or simulated N fertilizers were supplied weekly according to the amount of precipitation in the study site.

Determining Plant Characteristics

The leaf length is the maximum value along the midrib, while the width is the maximum value perpendicular to the midrib [19-20, 35]. Leaf length and leaf width were measured using a ruler [19-20]. The relative chlorophyll and N concentrations in the leaves were estimated with a handheld 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.

SLA was computed using the ratio of the leaf area to the corresponding leaf dry weight (cm\(^2\) g\(^{-1}\)) according to previous studies [16, 19-20].
Single leaf wet weight was determined using an electronic balance with an accuracy of 0.001 g \[19-20\].

Leaf thickness was calculated through the overlap of five leaves using a Vernier caliper with an accuracy of 0.01 mm \[19-20\].

The pH values of original nutrition soil samples and those under growth of *R. typhina* were all measured using a soil acidity meter in situ (ZD instrument – ZD-06, P. R. China) \[19\].

**Statistical Analysis**

Data were evaluated to determine the deviations from normality and homogeneity of variance before data analysis. Differences among various dependent variables were assessed using analysis of variance. Statistically significant differences were set at \( P \) values equal to or lower than 0.05. Correlation patterns among various dependent variables were determined by correlation analysis using IBM SPSS Statistics (version 22.0).

**Results**

Soil pH decreased under the growth of *R. typhina* but not substantially (Fig. 1, \( P > 0.05 \)). Soil pH decreased under all N fertilization (Fig. 1) and significantly decreased under medium N and high N fertilization (Fig. 1, \( P < 0.05 \)).

Leaf width, leaf chlorophyll and N concentrations (\( P > 0.05 \)), and single leaf wet weight (\( P < 0.05 \)) of *R. typhina* were in the order of medium N > high N > low N > CK (Table 1). Medium N and high N fertilization increased leaf length and leaf thickness of *R. typhina* and medium N displayed greater effects than those of high N fertilization, but this change was not pronounced (Table 1, \( P > 0.05 \)). SLA increased with increasing concentrations of N fertilizers (Table 1, \( P > 0.05 \)).

Correlation patterns among leaf functional traits of *R. typhina* were observed through correlation analysis (Table 2). In particular, leaf length was positively correlated with leaf width, single leaf wet weight, and leaf thickness (Table 2, \( P < 0.001 \)). Leaf width was positively correlated with single leaf wet weight and leaf thickness (Table 2, \( P < 0.01 \)). Leaf chlorophyll concentration was positively correlated with leaf N concentration (Table 2, \( P < 0.0001 \)). Single leaf wet weight was positively correlated with leaf thickness (Table 2, \( P < 0.001 \)).

**Discussion**

Previous studies showed that invasive species could increase soil pH \[25-26\] because of the preferences of plant N required \[27-28\], and/or the alkaline substances in the litters and/or root exudations of invasive species \[28-29\]. Soil pH decreased under the growth of *R. typhina*, although not substantially in this study (Fig. 1). This result is inconsistent with our hypothesis and with the results of previous studies \[25-26\]. The soil acidification mediated by *R. typhina* may be due to the positive effects on soil ammonium concentration and negative affects on soil nitrate concentration \[34\]. Similar results were also revealed in other studies \[29, 37-38\].

It can be speculated that the decreased soil pH values mediated by invasive species could enhance phosphor (P) solubility in soil \[39-40\], and higher P availability could facilitate further encroachment by invasive species \[39, 41\]. Previous studies also have reported some inconsistent results of the effects of invasive species on soil pH. For example, some studies founded that invasive species could trigger soil acidification \[29, 36-38\]. Si et al. \[11\]

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**Table 1. Differences in leaf functional traits of *R. typhina* under different treatments. Data with different letters in a vertical row indicate a significant difference (\( P < 0.05 \)). “ns” means not significant difference (\( P > 0.05 \)). Abbreviations: LL, leaf length (cm); LW, leaf width (cm); LCC, leaf chlorophyll concentration (SPAD); LNC, leaf N concentration (mg g\(^{-1}\)); SLA, specific leaf area (cm\(^2\) g\(^{-1}\)); SLWW, single leaf wet weight; LT, leaf thickness (mm).**

<table>
<thead>
<tr>
<th></th>
<th>LL</th>
<th>LW</th>
<th>LCC</th>
<th>LNC</th>
<th>SLA</th>
<th>SLWW</th>
<th>LT</th>
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<tbody>
<tr>
<td>CK</td>
<td>5.50±0.28 ns</td>
<td>1.64±0.05 ns</td>
<td>38.99±2.45 ns</td>
<td>3.30±0.17 ns</td>
<td>422.23±63.64 ns</td>
<td>0.03±0.00b</td>
<td>0.20±0.01 ns</td>
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<tr>
<td>Low N</td>
<td>5.37±0.64 ns</td>
<td>1.79±0.16 ns</td>
<td>39.89±0.68 ns</td>
<td>3.37±0.05 ns</td>
<td>458.07±37.50 ns</td>
<td>0.04±0.01ab</td>
<td>0.20±0.01 ns</td>
</tr>
<tr>
<td>Medium N</td>
<td>6.87±1.07 ns</td>
<td>2.26±0.24 ns</td>
<td>41.01±0.38 ns</td>
<td>3.43±0.03 ns</td>
<td>477.18±25.47 ns</td>
<td>0.08±0.02a</td>
<td>0.27±0.03 ns</td>
</tr>
<tr>
<td>High N</td>
<td>6.54±0.20 ns</td>
<td>1.89±0.18 ns</td>
<td>40.78±0.15 ns</td>
<td>3.41±0.01 ns</td>
<td>588.50±27.05 ns</td>
<td>0.07±0.01ab</td>
<td>0.23±0.01 ns</td>
</tr>
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</table>
Wang C., et al. also founded that low-cover classes of the invasive plant *Wedelia trilobata* significantly increase soil pH, but high-cover classes do not affect soil pH significantly. Thus, the effects of invasive species on soil pH may vary by species and the difference in the effects of plant invasion on soil pH may be mainly due to the difference in the chemical composition of the leaching substances of plant litters and root exudates.

Evidence suggests that the addition of N fertilizers contributes to soil acidification due to the release of free H\(^+\) through the nitrification process, which results in a gradual decrease of soil pH [30-31]. This study also showed that soil pH decreased in the presence of all N fertilizers (Fig. 1). The result is consistent with our hypothesis and those reported in previous studies [6-8].

N has been found to be the most essential factor that determines plant growth [42]. Thus, plants can present stimulated growth and physiological performance under elevated N supply mainly via eliminating N deficiency [9-10]. Generally, N fertilization could increase leaf size, leaf chlorophyll and N concentrations, and SLA [15, 32]. This study also revealed that leaf width, leaf chlorophyll and N concentrations, SLA, and single leaf wet weight of *R. typhina* increased in the presence of all N fertilizers; medium N and high N also increased leaf length and leaf thickness of *R. typhina* (Table 1). The result is consistent with our hypothesis and those reported in previous studies [6-8].

Numerous studies have shown that leaf size was positively correlated with SLA because leaves with high SLA provide low structural investment, but leaves with low SLA likely allocate more biomass on leaf structures [15, 17-18]. However, SLA was unrelated to other leaf traits (including leaf size) significantly in this study (Table 2). This result is inconsistent with previous studies [15, 17-18]. Previous studies also have reported some inconsistent results of the correlations between leaf size and SLA, including positive [45], negative [20, 46], unrelated [19, 47], or variable among habitats [48]. Based on this, we believe that there is species specificity for the relationship among leaf functional traits. The main aim of this study was to investigate the effects of a gradient of simulated N deposition on leaf functional traits of the controversial invader *R. typhina*.

*R. typhina* can trigger soil acidification, possibly due to the positive effects of *R. typhina* on soil ammonium concentration and negative affects on soil nitrate

<table>
<thead>
<tr>
<th>LL</th>
<th>LW</th>
<th>LCC</th>
<th>LNC</th>
<th>SLA</th>
<th>SLWW</th>
<th>LT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>r</strong></td>
<td>1.00</td>
<td>0.83***</td>
<td>0.00</td>
<td>-0.01</td>
<td>0.48</td>
<td>0.83***</td>
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<td><strong>P</strong></td>
<td>0.0008</td>
<td>0.9925</td>
<td>0.9862</td>
<td>0.1133</td>
<td>0.0008</td>
<td>0.0005</td>
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<tr>
<td>LW</td>
<td><strong>r</strong></td>
<td>1.00</td>
<td>0.13</td>
<td>0.7363</td>
<td>0.3412</td>
<td>0.0050</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.6788</td>
<td>&lt;0.0001</td>
<td>0.7319</td>
<td>0.2120</td>
<td>0.6542</td>
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<tr>
<td>LCC</td>
<td><strong>r</strong></td>
<td>1.00</td>
<td>1.00**</td>
<td>-0.11</td>
<td>0.39</td>
<td>0.14</td>
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<tr>
<td><strong>P</strong></td>
<td>1.00</td>
<td>-0.13</td>
<td>0.39</td>
<td>0.13</td>
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</tr>
<tr>
<td>LNC</td>
<td><strong>r</strong></td>
<td>0.6766</td>
<td>0.2146</td>
<td>0.6982</td>
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<tr>
<td><strong>P</strong></td>
<td>0.1123</td>
<td>0.1455</td>
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<tr>
<td>SLA</td>
<td><strong>r</strong></td>
<td>1.00</td>
<td>0.48</td>
<td>0.45</td>
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<tr>
<td><strong>P</strong></td>
<td>0.86***</td>
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<tr>
<td>SLWW</td>
<td><strong>r</strong></td>
<td>1.00</td>
<td>0.86***</td>
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<tr>
<td><strong>P</strong></td>
<td>0.0003</td>
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<tr>
<td>LT</td>
<td><strong>r</strong></td>
<td>1.00</td>
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<tr>
<td><strong>P</strong></td>
<td>0.0000</td>
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Table 2. Relationship among leaf functional traits of *R. typhina*. ** and *** indicate significant differences at 0.01 and 0.001 probability levels, respectively. Abbreviations have the same meanings as described in Table 1.
concentration. N fertilization can mediate a reduction in soil pH due to the release of free H⁺ via the nitrification process. All N fertilizers have a fertilizing effect on leaf width, leaf chlorophyll and N concentrations, SLA, and single leaf wet weight of *R. typhina*. Meanwhile, medium N and high N fertilization also display positive effects on leaf length and leaf thickness of *R. typhina*. This implies that *R. typhina* leaves may possess higher resource capture ability as well as a higher relative growth rate by reducing material investment per unit area under exogenous N fertilization. Then again, medium N fertilization exerts stronger fertilizing effects on leaf length, leaf width, leaf chlorophyll and N concentrations, single leaf wet weight, and leaf thickness of *R. typhina* than those of high N fertilization. The phenomenon may be caused by the unexpected reverse phenomena on leaf growth of *R. typhina* mediated by excess N fertilization. Based on this, leaf growth of *R. typhina* may be presumably attenuated with increasing amounts of anthropogenic N deposited into ecosystems in the future, and thereby pose pronounced effects on its further invasion.

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