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Complex effects of different types of acid rain on root growth of *Quercus acutissima* and *Cunninghamia lanceolata* saplings

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Abstract

Background: Soil acidification caused by acid rain (AR) can damage plant roots, which in turn negatively impacts plant health. In response to changing AR types, research efforts to elucidate their specific impacts on plants have become intense.

Methods: For this study, we investigated the effects of simulated sulfuric, nitric, and mixed AR on the root systems of *Quercus acutissima* Carr. and *Cunninghamia lanceolata* (Lamb.) Hook. under different acidity levels.

Results: As the AR S/N ratio and pH decreased, the height growth rate (HGR), basal diameter growth rate (DGR), total root length (TRL**)** and total root surface area (TRS) of *C. lanceolata* decreased, whereas the TRL and TRS of *Q. acutissima* remained the same. When the NO_3^- concentration in AR was increased, the root activity, superoxide dismutase (SOD) and catalase (CAT) activities of *C. lanceolata* roots revealed a downward trend; however, the root activity of *Q. acutissima* and the peroxidase (POD) activity of *C. lanceolata* roots revealed an upward trend. Further, redundant analysis and structural equation models indicated that AR pH had a greater impact on the HGR of *Q. acutissima* than that of *C. lanceolata*, while the impact of the AR S/N ratio on *C. lanceolata* growth rates was greater than that of *Q. acutissima*.

Conclusions: Our results suggested that the root systems of different tree species had variable responses to AR, and the AR S/N ratio was an important factor affecting plant root growth. This might facilitate new strategies for the cultivation and protection of plantations in the future.

Keywords: Sulfuric and nitric acid rain, Root growth, Root morphology, Root activity, Antioxidant enzyme activity

Introduction

Over the last few decades, in conjunction with its rapid economic growth, China has established the largest plantation area in the world (Tang et al. 2015). *Quercus acutissima* Carr. and *Cunninghamia lanceolata* (Lamb.) Hook. are the dominant tree species in China, which have important economic and ecological value, with multiple uses beyond timber (Kang et al. 2018; Zhang et al. 2004). However, with increasing acid rain (AR), many plantations suffer from its deleterious effects (Sun et al. 2016).

AR is a progressively serious environmental issue caused by anthropogenic urbanization and industrialization (Zhang et al. 2017), which not only has negative impact on ecosystems, but also significantly threatens human health and survival (Ma et al. 2021). In addition to Europe and North America, China is the third largest AR-afflicted region worldwide (Ren et al. 2018). The primary sources of AR include sulfur dioxide (SO_2) and

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nitrogen oxide (NO_x) (Lv et al. 2014), which dissolve in water to form sulfuric acid rain (SAR) and nitric acid rain (NAR) (Zhang et al. 2007). The exact composition of AR varies from region to region, among which SAR was once the main type of AR in China due to coal combustion (Liu et al. 2020; Ma et al. 2019). In view of the more stringent control of sulfur emissions from factories since 1990 (Chan and Yao 2008), in conjunction with increased vehicular exhaust emissions caused by rapid economic development (Liu et al. 2018c), the SAR in China is progressively transforming to NAR (Liu et al. 2020).

Root systems facilitate plant growth via mechanical support, nutrient foraging, gas exchange, through the recruitment of beneficial microbes, etc. (Bardgett et al. 2014), which allows plants to endure various biological and abiotic stresses (Saleem et al. 2018), such as salinity, acidity, drought, and pests. Concurrently, root density and distribution are intimately linked to the soil environment, where root density influences the growth of aboveground plant components (Simpson et al. 2020). However, broadleaf and coniferous species possess unique root characteristics, thus, their tolerance for and responses to AR are variable (Ren et al. 2018). Research shows that AR increases the accumulation of reactive oxygen species in roots (Ju et al. 2017b), reduces root activities (Liu et al. 2018b), limits the utilization of nutrients and inhibits root growth (Zhang et al. 2016a) by altering soil chemistry and cation adsorption (Xu and Ji 2001). Besides, as the AR in China shifts from SAR to NAR, its NO_3^- concentration gradually increased, which can fertilize plants to some extent (Sparks 2009). However, these higher NO_3^- concentrations can affect the nitrogen metabolism associated with genes and associated proteins (Liu et al. 2013), further influences amino acid biosynthesis and disrupts the metabolic balance between nitrogen and carbon (Zhang et al. 2020). Thus, the influences of different types of AR on plants are complex and variable. Accordingly, a growing number of researchers have committed to the study of different types of AR, with most of them focusing on the recovery of acidified soil (Liu et al. 2020), decomposition of litter (Liu et al. 2017), microbial communities (Li et al. 2021) and the physiology of aboveground components (Yao et al. 2016), while few investigations have set their focus on root physiology (Huang et al. 2019; Liu et al. 2018b). Although we have previously explored the fine root biomass and fine root elements of subtropical plantation in response to different types of AR (Liu et al. 2018c), the data remain limited in regard to the complex effects of different types of AR on the root growth of *Q. acutissima* and *C. lanceolata* under pot experiment.

To investigate the specific impacts of different types of AR on *Q. acutissima* (broadleaf) and *C. lanceolata*

(conifer) root growth, AR simulation tests using potted plants were developed. Our objective was to explore the effects of different S/N ratios and acidities on the root systems of these two species, which included total root length, total root surface area, average root diameter, root activity, and the activities of antioxidant enzymes in contrast to a control. In accordance with earlier studies, we proposed our hypotheses as follows: (1) AR inhibits the root growth of *Q. acutissima* and *C. lanceolata* saplings; (2) this inhibition is exacerbated by higher concentrations of NO_3^-; (3) the root systems of different tree species have variable responses to higher AR acidity and NO_3^- concentrations.

Methods

Plant material and treatments

This study was conducted from March 2015 to April 2016 in a greenhouse at the Xiashu Ecological Station (31° 7′ N, 119° 12′ E) of Nanjing Forestry University, in Jiangsu Province, China. The research samples included 1-year old *Q. acutissima* and *C. lanceolata* seedlings of uniform height. The average height of the *Q. acutissima* saplings was 31.23 ± 2.63 cm, with ground diameters of 2.75 ± 0.53 cm, whereas the average height of the *C. lanceolata* saplings was 27.15 ± 1.33 cm, with ground diameters of 5.03 ± 0.28 cm. Following the preparation of plastic pots (Ø20 cm × 25 cm high) that contained yellow–brown soil (as described by the Chinese Soil Classification System) (Li et al. 2020) with a pH of 6.31 ± 0.01, which was collected from nearby plantations, we transplanted the saplings into the pots.

The physical and chemical soil properties were as follows: total carbon content (0.78 g/kg); total nitrogen content (0.05 g/kg); total phosphorus content (0.5 g/kg); total potassium content (14.18 g/kg); ammonium nitrogen content (15.3 mg/kg); available phosphorus content (34.2 mg/kg); available potassium content (121.39 mg/kg); and electric conductivity (0.24 mS/cm). Following transplantation, the saplings were allowed 2 months of recovery time, during which they were hydrated with distilled water. Additionally, the indoor environmental conditions of the greenhouse were as follows: temperature 18–35 ℃, relative humidity 40–80%, light/dark period 14/10 h, light intensity 700–1000 mol/(m^2 s).

After 2 months recovery time, 192 uniform saplings of tree species (with only slight differences) were selected for the simulation experiment. By mixing 0.5 mol/l H_2SO_4 and 0.5 mol/l HNO_3 in different proportions, 16 treatments including 15 AR treatments and a control group (ck) were established. The specific formulations were as follows: ck (distilled water, pH 7.0), S1 (S/N=1:0, pH=4.5), S2 (S/N=1:0, pH=3.5), S3 (S/N=1:0, pH=2.5), S4 (S/N=5:1, pH=4.5), S5 (S/N=5:1,

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pH = 3.5), S6 (S/N = 5:1, pH = 2.5), S7 (S/N = 1:1, pH = 4.5), S8 (S/N = 1:1, pH = 3.5), S9 (S/N = 1:1, pH = 2.5), S10 (S/N = 1:5, pH = 4.5), S11 (S/N = 1:5, pH = 3.5), S12 (S/N = 1:5, pH = 2.5), S13 (S/N = 0:1, pH = 4.5), S14 (S/N = 0:1, pH = 3.5), and S15 (S/N = 0:1, pH = 2.5).

The total simulated AR was 670.38 mm, whereas the monthly simulated AR was 55.865 mm according to the average annual precipitation of 1117.29 mm and AR frequency of 60% (Liu et al. 2017). Based on the 314 cm^2 pot area, the simulated monthly AR volume for each pot was 1754.16 ml. Starting in May 2015, each sapling was sprayed with 438.54 ml of simulated AR four times per month until April 2016 (Liu et al. 2018b; Ma et al. 2021). On April 30, 2016, 80 saplings of each tree species were selected for the determination of each index.

Growth measurement
The sapling heights, from the bases of the stems to the terminal buds, were quantified using a tape measure. The stem basal diameters were measured using a Vernier caliper at the bases of the stems. The initial measurements were taken on April 30, 2015, and the final measurements were obtained on April 30, 2016. The relative growth rates of the basal diameter and height were determined by the equations described by Ma et al. (2021).

Determination of root morphology
The roots of each seedling were rinsed with deionized water and scanned using an Epson Expression 12000XL scanner (Japan). The images were analyzed using Win-RHIZO (WinRHIZO Pro2016) to determine the total root length (TRL), total root surface area (TRS), and average root diameter (AD) (Zhou et al. 2019).

Malondialdehyde content of roots
Malondialdehyde (MDA) was extracted from root tissues using 5% trichloroacetic acid (TCA) solution and reacted with a 0.6% thiobarbituric acid (TBA) solution and was expressed as μg/g. The specific method was followed as described by Wang et al. (2020).

Root activity
The root activity was measured using the triphenyl tetrazolium chloride (TTC) method, and expressed as the deoxidization capacity (μg/g h). The specific method was in reference to Zhang et al. (2015).

Antioxidant activities
To prepare a crude enzyme extract, 2–3 g of root tissues were placed in a 5-ml frozen phosphate buffer (50 mM, pH 7.8) for homogenization. An ultraviolet spectrophotometer was employed to elucidate the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and were expressed as unit mg/protein min (Ma et al. 2021). The specific methods were described by Khan et al. (2017).

Statistical analysis
One-way ANOVA was employed to investigate variations in the growth rates and root traits of *Q. acutissima* and *C. lanceolata* among the treatments. Moreover, two-way ANOVA was utilized to assess the main impacts and interactivity of the AR S/N ratio and pH on the growth rates and root traits (SPSS 26.0 Inc., Chicago, Ill., USA).
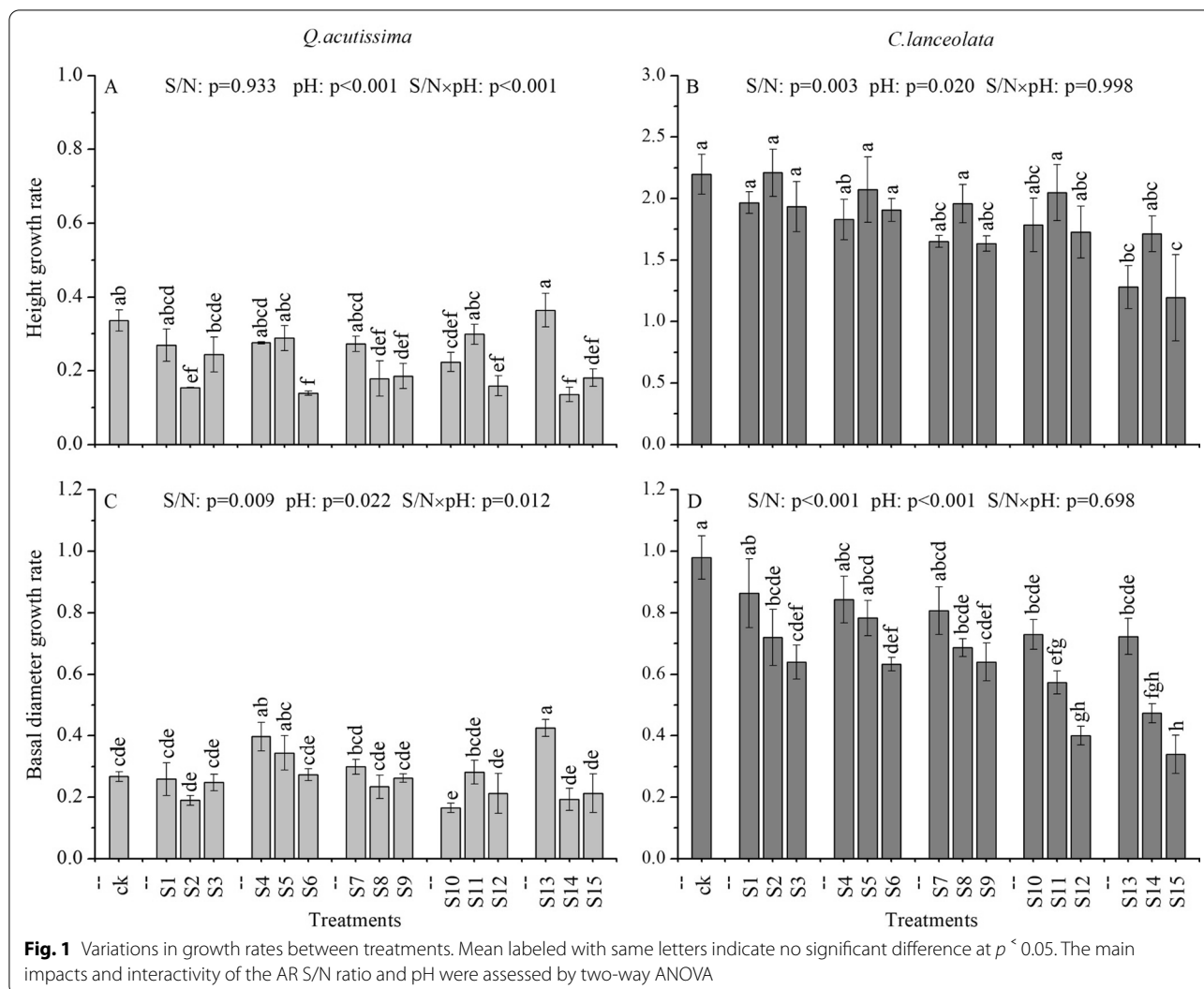
Redundant analysis (RDA) was utilized to reveal the relationships between the simulated AR pH, S/N ratio, growth rate, root morphologies, and root physiological properties, which were visualized using Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA). AMOS 22.0 (SPSS Inc., Chicago, Ill., USA) was employed to develop structural equation models to test whether the AR S/N ratio and pH directly or indirectly impacted the growth rates and root morphologies through the modification of the physiological properties of the roots.

Results
Growth rate
The AR pH significantly impacted the height and basal diameter growth rates of *Q. acutissima* and *C. lanceolata* ($p < 0.05$), which was the same for the AR S/N ratio ($p < 0.05$), except for the height growth rate of *Q. acutissima*. In contrast to *C. lanceolata*, the interaction of the AR S/N ratio and pH significantly impacted the growth of *Q. acutissima* ($p < 0.05$) (Fig. 1).

When the AR pH was 3.5, the height growth rates of *Q. acutissima* treated with S2, S8, and S14 were lower than the ck ($p < 0.05$). As the pH dropped to 2.5, the S6, S9, S12, and S15 height growth rates of *Q. acutissima* were also below the ck ($p < 0.05$) (Fig. 1A). However, the basal diameter growth rate of *Q. acutissima* treated with S4 and S13 were greater than the ck ($p < 0.05$) (Fig. 1C). Further, the height growth rates of *C. lanceolata* initially increased and then decreased with lower pH, where only the S13 and S15 treatments were below the ck ($p < 0.05$) (Fig. 1B), while the basal diameter growth rate continued to decrease. All the other treatments were below the control except for S1, S4, S5, and S7 treatments ($p < 0.05$) (Fig. 1D). Both the height and basal diameter growth rates of *C. lanceolata* exhibited a downward trend as the proportion of nitrogen was increased, except for the height growth rates of the S10, S11, and S12 treatments, and basal diameter growth rate of the S5 treatment (Fig. 1B, D).

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Fig. 1 Variations in growth rates between treatments. Mean labeled with same letters indicate no significant difference at *p* ˂ 0.05. The main impacts and interactivity of the AR S/N ratio and pH were assessed by two-way ANOVA

Root morphology

Compared with the ck, the total root length, total root surface area, and root average diameter varied with the AR treatments (Fig. 2). The AR S/N ratio significantly affected the total root length of *Q. acutissima* ($p < 0.05$), while AR pH had a strong effect on total root surface area ($p < 0.05$). The responses of the total root length and total root surface area of *C. lanceolata* to the AR S/N ratio and pH were notable ($p < 0.05$). However, the AR S/N ratio, pH, and their interactions had negligible effects on the root average diameter of the two species.

With higher AR nitrogen proportions, the total root length and total root surface area of the two species exhibited variable decreasing tendencies. Moreover, the total root length and surface area of both species decreased as the AR acidity increased, except for the total root length of the S10 and S11 treatments for *Q. acutissima*. In addition, the total root length of *Q. acutissima*

under the S12, S14, and S15 treatments and total root surface area under the S8, S9, S12, S14 and S15 treatments were below the ck ($p < 0.05$) (Fig. 2A, C). When the AR pH was 3.5 or 2.5, most of the total root length and total root surface area of *C. lanceolata* were lower than those of the ck, except for the S5 and S11 treatments ($p < 0.05$) (Fig. 2B, D). The root average diameter of the two tree species differed insignificantly between treatments (Fig. 2E, F).

Malondialdehyde content of roots

The AR S/N ratio and pH influenced the malondialdehyde (MDA) content of *Q. acutissima* and *C. lanceolata* to a considerable degree ($p < 0.05$), and we observed significant interactive effects ($p < 0.05$). With higher AR acidity, the MDA content of *Q. acutissima* increased while that of *C. lanceolata* initially increased and then decreased, except when the S/N ratio was 0:1. When the
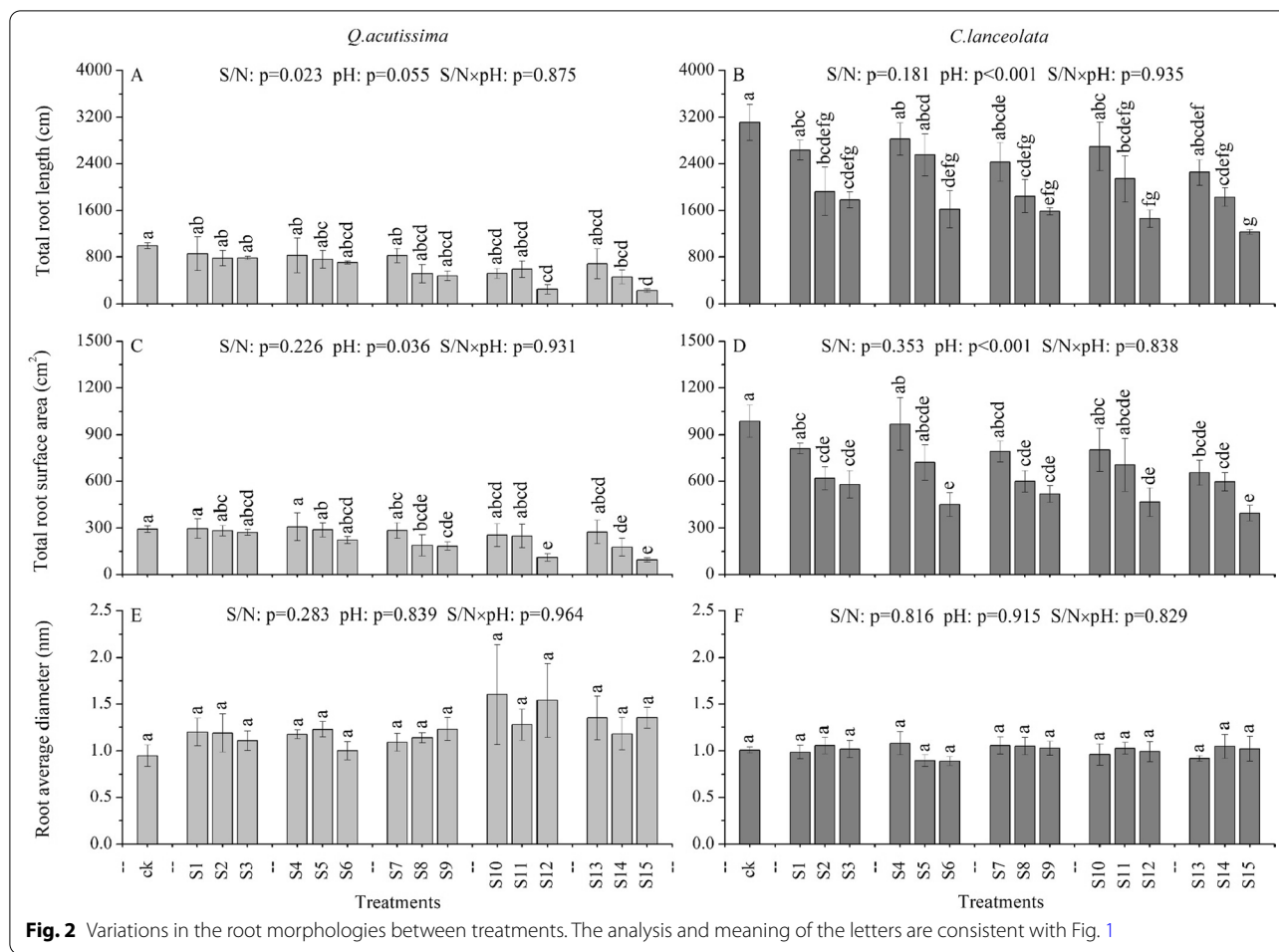
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Fig. 2 Variations in the root morphologies between treatments. The analysis and meaning of the letters are consistent with Fig. 1

AR S/N ratios were 1:0 and 5:1, the MDA contents of *Q. acutissima* and *C. lanceolata* under all AR treatments, except for S4 treatment, exceeded the corresponding ck ($p < 0.05$). However, when the AR S/N ratios were 1:5 and 0:1, only the MDA content of *Q. acutissima* under the S12 treatment, and the MDA contents of *C. lanceolata* under the S11 and S15 treatments were higher than the corresponding ck ($p < 0.05$) (Fig. 3).

Root activity

The AR S/N ratio and pH significantly affected the root activities of *Q. acutissima* and *C. lanceolata* ($p < 0.05$) and demonstrated considerable interactive effects ($p < 0.05$). With the increased proportion of nitrogen in AR, the root activity of *Q. acutissima* showed a gradually increasing trend, while that of *C. lanceolata* was the converse. When the AR S/N ratios were 1:0 and 5:1, the root activity of *Q. acutissima* exhibited a variable tendency to decrease after increasing with higher acidity, while that of *C. lanceolata* was reduced. When the AR S/N ratio was 1:1, the root activity of *Q. acutissima* decreased

continuously with higher acidity, while that of *C. lanceolata* initially increased and then decreased. When the AR S/N ratios were 1:5 and 0:1, the root activity of the two species initially increased and then decreased with higher acidity. Furthermore, when the AR S/N ratio was 0:1 and pH was 3.5, the root activity of *Q. acutissima* was higher than that of the ck ($p < 0.05$) (Fig. 4).

Antioxidant enzyme activities

The S/N ratio of the AR had no significant effect on the SOD activity of *Q. acutissima*; however, its interaction with the pH significantly influenced its activity ($p < 0.05$). Most of the SOD activity of *Q. acutissima* roots did not change significantly under AR treatments compared with the ck. When the S/N ratio was changed from 1:5 to 0:1, the SOD activity of *Q. acutissima* roots decreased significantly under the pH 2.5 treatment ($p < 0.05$). The main and interactive effects of the AR S/N ratio and pH on the SOD activity of *C. lanceolata* roots reached significant levels ($p < 0.05$). With higher AR NO_3^- concentrations,
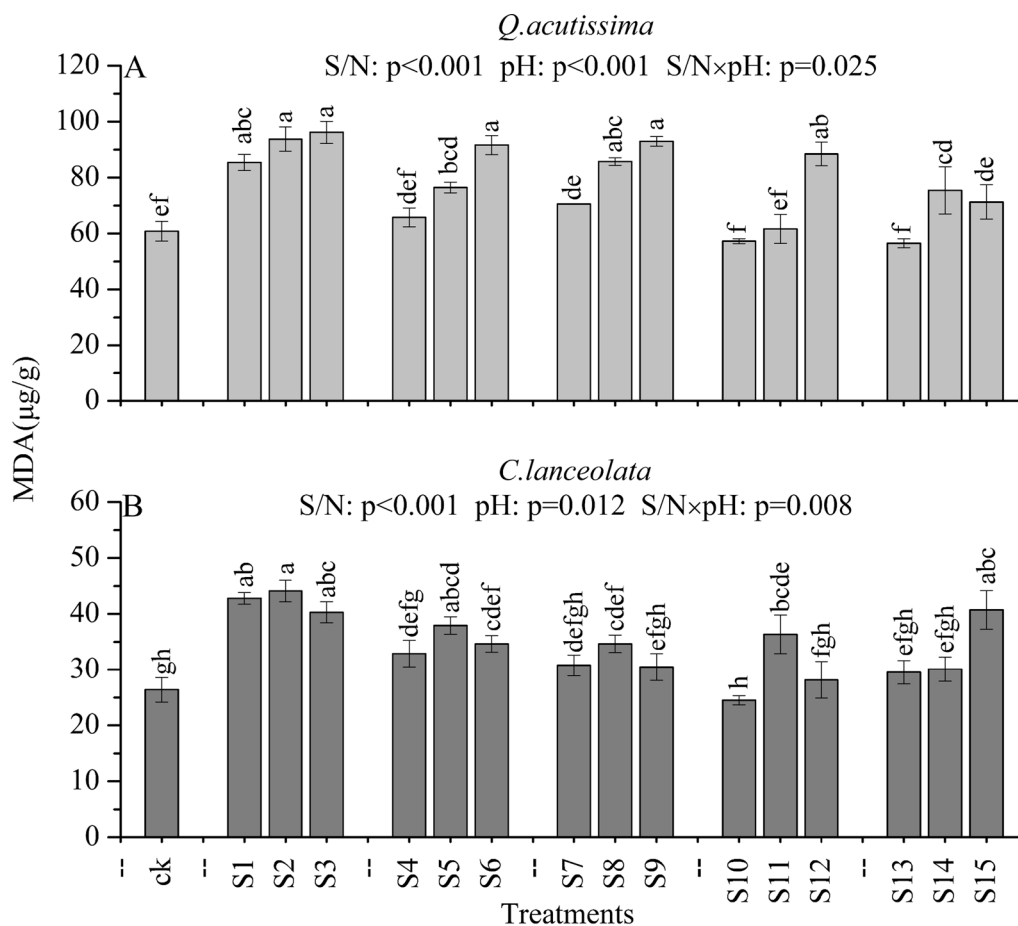
Fig. 3 Variations in root MDA contents between treatments. The analysis and meaning of the letters are consistent with Fig. 1

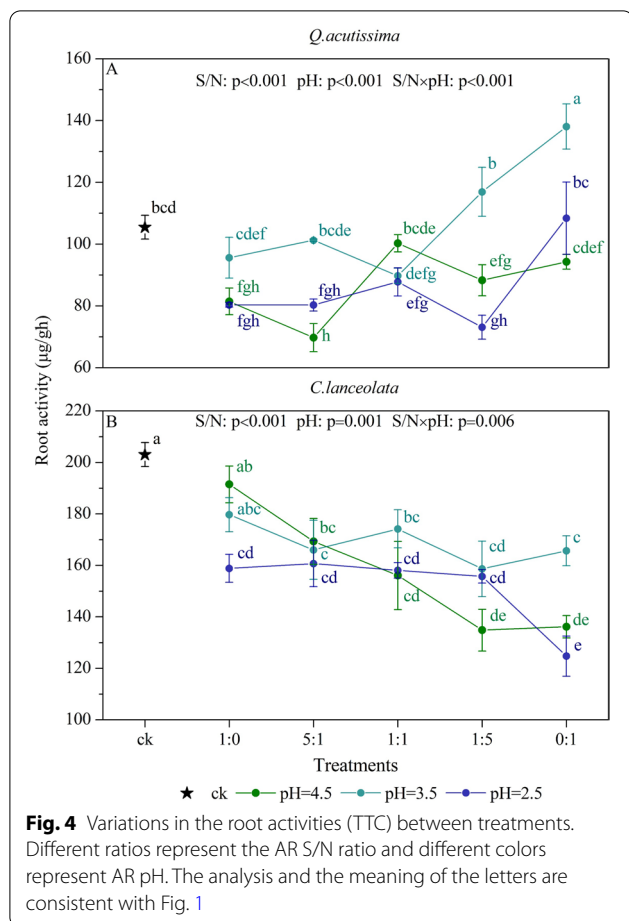the SOD activity of *C. lanceolata* roots showed a downward trend (Fig. 5A, B).

There was no significant difference in the POD activity between the S/N ratio and pH in the *Q. acutissima* roots under the AR treatments. However, in contrast to the ck, the root POD activity of *Q. acutissima* was higher ($p < 0.05$) under most AR treatments, except when the AR pH was 4.5 and the S/N ratios were 1:0 and 1:1; when the AR pH was 3.5 and the S/N ratios were 1:5 and 5:1; and when the AR pH was 3.5 and the S/N ratio was 0:1. Furthermore, when the S/N ratio was altered from 1:5 to 0:1, the POD activity of *Q. acutissima* roots decreased significantly under the pH 2.5 treatment ($p < 0.05$). The POD activity of *C. lanceolata* roots had a significant response to the AR S/N ratio and its interaction with pH ($p < 0.05$), and gradually increased with higher AR NO_3^- concentrations. The POD activity of *C. lanceolata* roots under NAR stress was higher than the ck ($p < 0.05$), except when the S/N ratio was 1:5 and the pH was 3.5 (Fig. 5C, D).

The main and interactive effects of the S/N ratio and pH of the AR on the CAT activities of *Q. acutissima* and

C. lanceolata roots were significant ($p < 0.05$). When the S/N ratio was altered from 1:5 to 0:1, the CAT activity of *Q. acutissima* roots decreased significantly under each pH treatment ($p < 0.05$). When the AR pH was 4.5, and the AR S/N ratio changed from 5:1 to 1:5, the CAT activity of *Q. acutissima* roots initially decreased and then increased, while the opposite was the case when the AR pH was 3.5 and 2.5. Further, the CAT activity of *C. lanceolata* roots revealed the variable tendency of decreasing with higher NO_3^- concentrations in the AR under all pH levels (Fig. 5E, F).

Redundant analysis results

Using the AR NO_3^- concentrations, pH, and the physiological characteristics of roots as explanatory variables, redundant analysis was performed on the sapling growth rates and root morphologies. Among them, Axis 1 explained 41.68% of the variation in the *Q. acutissima* dataset and 48.54% of the variation in the *C. lanceolata* dataset, whereas Axis 2 accounted for 1.55% of the

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Fig. 4 Variations in the root activities (TTC) between treatments. Different ratios represent the AR S/N ratio and different colors represent AR pH. The analysis and the meaning of the letters are consistent with Fig. 1

variation in the *Q. acutissima* dataset, and 3.78% of the variation in the *C. lanceolata* dataset.

As depicted in Fig. 6A, a high total root surface area, total root length with height growth rate, and basal diameter growth rate were found at the left side of coordinate axis, which were related to the AR NO_3^- concentration and pH. The CAT, POD, SOD, and root average diameter increased along the *y*-axis, whereas the TTC and MDA decreased. As shown in Fig. 6B, a high total root length, total root surface area, and basal diameter growth rate were found in the left quadrant, which were related to the AR pH. The CAT, SOD, TTC, MDA, and height growth rate decreased along the *y*-axis, whereas the POD and AR NO_3^- concentration increased.

Structural equation model results
The structural equation modeling of *Q. acutissima* and *C. lanceolata* was estimated by Amos (Fig. 7). Model A and Model B were the structural equation models of the effects of NO_3^- and pH on the aboveground growth rates and root morphologies of *Q. acutissima* and *C. lanceolata*, respectively.

Model A revealed that the direct effects of the AR pH on the *Q. acutissima* sapling height growth rate (0.46, $p < 0.001$) and total root surface area (0.39, $p < 0.01$) were significant, while the AR NO_3^- concentration had a significant direct effect on the total root length (-0.20, $p < 0.01$) and total root surface area (-0.37, $p < 0.01$) (Fig. 7A). Due to the indirect influences of induced root system property changes, the standardized total effects of the AR pH on the *Q. acutissima* sapling height growth rate and basal diameter growth rate were 0.508 and 0.299, respectively. The total effect of the AR NO_3^- concentration on the *Q. acutissima* sapling height growth rate and basal diameter growth rate was 0.000 and -0.094, respectively (Table 1).
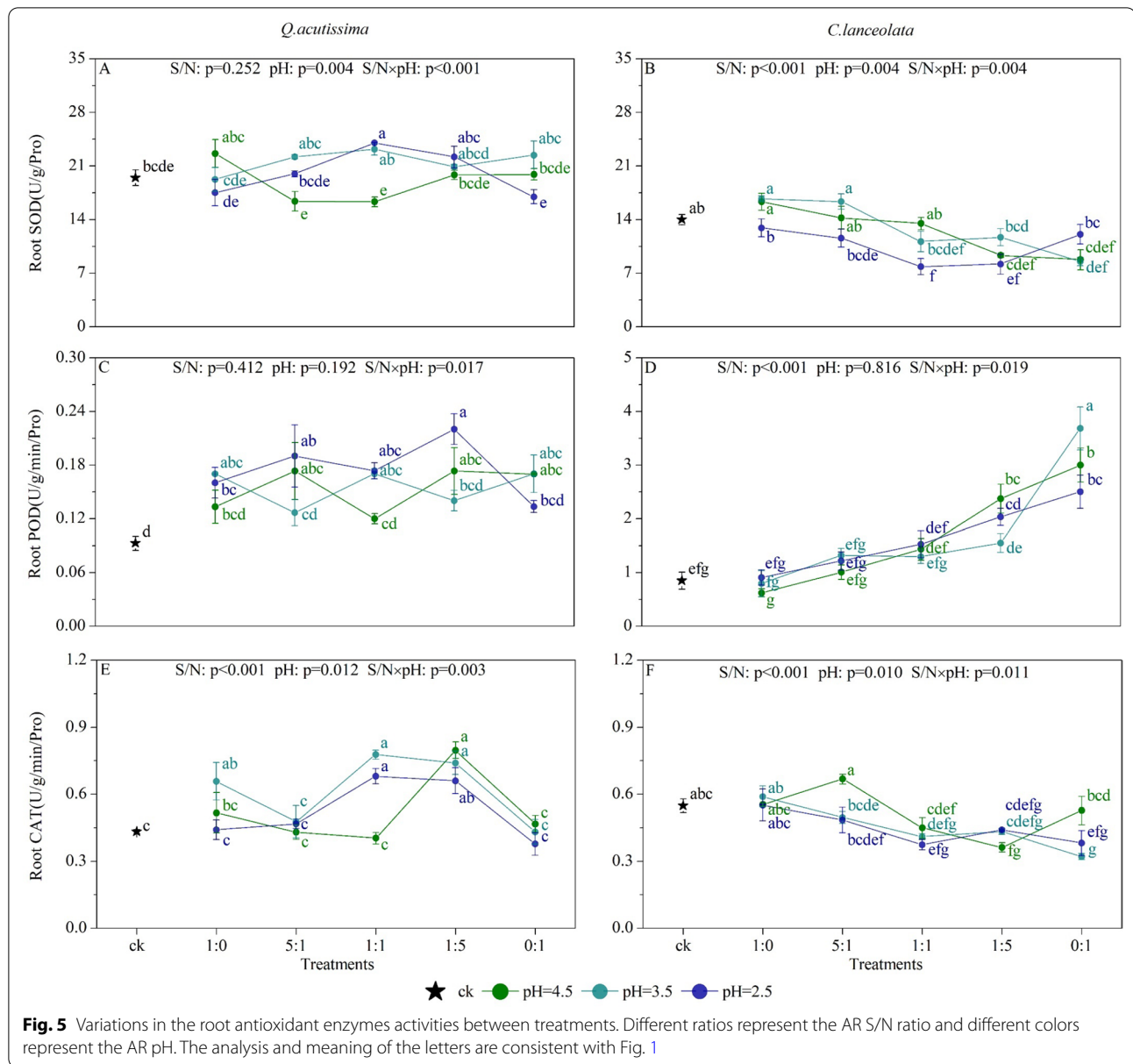
Model B showed that the direct effects of the AR pH on the *C. lanceolata* sapling basal diameter growth rate (0.66, $p < 0.001$) and total root surface area (0.69, $p < 0.001$) were significant, while the AR NO_3^- concentration had a substantial direct impact on the basal diameter growth rate (-0.51, $p < 0.01$) (Fig. 7B). Due to the indirect effects of induced changes to root system properties, the standardized total effect of the AR pH on the *C. lanceolata* sapling height growth rate and basal diameter growth rate was 0.027 and 0.617, respectively. The total effects of the AR NO_3^- concentrations on the *C. lanceolata* sapling height and basal diameter growth rates were -0.482 and -0.533, respectively (Table 1).

Discussion
Plants utilize NO_3^- as an important strategy to facilitate their absorption of nitrogen (Shi et al. 2014; Wang et al. 2019). Although the strong oxidation of NO_3^- can damage plant cell structures (Mo et al. 2008), the transformation of nitrogen can produce certain benefits (in terms of fertilization) for plant growth (Ouyang et al. 2008; Qiu et al. 2015). Consequently, various types of AR have complex and variable impacts on the root systems of plants.

Complex effects of different types of AR on growth rates of different tree species
The relative growth rates of the basal diameters and heights of plants are critical indicators that reflect their growth under environmental stress (Liu et al. 2018a). This argument supported our first hypothesis that AR will inhibit plant growth and lead to plant poisoning, when pH values attain a certain damaging threshold (Ju et al. 2017b). Since deciduous species are more sensitive to AR than evergreen species (Du et al. 2017), *C. lanceolata* exhibited a higher acid tolerance than did *Q. acutissima*. This was most clearly illustrated by the AR with the highest acidity (pH 2.5), which significantly inhibited the height growth rate of *Q. acutissima*, while the inhibitory

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Fig. 5 Variations in the root antioxidant enzymes activities between treatments. Different ratios represent the AR S/N ratio and different colors represent the AR pH. The analysis and meaning of the letters are consistent with Fig. 1
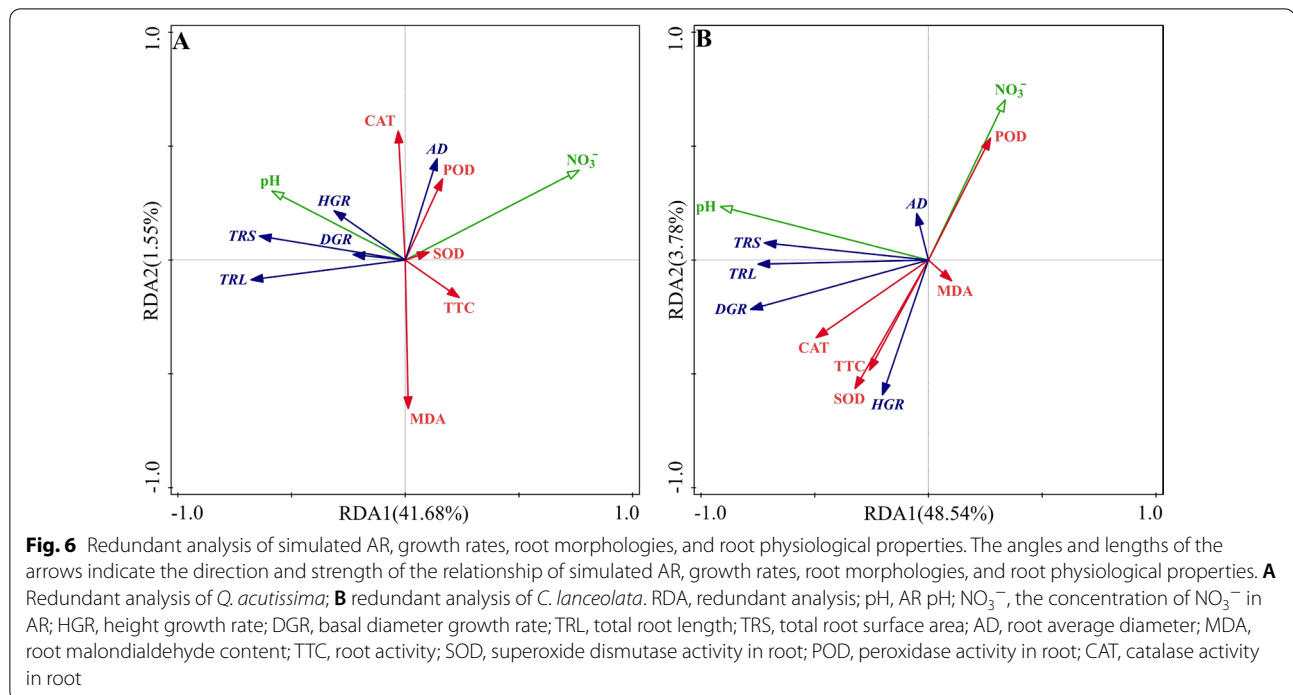
effect of high acidity AR on the height growth rate of *C. lanceolata* was reflected only in pure NAR.

Researchers found that different tree species had variable nitrogen uptake characteristics (Rollwagen and Zasoski 1988), where most conifers had a preference for ammonium nitrogen uptake in contrast to hardwoods (Kronzucker and Siddiqi 1997; Malagoli et al. 2000). This explained why NO_3^- did not have a fertilizing effect on the basal diameter growth rate of *C. lanceolata* compared with *Q. acutissima*. Further, in contrast to previous research on broadleaf trees (Chen et al. 2013; Lee et al. 2006), we found that highly acidic NAR had a more potent inhibitory effect on the basal diameter growth

rate of *C. lanceolata* than did SAR and MAR. Since the hydroxyl exchange capacity of NO_3^- was lower than that of SO_4^{2-}, NAR promoted soil acidification to a greater degree than did SAR (Lindberg et al. 1990). Combined with the nitrogen absorption characteristics of tree species, we inferred that the NAR induced stronger H^+ stress on the aboveground growth of *C. lanceolata* than on *Q. acutissima*.

Complex effects of different types of AR on root morphologies of different tree species

The inhibition of plant growth due to AR not only affects the aboveground plant components that are directly

Fig. 6 Redundant analysis of simulated AR, growth rates, root morphologies, and root physiological properties. The angles and lengths of the arrows indicate the direction and strength of the relationship of simulated AR, growth rates, root morphologies, and root physiological properties. **A** Redundant analysis of *Q. acutissima*; **B** redundant analysis of *C. lanceolata*. RDA, redundant analysis; pH, AR pH; NO_3^-, the concentration of NO_3^- in AR; HGR, height growth rate; DGR, basal diameter growth rate; TRL, total root length; TRS, total root surface area; AD, root average diameter; MDA, root malondialdehyde content; TTC, root activity; SOD, superoxide dismutase activity in root; POD, peroxidase activity in root; CAT, catalase activity in root

(See figure on next page.)
Fig. 7 Structural equation model of NO_3^- and pH effects on aboveground tree growth rate and root morphologies. **A** Structural equation model of *Q. acutissima* ($x^2 = 12.033$; $df = 10$, $p = 0.283 > 0.05$; GFI $= 0.951 > 0.900$; RMSEA $= 0.068 < 0.080$). **B** Structural equation model of *C. lanceolata* ($x^2 = 12.627$; $df = 11$, $p = 0.318 > 0.05$; GFI $= 0.951 > 0.900$; RMSEA $= 0.058 < 0.080$). Numbers on lines are standardized path coefficients. The widths of lines indicate the strength of the causal influence. Solid lines represent the direct effects on plant growth rates and root growth, dotted lines represent the effects on root physiologies. pH, AR pH; NO_3^-, the concentration of NO_3^- in AR; HGR, height growth rate; DGR, basal diameter growth rate; TRL, total root length; TRS, total root surface area; TTC, root activity; SOD, activity of superoxide dismutase in root; POD, activity of peroxidase in root; CAT, activity of catalase in root

exposed to AR, but also leads to the degradation of soil fertility and increases the vulnerability of plants to toxic metals (Du et al. 2017). Root morphology (e.g., root length, surface area, number of root tips, etc.) can reflect the growth of plant roots under environmental stress (Forino et al. 2012), such as acidity. Our research revealed that all highly acidic AR (pH = 2.5) significantly inhibited the total root length and total surface area of *C. lanceolata*; however, the inhibitory effects of highly acidic AR on the total root length and total surface area of *Q. acutissima* was primarily reflected in the NAR. Not only did our experimental results prove our first hypothesis, previous studies on soybean and rice root systems also confirmed the inhibitory effects of highly acidic AR on root morphologies (Sun et al. 2013; Zhang et al. 2016a).

Consistent with our second hypothesis and research on *Horsfieldia mer* (Huang et al. 2019), but contradictory to some research (Ju et al. 2020), the inhibition of simulated AR on the root growth of the two tree species under study increased with higher NO_3^- concentrations.

Studies have shown that excessive nitrogen supplies can inhibit root growth and elongation, which verified our results above. Furthermore, although the NAR reduced the mineralization of soil carbon, nitrogen, and phosphorus (Liu et al. 2018b), and had a particular fertilizing effect on plants, the degree of soil acidification was increased due to NO_3^-, and the roots were significantly stressed by acid and aluminum poisoning (Liu et al. 2018c). Additionally, the potent oxidative properties of NO_3^- caused additional damage to plant roots (Mo et al. 2008).

Complex effects of different types of AR on the root physiology of different tree species

The lipid peroxidation product malondialdehyde (MDA) can serve as an indicator of oxidative damage (Wang et al. 2007); thus, it may be employed to elucidate fluctuations in plant growth and functionality under environmental stress (Sánchez-Pardo et al. 2012). In alignment with a previous study (Xia et al. 2017), most of the MDA
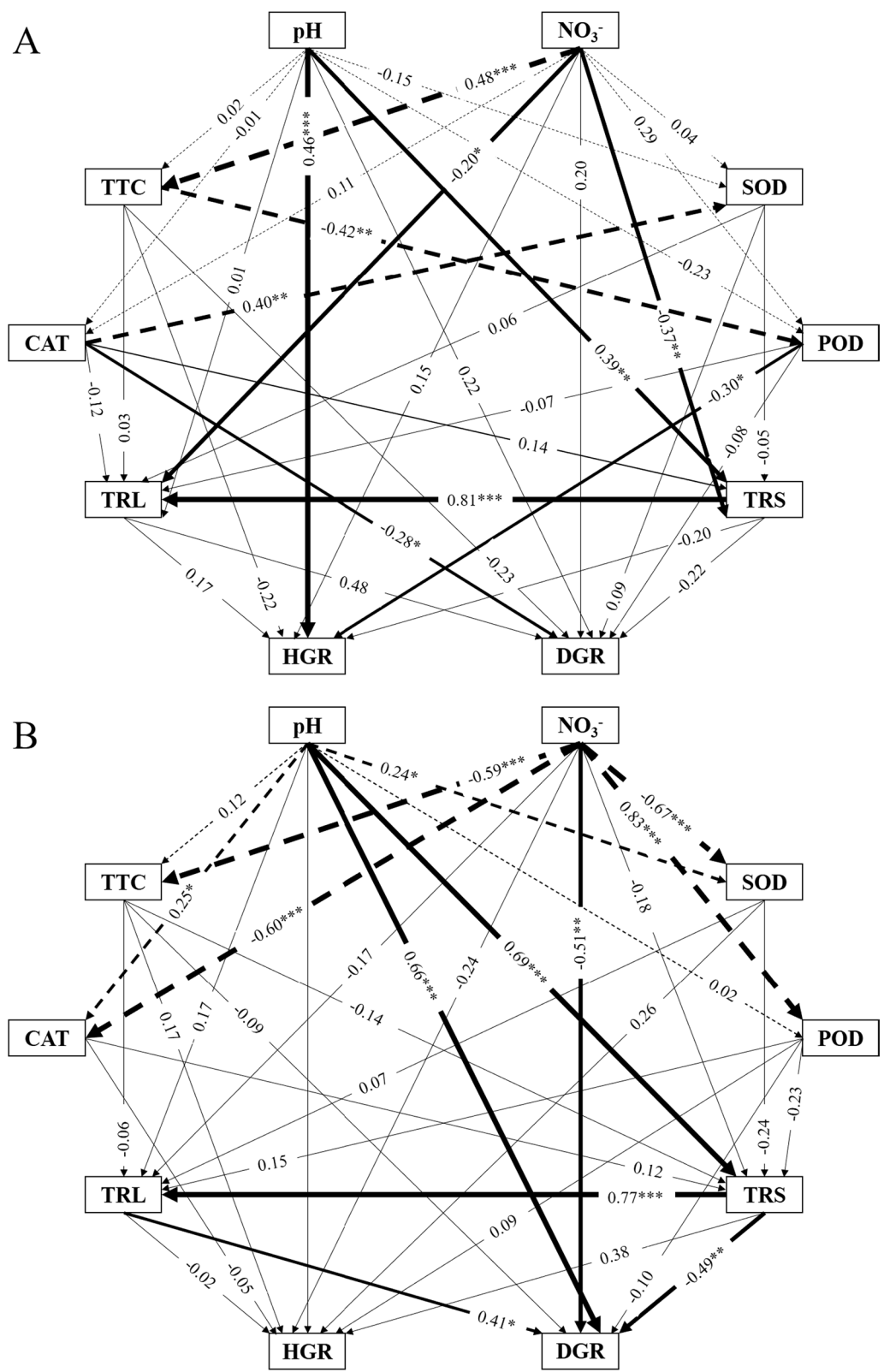
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Fig. 7 (See legend on previous page.)

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Table 1 Direct, indirect, and total impact tables of AR pH and NO_3^- concentrations on height growth rate and basal diameter growth rate of *Q. acutissima* and *C. lanceolata* saplings

	Q. acutissima		C. lanceolata	
	HGR	DGR	HGR	DGR
NO_3^-				
SDE	0.148	0.204	− 0.241	− 0.513
SIE	− 0.148	− 0.299	− 0.241	− 0.020
STE	0.000	− 0.094	− 0.482	− 0.533
pH				
SDE	0.458	0.217	− 0.277	0.662
SIE	0.050	0.081	0.303	− 0.045
STE	0.508	0.299	0.027	0.617

This table shows the standardized direct, indirect, and total effects of the concentration of NO_3^- in AR and AR pH on the height and basal diameter growth rates of *Q. acutissima* and *C. lanceolata* saplings

pH, AR pH; NO_3^-, the concentration of NO_3^- in AR; HGR, height grow rate; DGR, basal diameter growth rate; SDE, standardized direct effects; SID, standardized indirect effects; STE, standardized total effects

content of *Q. acutissima* and *C. lanceolata* roots under AR stress exceeded that of their corresponding ck, which further verified that AR has destructive effects on the physiology and growth of plant roots. A study on the resistance of *Arabidopsis* to different types of AR found that the SAR treatment had the highest MDA content (Qiao et al. 2018). This was consistent with the MDA trend of *Q. acutissima* roots in our study. However, the high acidity of NAR significantly increased the MDA content of *C. lanceolata* roots. The tree species formed the characteristic of the selective absorption of nitrogen during the process of long-term adaptation. Tree species growing in neutral or alkaline soils are more predisposed to absorb NO_3^--N (Zhang and Bai 2003), which relieves NAR stress damage to a certain degree.

The differences in AR tolerance caused by the nitrogen absorption strategies of tree species were also reflected in our research on root activity. With higher NO_3^- concentrations in the simulated AR, the root activities of *Q. acutissima* increased, while for *C. lanceolata* they decreased. Since different tree species have variable responses to nitrogen deposition (Thomas et al. 2009), and *Q. acutissima* is sensitive to nitrogen absorption (Ma et al. 2021; Yu et al. 2019), the fertilization effect of NO_3^- in our study was exhibited most prominently in the *Q. acutissima* root systems, while the positive effects of fertilization by NO_3^- on *C. lanceolata* roots were lower than the negative effects of H^+ stress. Root activity reflects the metabolism of root systems and is an important physiological indicators for the assessment of water and nutrient uptake (Islam et al. 2007). Most studies on the effects of AR on root activity revealed that they decreased under

lower AR pH (Liang and Wang 2013; Sun et al. 2013; Zhang et al. 2016b). Surprisingly, under the influence of pure NAR, the root activities of *Q. acutissima* and *C. lanceolata* treated with pH 3.5 were significantly higher than the other pH treatments. Due to the significant addition of NO_3^- in the NAR, the nitrogen metabolism in plant roots were improved (Qiao et al. 2018) and their threshold tolerance for acidity was increased, while the acidity-induced stress within this tolerance range had a certain growth-promoting effect.

To alleviate the damage from reactive oxygen species (ROS) that is inflicted on plants under stress, they generate multiple antioxidant enzymes, such as SOD, POD, and CAT (Kazemi et al. 2010). The generation and accumulation of ROS are also related to particular tree species (Chen et al. 2013). In our study, when the AR (pH = 2.5) type changed from 1:5 NAR to pure NAR, the activities of SOD, POD, and CAT in the *Q. acutissima* root system were significantly decreased. On the one hand, highly acidic NAR initiates a significant increase of H^+ in plant cells (Liang et al. 2015). Excessive H^+ will lead to the superabundant accumulation of ROS, such as hydrogen peroxide and superoxide anions, which will in turn induce oxidative stress in plants (Ahmad et al. 2010; Gill and Tuteja 2010). On the other hand, excessive NO_3^- accelerates soil acidification (Lindberg et al. 1990), and its strong oxidation will also damage plant organelles (Mo et al. 2008). Therefore, under the influence of two these aspects, the ROS level of the *Q. acutissima* root system exceeded the standard, cell structures were seriously damaged, and the activities of the antioxidant system were inhibited. Compared with *Q. acutissima* roots, the antioxidant enzyme activities of *C. lanceolata* roots were more closely related to the NO_3^- concentration of AR. The POD oxidation reaction is dominant in protecting plant cells from the influence of high oxygen concentrations, when cellular oxygen concentrations become too high (Bezerril Fontenele et al. 2017). The POD activity of *C. lanceolata* roots under NAR stress was significantly higher than that of the ck in our study, which demonstrated that the impact of NAR on the oxidative stress of *C. lanceolata* roots was primarily reflected through increased intercellular oxygen levels. Furthermore, consistent with previous work (Qiao et al. 2018), the CAT activity of the MAR treatment in this study was significantly higher than that of SAR at pH 2.5. However, when the AR S/N was shifted from 5:1 to 1:5, the change of CAT activity in *Q. acutissima* roots at pH 4.5 was opposite to that of pH 3.5 and 2.5. We attributed this to the acid tolerance threshold of *Q. acutissima*, where the AR at pH 4.5 was still within its tolerance range. However, with higher NO_3^- concentration, its strong oxidizing properties still boosted the accumulation of active

oxygen (Ahmad et al. 2010), which in turn increased its CAT activity at a S/N ratio of 1:5.

Variations between tree species in response to different types of AR

Various plants have different tolerances for AR (Ren et al. 2018), and studies have found that the abundance of H^+ caused by AR is the main factor that threatens the growth of trees. The trees absorb H^+ into plant cells through roots, which induces changes in intracellular free radicals that have adverse effects on root growth (Zhou et al. 2020). Consistent with the above results, redundancy analysis and structural equation modeling showed that AR pH had a positive standardized total effect on growth rates of *Q. acutissima* and *C. lanceolata* (the higher the acidity of the AR, the greater the negative impact). In contrast to *C. lanceolata*, AR pH had a strong correlation with the height growth rate of *Q. acutissima*, and primarily affected seedling height growth through direct influence. As a broadleaved tree species, *Q. acutissima* leaves are more vulnerable and sensitive to AR (Du et al. 2017), as excessive hydrogen ions can severely inhibit its photosynthesis (Du et al. 2020), and directly lead to the slow growth of seedling height. However, since *C. lanceolata* is a tree species that grows well in acidic soil, it has higher acid tolerance (Zhang and Bai 2003), which weakens the influence of AR pH to a certain extent. This result partially supports our third hypothesis mentioned above.

With the change in AR types, the NO_3^- concentration of AR has gradually attracted increased attention (Liu et al. 2019). Redundancy analysis and structural equation model indicated that the NO_3^- concentrations in AR had a negative standardized indirect effect on the growth rates of both *Q. acutissima* and *C. lanceolata*. This revealed that excessive AR NO_3^- concentration can inhibit root activity, destroy antioxidant systems through its strong oxidation, and then weaken the height growth of seedlings (Liu et al. 2018b). However, in contrast to *Q. acutissima*, the NO_3^- concentrations in AR had a strong correlation with the height growth rate of *C. lanceolata*. In addition to its negative indirect effects, the NO_3^- concentrations in AR also directly inhibited the height growth of *C. lanceolata* seedlings. As discussed above, this may be related to the nitrogen utilization characteristics of trees (Thomas et al. 2009). Excessive NO_3^- concentrations in AR affects the nitrogen absorption and utilization in *C. lanceolata*, however, they have a certain fertilizing effect on *Q. acutissima*. This result further supported our third hypothesis. In short, our results indicated that changing AR types further complicate the challenges faced in the development of different plantations.

Furthermore, this study analyzed only the responses of the root systems of *Q. acutissima* and *C. lanceolata* to various types of simulated AR under brief potting tests; thus, there remains a lack of long-term natural environmental research. For future studies, we aim to establish a long-term field experiment combined with root turnover, to further explore the impacts of different simulated AR types on the root systems and growth of different tree species.

Conclusion

During simulated AR stress, as the concentration of NO_3^- increased in AR, the inhibitory effect of AR on the root growth of *Q. acutissima* and *C. lanceolata* saplings gradually exacerbated. The root systems of *Q. acutissima* and *C. lanceolata* saplings had variable responses to higher AR acidity and NO_3^- concentrations. Meanwhile, the AR pH had a direct potent inhibition on the height growth rate of *Q. acutissima* in contrast to *C. lanceolata*, while the AR S/N ratio affected the growth rate of *C. lanceolata* to a greater degree than *Q. acutissima* by directly inhibiting and indirectly impacting the physiological attributes of the roots. In brief, highly acidic AR and elevated NO_3^- concentrations had the capacity to alter the physiological characteristics of roots. Although the degree of these changes and their positive or negative effects may vary with tree species, they ultimately inhibit the growth of trees. Compared with *C. lanceolata*, *Q. acutissima* was less affected by AR with high NO_3^- concentrations.

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