Nitrogen addition increases root biomass allocation and changes functional traits of two dominant tree species in North China

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Abstract

Aims

Nitrogen (N) is one of the limiting nutrients for plant growth in terrestrial ecosystems. Numerous studies that have explored the effects of N addition on the eco-physiological traits and biomass production of plants, but the underlying mechanism of N deposition on biomass allocation has not been clarified, especially for urban greening trees.

Methods

A greenhouse simulated experiment was conducted by two dominating urban street trees in North China, including conifer *Pinus tabuliformis* and broadleaved *Fraxinus chinensis*. We set up three levels of N addition: ambient, low N addition, and high N addition (0, 3.5, and 10.5 gN m^{-2} $year^{-1}$) and determined the biomass distribution, plant functional traits, and soil nutrient traits of the two trees.

Results

Our results showed that N addition had positive effects on the aboveground and belowground biomass of *P. tabuliformis*, which also shifted its functional traits to fast. While *F. chinensis* only increased root biomass distribution and root acquisitive traits as N increased. Furthermore, N addition increased the soil N and phosphorus contents of both two trees and improved the root nutrient uptake capacity, resulting in the increase of root-shoot ratio. We found that optimal partitioning theory could better explain that trees would invest more resources in roots in the poor-resource area.

Conclusion

Trees changed their root structure and increased root biomass allocation to adapt to the high N deposition environment. Our findings highlight the importance of plant functional traits in driving the responses of biomass allocation to environmental factors for urban greening-dominated tree species.

Introduction

Nitrogen (N), as a primary element in terrestrial ecosystems, controls plant growth and productivity (Galloway, 2005; Tegeder and Masclaux-Daubresse 2018). During recent decades, with the increasing frequency of human activities, such as fossil fuel combustion and fertilizer production and usage, global atmospheric N deposition has increased rapidly and become the focus of attention (Galloway et al. 2008; Liu et al. 2021). Generally, elevated N deposition increases soil N availability, promoting photosynthesis of leaf and root growth, as well as contributing to plant biomass and productivity (Liu et al. 2013; Ren et

al. 2019). However, excessive N deposition causes “N saturation” of ecosystem, leading to soil acidification and nutrient loss, which threatens plant photosynthetic capacity and growth (Bueno et al. 2019). During the past 30 years, the N deposition rate in China has more than doubled, especially in urban ecosystem (Chen et al. 2012; Huang et al. 2022). Urban greening tree species, as an indispensable part of urban ecosystem, play an important role in alleviating urban environmental pressure and providing ecosystem services, which have been also affected by the global climate changes (Chen et al. 2022). Thus, it is crucial to explore the response of different trees to N deposition, which contributes to select suitable species, developing sustainable management policies, improve the functional urban green space, and create multiple ecosystem service values.

Plant aboveground and belowground biomass allocation reflects plant evolution strategies for resource acquisition, growth patterns, and competitive capacity (Poorter et al. 2012; Li et al. 2019). In general, it has a strong variability due to the species and its growth environment (Yang et al. 2009). Previous studies have demonstrated that N deposition could change the plant biomass allocation and growth pattern (Li et al. 2019). The ratio-based optimal partitioning theory, as one of the plant biomass allocation strategies, shows that plants allocate more biomass proportionally to capture the limiting resource in response to environmental fluctuation (Poorter et al. 2012; Yue et al. 2020), which can better explain the effect of N addition on biomass allocation in a short term (Bernacchi et al. 2010). For example, N input could increase the root mass fraction of invasion *Solidago canadensis* to better access nutrients from the soil in the poor-resource environment (Ren et al. 2019). On the contrary, plants invest more biomass in aboveground architectures to promote photosynthesis as N addition when growing in abundant nutrient environments (Poorter et al. 2012). However, it’s unclear how N deposition affects the biomass allocation of different tree species, and whether the optimal allocation theory is suitable for this situation remains to be explored.

Functional traits can indicate the capacity of plant to capture resources and adaptability to environmental changes, which are considered the potential covariates to understand biomass allocation (Mensah et al. 2016; Zhang et al. 2022). Generally, a suite of correlated functional traits has been divided into acquisitive and conservative traits, known as the plant economics spectrum strategy (Roumet et al. 2016). Plants with fast traits like specific leaf area, leaf N content, and root length, are easily accessible to resources. Slow traits such as leaf thickness and root tissue density tend to efficiently preserve resources for long-lived plants or in poor-resource habitats (Pérez-Harguindeguy et al. 2013). It has been noted that N addition increased soil nutrient content, leading to changes in plant functional traits, which shift to a fast acquisitive strategy and get resources more quickly (Trocha et al. 2017). However, the response of aboveground and belowground traits to N deposition may be different due to the different environmental selection pressures (Mao et al. 2018). There were synergistic, antagonistic, or uncorrelated relationships between aboveground and belowground traits in different environments (Poorter et al. 2012; Zhang et al. 2022). Therefore, it is necessary to explore the response of plant aboveground and belowground functional traits to N deposition, so as to fully understand the response mechanisms of species to environmental changes.

Due to rapid economic development, urban green spaces receive a higher level of N deposition compared to the natural forest ecosystems, especially in North China, (Davidson et al. 2009). However, few studies have explored the underlying mechanism of N inputs on the growth patterns and functional traits of different urban greening trees. *Pinus tabuliformis* and *Fraxinus chinensis*, as the dominant conifers and deciduous trees of urban ecosystem in North China, have important ecological and economical values (Chen et al. 2017). Therefore, we selected the two tree species seedlings in a greenhouse under three N addition treatments for six months. We investigated the biomass and functional traits correlated with plant growth and nutrient uptake at the end of the cultivation. Specifically, we put forward the hypotheses that (1) N addition will promote the growth of *P. tabuliformis* and *F. chinensis*, both for aboveground and belowground parts. (2) The biomass allocations of the two target tree species tend to optimal partitioning, and trees will provide more resources for roots as N addition. (3) N addition affects the aboveground and belowground traits of *P. tabuliformis* and *F. chinensis*, which is consistent with the plant biomass allocation strategy. (4) N also changes the nutrient traits of soil and tree species, which can drive variation in plant biomass allocation.

Materials and methods

Study site

We carried out the experiment from February to October 2022 in the greenhouse of Nankai University, Tianjin, China (38.986° N, 117.357° E). This area has a temperate monsoon climate with the annual average temperature of 13.4 ℃, annual average precipitation of 580.1 mm, and annual average relative humidity of 59.0%. Recently, N deposition has increased rapidly in China, and the total amount of N deposition in Tianjin was up to 35 g N m^{-2} $year^{-1}$ (Yu et al. 2019).

Experiment design

We selected 36 cylindrical pots in the greenhouse, which had a height of 40 cm and a base radius of 19 cm. The soil we collected was the weathered soil from the forests of *Pinus tabuliformis* and *Fraxinus chinensis* at Nankai University. After removing stones and other impurities, we mixed the soil homogeneously and each pot contained three-quarters of the soil, which weighed about 30 kg. The soil PH was 7.75 ± 0.06, soil C and N contents were 10.60 ± 0.61 mg g^{-1} and 0.87 ± 0.13 mg g^{-1}, and ammonium and nitrate N contents were 6.02 ± 0.96 μg g^{-1} and 13.21 ± 0.98 μg g^{-1}, respectively. One-year-old bare rooted seedlings of the two tree species (*P. tabuliformis* and *F. chinensis*) were transplanted into the pots. Then, all pots were irrigated twice a week to keep the soil surface moist until the seedlings were adapted to the greenhouse conditions.

At the end of the acclimation period, we conducted a two-factorial experiment, including two tree species and three N addition treatments [no N, low N (3.5 g N m^{-2} $year^{-1}$), and high N addition (10.5 g N m^{-2} $year^{-1}$)], with six replicates. The amount of N addition was based on the total amount of N deposition and its triple in Tianjin. The aqueous solution of NH_4NO_3 salts was used for the different N addition

gradients, added on the soil surface once a month. Meanwhile, the no N addition group applied the same amount of water every time. In addition, we also watered the pots twice a week. The mean temperature of the greenhouse was 24.37 ± 0.06°C and mean humidity was 64.67 ± 0.18% RH.

Measurements of tree aboveground and belowground traits and biomass

The saplings were cultivated for six months. At the end of the experiment, we measured the tree aboveground and belowground traits based on the standard methods of Pérez-Harguindeguy et al. (2013), which were universally validated as appropriate predictive indicators to reflect plant resource acquisition, utilization, and storage strategies, including water, light, and nutrients (Table S1; Hao et al. 2020). Plant height (PL) was measured as vertical distance between the highest leaf of the tree in the natural state and pot topsoil level. Then, five whole and expanded leaves of each plant were selected randomly to scan and measure leaf area (LA) by ImageJ software and measured leaf thickness (LT) by vernier. We also weighed the fresh mass of the scanned leaves and dried them at 70 ◦C for 72 h to a constant weight and weighed. Afterwards, we calculated the specific leaf area (SLA; LA/leaf dry mass), leaf dry matter content (LDMC; leaf dry/fresh mass), and leaf tissue density (LTD; leaf dry mass/volume).

Subsequently, we took the plants out of the pots to ensure the integrity of the roots, and then washed the root samples with water to remove the surface soil. The saplings were carefully divided into the aboveground and belowground parts by cutting at the growing point. The fresh root samples were scanned and analysed by the WinRhizo root analysis system to obtain total root length (RL), root area (RA), average root diameter (RD), and root tips. Meanwhile, we dried the root samples and calculated specific root length (SRL; RL/root dry mass), specific root area (SRA; RA/root dry mass), branching intensity (BRI; root tips/RL), and root tissue density (RTD; root dry mass/volume). All samples were also oven dried to a constant weight at 70 ◦C for 72 h, which weighed and calculated the aboveground, belowground, and total biomass (AGB, BGB, Tbio) and root-shoot ratio (R/S).

Measurements of tree and soil major nutrient elements

After removing seedlings, the rhizosphere soils were collected from the pots and then air dried and screened. Similarly, dried plant samples were also ball-milled to fine powders. The soil and tree leaf and root carbon and nitrogen content (SCC, LCC, RCC and SNC, LCC, LNC) were measured using the Vario MAX C/NMacro Elemental Analyzer and then calculated LCC/LNC and RCC/RNC. In addition, the soil ammonium (SNH) and nitrate (SNO) N contents were extracted in 2 mol/L KCl solution and determined by the Bran-Luebbe Flow Injection Analyzer. The soil phosphorus content (SPC) was digested by H_2SO_4-$HClO_4$ solution and determined by the Bran-Luebbe Flow Injection Analyzer.

Statistical analysis

Prior to the statistical analyses of variance (ANOVA), we tested the Kolmogorov-Smirnov normality distribution test and the Bartlett homogeneity test of variances. All data were normalized using log-transformation before analysis when necessary.

One-way ANOVA was performed to test the effects of N addition on plant biomass, functional traits, and soil nutrient elements. The LSD multiple comparison test was employed to compare means and the significance level was $p < 0.05$. The statistical analyses were performed using SPSS 22.0. Furthermore, to assess the relationship between aboveground and belowground biomass, we performed linear regressions and calculated the slope and intercept. We also used Pearson's correlation analysis to test the connection between plant and soil traits. All of these analyses and figures were constructed using the package "psych", "car", and "vegan" in R version 4.1.1 (R Core Team, 2021).

To explore the underlying mechanisms of N addition in soil and plant nutrient elements and root-shoot ratio, structural equation modelling (SEM) analysis was used based on the regression analysis and previous experience. In this model, N addition was regarded as an exogenous variable, soil (SNC, SCC, and SPC) and tree (LNC, LCC, RNC, and RCC) nutrient traits were treated as endogenous variables, and R/S was considered as a response variable. All variables were standardized to satisfy the assumptions of normality and linearity. After removing non-significant pathways ($p > 0.05$) and collinear variables, we used the Fisher's C test to evaluate the goodness of SEM and ensure its $p > 0.05$ with the lowest AIC value. The SEM analyses were performed using the package "piecewiseSEM" in R.

Results

Aboveground, belowground, and total biomass and their biomass allocation

N addition significantly increased the total biomass of both *P. tabuliformis* and *F. chinensis* (Table S2). Compared to the no N groups, low N and high N addition enhanced Tbio by 32.8% and 45.3% for *P. tabuliformis* and 33.1% and 47.4% for *F. chinensis*, respectively (Fig. 1). Specifically, N addition increased the AGB and BGB of *P. tabuliformis*, while only increasing the BGB of *F. chinensis*. There was no significant variation in the AGB of *F. chinensis*.

Furthermore, consecutive N addition had a significant effect on aboveground and belowground biomass allocation. The R/S of both *P. tabuliformis* and *F. chinensis* increased with N increasing (Fig. 1; Table S2). As for *P. tabuliformis*, N addition increased R/S of 6.7% under low N treatment and 36.7% under high N treatment relative to no N treatment. While the R/S of *F. chinensis* increased by 28.6% and 62.0%. These variations indicated that the positive effect of N addition on belowground biomass allocation was more significant for the two target tree species.

Our results showed that there were positive relationships between AGB and BGB of both the two trees (Fig. 2). The scaling slopes for the ratio of AGB to BGB of *P. tabuliformis* were 0.40, 0.41, and 0.44 in the no, low, and high N groups, and the correlation coefficients were 0.82, 0.12, and 0.64, respectively. Despite there being no correlation in low N treatment, it was obvious that aboveground and belowground biomass allocation of *P. tabuliformis* showed an isometric relationship as N increased. For *F. chinensis*, the scaling slopes for the ratio of AGB to BGB were 1.64, 0.68, and 0.79, and the correlation coefficients were 0.60, 0.74, and 0.93 under no, low, and high N treatment, respectively. These results confirmed the biomass

optimal partitioning hypothesis, which performed N addition significantly enhanced belowground biomass allocation of *F. chinensis*.

Aboveground and belowground traits

N addition had significantly affected the aboveground traits of *P. tabuliformis*, increasing the tree height, SLA, and LDMC. However, only high N increased the LDMC and LTD of *F. chinensis*, while there were no significant differences among other aboveground traits. (Fig. 3; Table S3). As for belowground traits, N addition had significant effects on the belowground traits of both *P. tabuliformis* and *F. chinensis* (Table S4). N increased the SRL, RA, and SRA of *P. tabuliformis*, while reducing its RTD. Meanwhile, there were also positive correlations between N and RL, SRL, RA, and SRA of *F. chinensis*, while the negative correlation between N and RD (Fig. 4).

Besides, we observed a synergy relationship between aboveground and belowground traits of *P. tabuliformis* in response to N addition (Fig. 5). Both tree height and SLA were positive with SRL and SRA, and N addition increased all of these functional traits. However, there was no obvious correlation between the aboveground and belowground traits of *F. chinensis*.

Relationship between tree biomass and traits

Our results of Pearson's correlation analysis indicated that there were significant associations between tree biomass and aboveground and belowground traits of *P. tabuliformis* (Fig. S1a). We found that PL and SLA were positive with AGB, BGB, Tbio, and R/S, while LDMC and LTD were positive with AGB and Tbio. Likewise, SRL was positive with AGB, BGB, Tbio, and R/S, and SRA was positive with BGB, Tbio, and R/S. However, RTD was negatively correlated with AGB, BGB, and Tbio. As for *F. chinensis*, only belowground traits were significantly correlated with biomass (Fig. S1b). RL and RA were positively related to BGB, Tbio, and R/S, while RD was negative with AGB, BGB, and Tbio.

Nutrient elements traits of trees and soil

N addition increased the RCC and RNC of *P. tabuliformis*, while decreasing the LCC/LNC and RCC/RNC. Similarly, LNC and RNC of *F. chinensis* were increased and the LCC/LNC and RCC/RNC were decreased as N increased (Fig. 6; Table S5). There was no significant difference between N and LCC and RCC. As for soil traits, N increased the SNC, SPC, SNH, and SNO in the rhizosphere soil of both two tree species, but didn't change the SCC (Table 1).

Table 1
Soil nutrients including carbon (C) nitrogen (N) and phosphorus (P) concentrations under different N treatments. Different lowercase letters indicate significant differences among N treatments at the same time at a significance level of *p*-value < 0.05.

Treatment	SCC (mg/g)	SNC (mg/g)	SNH (mg/kg)	SNO (mg/kg)	SPC (mg/g)
Pinus tabuliformis					
No N	13.63 ± 1.93	1.12 ± 0.04 a	6.52 ± 0.70 a	14.92 ± 1.06 a	7.84 ± 0.41a a
Low N	9.68 ± 0.88	1.18 ± 0.06 a	7.88 ± 0.26 b	15.68 ± 0.54 a	11.01 ± 1.39 b
High N	13.47 ± 0.18	1.38 ± 0.07 b	9.73 ± 2.36 c	16.84 ± 0.11 b	12.12 ± 0.69 b
Fraxinus chinensis					
No N	8.07 ± 0.17	0.67 ± 0.04 a	2.43 ± 0.15 a	12.18 ± 1.63 a	8.41 ± 0.27a a
Low N	8.33 ± 0.21	0.81 ± 0.02 b	4.75 ± 1.08 b	22.96 ± 1.45 b	8.48 ± 0.60a a
High N	8.20 ± 0.03	0.82 ± 0.04 b	4.20 ± 0.90 b	20.52 ± 0.15 b	10.06 ± 0.42 b

The first structural equation model explained 94% of the variance in R/S of *P. tabuliformis* (Fig. 7a). The result showed that N addition changed the soil traits (SNC, SPC) and tree nutrient elements (LNC, RNC, and RCC) and had direct inhibiting and indirect promoting effects on R/S (Table 2). Specifically, N addition indirectly increased R/S through positive effects on RNC and SNC and also promotes R/S as a whole. Moreover, N increased RNC via a direct effect and indirectly altering SNC. SPC had negative effects on LNC and N had direct promoting and indirect inhibiting effects on it.

Table 2
Direct, indirect and total effects on root-shoot ratio (R/S) on standardized values of statistically significant SEM paths ($p < 0.05$). Direction of relationship indicated by + (positive relationship) or - (negative relationship). NS indicate no significant relationships.

Predictor	Pathway to R/S	Effect
Pinus tabuliformis		
N	Direct	-0.37
	Indirect	0.68
	Total	0.31
SNC	Direct	0.47
	Indirect	0.27
	Total	0.74
RNC	Direct	0.60
	Indirect	NS
	Total	0.60
Fraxinus chinensis		
N	Direct	0.35
	Indirect	0.42
	Total	0.77
RNC	Direct	0.51
	Indirect	NS
	Total	0.51

The SEM2 explained 96% of the variance in R/S of *F. chinensis* (Fig. 7b). N addition was positive with soil traits (SNC, SPC) and tree nutrient elements (LNC, RNC). Meanwhile, N increased R/S through direct and indirect effects (Table 2). Specifically, N could promote RNC via a direct effect and indirect effects through increasing SNC and SPC, and finally indirectly increasing R/S.

Discussion

Effect of N deposition on aboveground and belowground biomass allocation

Many studies have confirmed that N has a positive effect on plant growth and biomass production, both in the aboveground and belowground parts (Hermans et al. 2006; Liu et al. 2022). Our results demonstrated that N addition increased the biomass of both *P. tabuliformis* and *F. chinensis*. However, the biomass allocation strategies of the two species were different. N addition promoted both AGB and BGB of *P. tabuliformis*, which proved our first hypothesis. While it only increased the belowground part for *F. chinensis*, not as expected. Generally, due to the higher variability of broadleaf species, their saplings allocate more woody biomass to roots than conifer species as soil resource changes (Annighöfer et al. 2022). Moreover, roots are relatively sensitive as soil N availability increased compared with the aboveground part (Kiba and Krapp 2016). According to the Second National Soil Survey of China, our native soil N content (0.87 ± 0.13 mg g^{-1}) lower than 1 mg g^{-1} was regarded as a poor nutrition level (Li et al. 2017). These meant *F. chinensis* would invest more resources in root biomass, leading to facilitate root extension and improve the resource-use efficiency in the soil (Davis et al. 2000). On the contrary, the aboveground material might be relatively less important and didn't change significantly as N increased.

Furthermore, we observed although N addition affected the R/S ratio of *P. tabuliformis*, there existed isometric relationships between AGB and BGB, with little variation in slope as N increased. These results indicated that the aboveground and belowground growth patterns of *P. tabuliformis* were consistent in the high N deposition environment, similar to Yang et al. (2009). But it's insufficient to substantiate our second hypothesis that the tree biomass allocation strategy tended to optimal partitioning. This may be because that *P. tabuliformis* has already adapted to the poor nutrient environment and plant biomass allocation was not closely related to short-term environmental fluctuations (Ren et al. 2019). *P. tabuliformis* tended to maintain a stable allocation strategy to meet the biomass needs of different plant organs. However, as for *F. chinensis*, exogenous N supplies increased the R/S ratio and decreased the slopes of linear relationship between AGB and BGB. The proportion of root biomass allocation increased with N addition. These results followed the ratio-based optimal partitioning theory that the biomass allocation pattern was induced by environmental change and proved our second hypothesis (Poorter et al. 2012). As mentioned above, soil N was a limiting factor in our experiment plot. N addition improved the soil N availability and promoted plant root growth to gain more nutrient resources, thereby leading the tree species to adapt to the poor-resource environment (Kiba and Krapp 2016).

Effect of N deposition on tree functional traits

Our experiment found that N addition affected both aboveground and belowground functional traits of *P. tabuliformis*. Under N fertilization, tree height and SLA of *P. tabuliformis* tended to be higher, which were related to plant photosynthesis (Heberling and Fridley 2016). These traits improved the capacity of conifers to capture light energy and promoted leaf photosynthetic efficiency (Zhang et al. 2022), which made *P. tabuliformis* better adapt to the high N deposition environment, thus increasing the aboveground biomass. Meanwhile, SRL and SRA of *P. tabuliformis* were increased and RTD was decreased as N increased. SRL and SRA belong to acquisitive traits, which were related to root resource acquisition capacity, while RTD tended to be conservative and was negative with root growth (Kramer-Walter et al. 2016; Ding et al. 2019). N addition changed the root architecture and shifted the resource strategy to the

fast pattern, improving the root resource acquisition efficiency and biomass in the poor nutrient environment. In addition, we also found that there were consistent between aboveground and belowground traits, similar to the plant biomass. The response of aboveground and belowground parts of *P. tabuliformis* to N deposition had synergetic effects, and showed the same growth strategy (Hu et al. 2019).

N had positive effects on RL, SRL, RA, and SRA but was negative with RD, while increased LDMC and LTD of *F. chinensis*, similar to the response to biomass allocation. Compared to conifers, broadleaf saplings had a lower root morphological similarity (monopodial growth) and more apparent variability when adapting to environmental changes (Luo et al. 2012). Therefore, the root traits of *F. chinensis* were more sensitive to N addition treatments. Moreover, according to optimal partitioning theory, tree species would pay more energy to root system in poor soil (Ding et al. 2019). These forced roots to shift to fast traits to acquire more nutrients, and were finally reflected in the root biomass. In contrast, the aboveground traits (LTD, LDMC) of *F. chinensis* tended to be conserved, with fewer resources invested in the growth of the aboveground parts (Roumet et al. 2016). Overall, plant aboveground and belowground traits showed opposite resource acquisition strategies, which was similar to that of Asefa et al. (2022). The above results confirmed our third hypothesis.

Effect of N deposition on nutrient traits of soil and tree species

N supply significantly increased soil N element availability (SNC, SNH, and SNO) of both *P. tabuliformis* and *F. chinensis*, and thus improved the LNC and RNC of the target trees, which was similar to our previous study (Zhang et al. 2022). Plants with higher LNC and RNC tended to possess greater capacities for foliar N storage and root uptake, indicated to improve photosynthesis and nutrient uptake (Butler et al. 2016). Meanwhile, N addition decreased the LCC/LNC and RCC/RNC, which had been shown in various ecosystems (Norby, 1998; Zhang et al. 2019). It is reported that evergreen and needleleaf species performed higher C: N ratio to guarantee survival in N limiting environment and at the slower position of the plant economic spectrum. However, they would transform towards low C: N ratios to synthesize protein faster and improve competitiveness as more N became available (Reich, 2014; Zhang et al. 2019), which was consistent with our results. Furthermore, we found that the SPC of *P. tabuliformis* and *F. chinensis* were significantly higher in the N supply treatments. N improved soil P availability and stimulates P uptake, so that plants could absorb nutrients more efficiently and grow better to acclimate to the nutrient limitation environment (Marklein and Houlton 2012).

Tree R/S ratio is a direct embodiment of biomass allocation strategy and growth pattern, responding to environmental changes (Li et al. 2019). Our study showed that SNC and RNC had multiple direct and indirect positive effects on R/S ratio of *P. tabuliformis*. These results suggested that soil N availability and root N uptake played important roles in the root growth of *P. tabuliformis*, which made roots absorb more resources and directly changed the plant biomass allocation pattern (Davis et al. 2000). Despite directly inhibiting the R/S ratio, N addition could indirectly increase it through increasing SNC and RNC, same as other studies (Yan et al. 2019). As for *F. chinensis*, on the one hand, N could indirectly increase

R/S ratio through its positive effects on RNC and SNC, similar to *P. tabuliformis*. On the other hand, N also increased RNC through increasing SPC, which might emphasize the responses of P to N addition (Li et al. 2016). N addition increased soil P availability and alleviated the P limitation to plant growth (Schreeg et al. 2014). Ultimately, soil P concentrations positively affected the RNC and improved R/S ratio. In general, we found that N deposition changed the biomass allocation mainly through improving RNC of the target conifer and broadleaf tree species, rather than LNC. This meant that compared to aboveground nutrient traits, the root nutrient traits were directly correlated to soil resource elements, and reflected in the R/S ratio (Zhang et al. 2022). Meanwhile, plants with higher RNC would change the root structure and better adapt to the poor soil nutrient environment, while LNC was less important (Ren et al. 2019; Yue et al. 2020). These results support the last hypothesis and reveal the mechanism of N addition on tree biomass allocations by altering nutrient traits.

Conclusion

This study demonstrates how N addition affects biomass allocation and functional traits of two urban greening-dominant tree species in North China. Our results suggested that the responses of conifers and broadleaf trees on N addition were different, due to species specificity. N increased the aboveground and belowground biomass of *P. tabuliformis*, and the relationship between them was isometric. This was also reflected in functional traits, showed a greater height, SLA, SRL, SRA, LNC, and RNC. However, only root biomass of *F. chinensis* responded to high N treatments, which also increased root biomass allocation. Root traits of *F. chinensis* were shifted to fast and positive with root biomass, while aboveground traits tended to be conservative with fewer resource inputs. These results were better explained by the optimal partitioning theory that plants would invest more resources in roots to adapt to the poor-resource areas. Furthermore, we also found that N addition improved soil N and P availability to change the root-shoot ratio of the two tree species, mainly by increasing the root N content. Compared to aboveground traits, root nutrient traits played a more important role in the poor-resource environment with high N deposition and finally changed the root structure and biomass allocation. Therefore, our finding provides a novel perspective into the influence mechanism of N deposition in the tree species functional traits and biomass allocation.

Declarations

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Authors' contributions Q.Z. and H.L. planned and designed the research; Q.Z., J.A., M.Y., and S.H. performed experiments and conducted fieldwork; Q.Z. analysed the data; Q.Z., J.Z., and H.L. wrote the manuscript. All authors made significant contributions to the drafts, which were eventually final approved for publication.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest The authors declare no Conflict of interest

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Figures

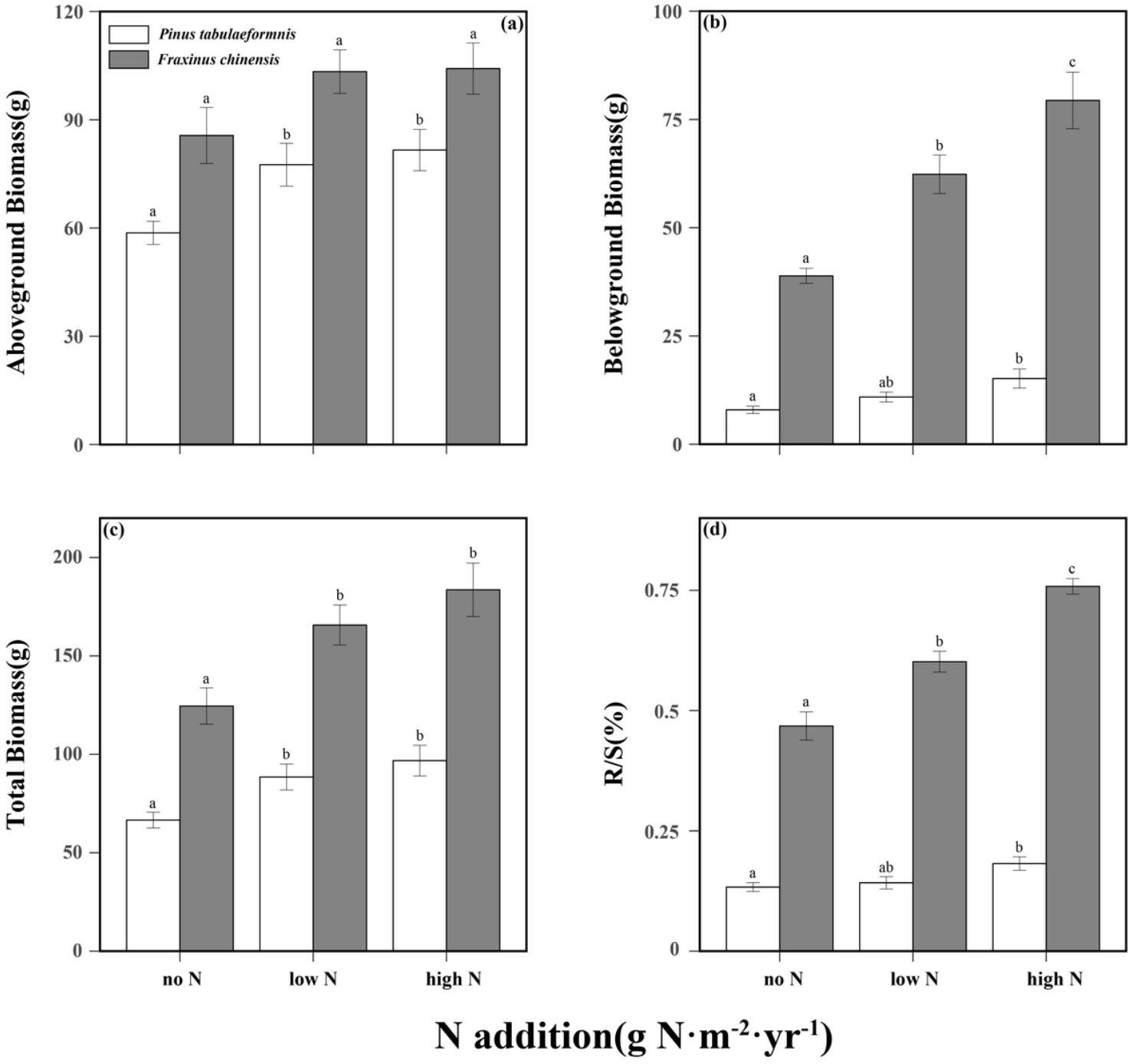

Figure 1

Aboveground biomass (a), belowground biomass (b), total biomass (c) and root-shoot ratio (R/S; d) of *Pinus tabuliformis* and *Fraxinus chinensis* under different N addition levels. Bars and error bars show means ± SE. Different lowercase letters indicate significant differences among N treatments at the same time at a significance level of *p*-value < 0.05.

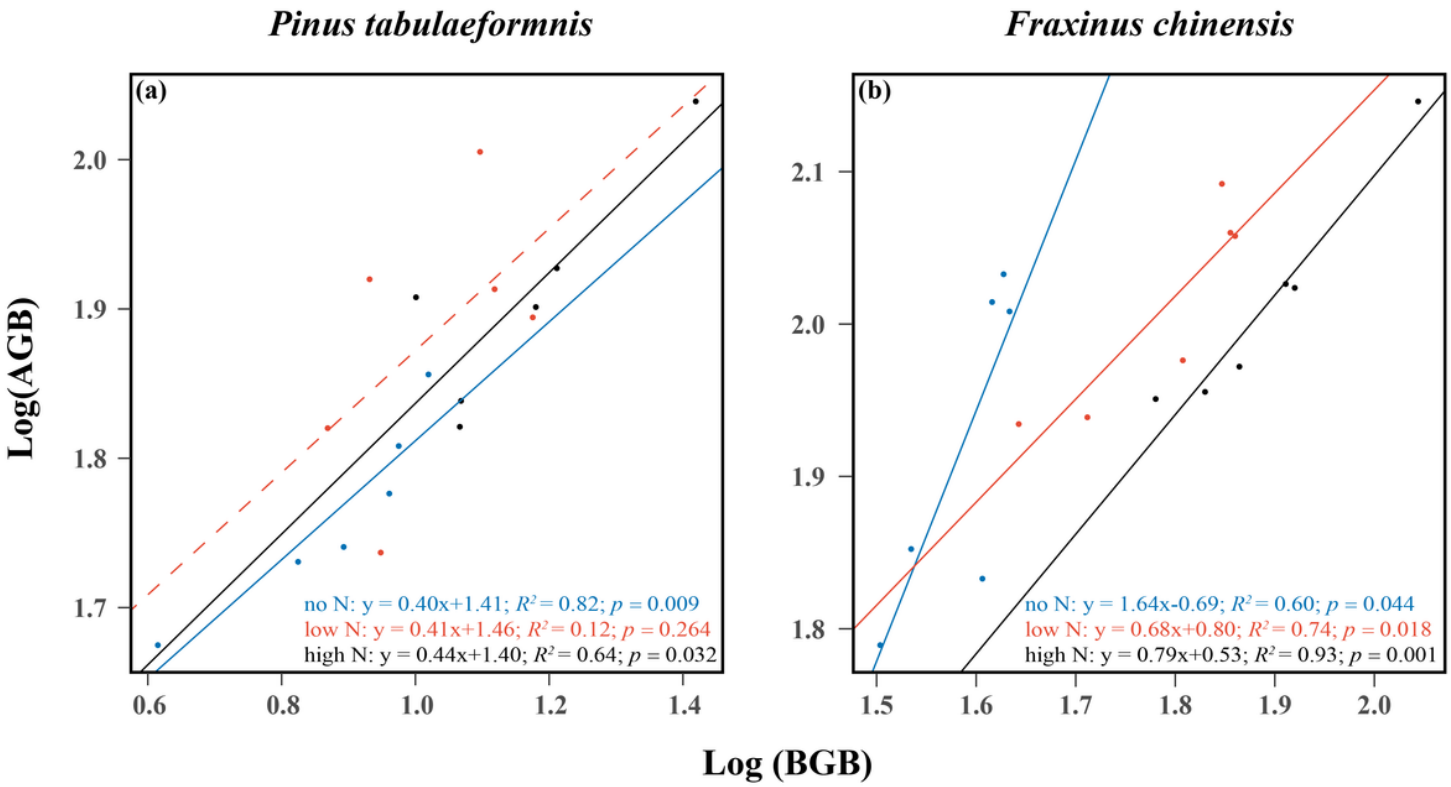

Figure 2

Relationships between aboveground biomass (AGB) and belowground biomass (BGB) of *Pinus tabuliformis* (a) and *Fraxinus chinensis* (b). The R^2 (coefficient of determination), *p*-values, and equations are obtained from the linear regression analyses. Different color lines denote the allocation relationships under different N addition levels. Solid lines indicate significant confidential intervals ($p < 0.05$), while dotted lines indicate insignificant confidential intervals.

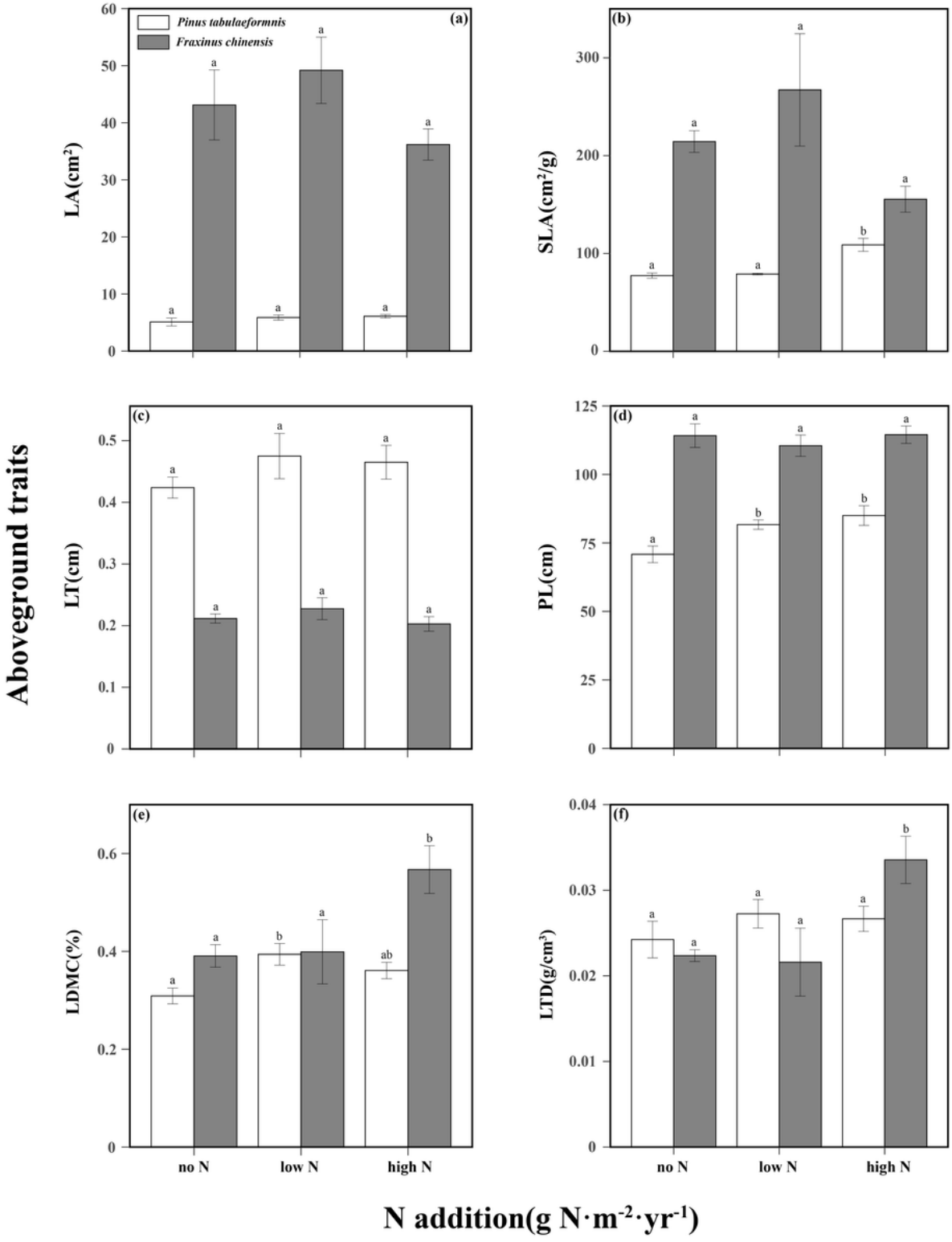

Figure 3

Aboveground traits including leaf area (LA), specific leaf area (SLA), leaf thickness (LT), plant height (PL), leaf dry matter content (LDMC), and leaf tissue density (LTD) of *Pinus tabuliformis* (a, c, e) and *Fraxinus chinensis* (b, d, f) under different N addition levels. Bars and error bars show means ± SE. Different lowercase letters indicate significant differences among N treatments at the same time at a significance level of *p*-value < 0.05.

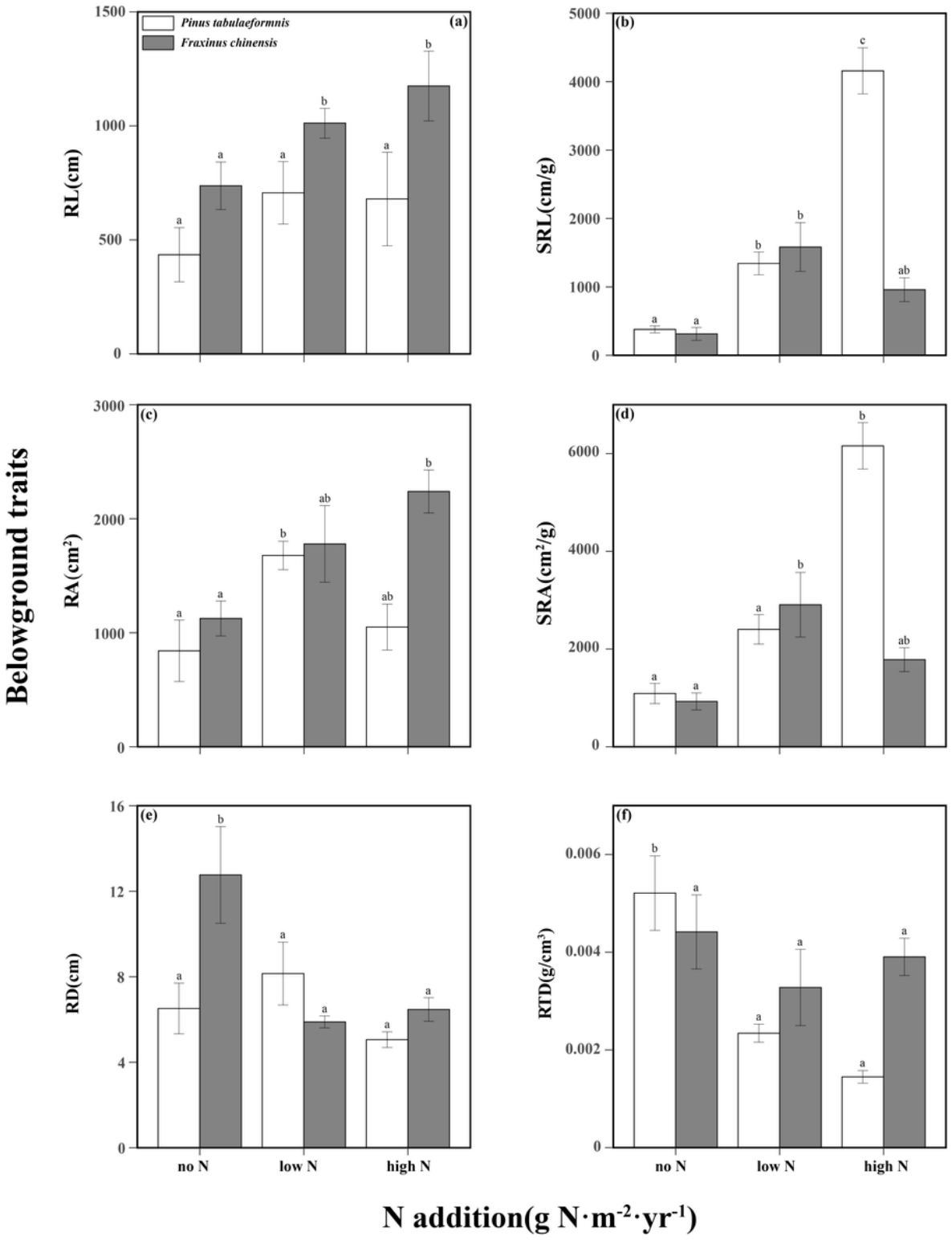

Figure 4

Belowground traits including root length (RL), specific root length (SRL), root area (RA), specific root area (SRA), average root diameter (RD), and root tissue density (RTD) of *Pinus tabuliformis* (a, c, e) and *Fraxinus chinensis* (b, d, f) under different N addition levels. Bars and error bars show means ± SE. Different lowercase letters indicate significant differences among N treatments at the same time at a significance level of *p*-value < 0.05.

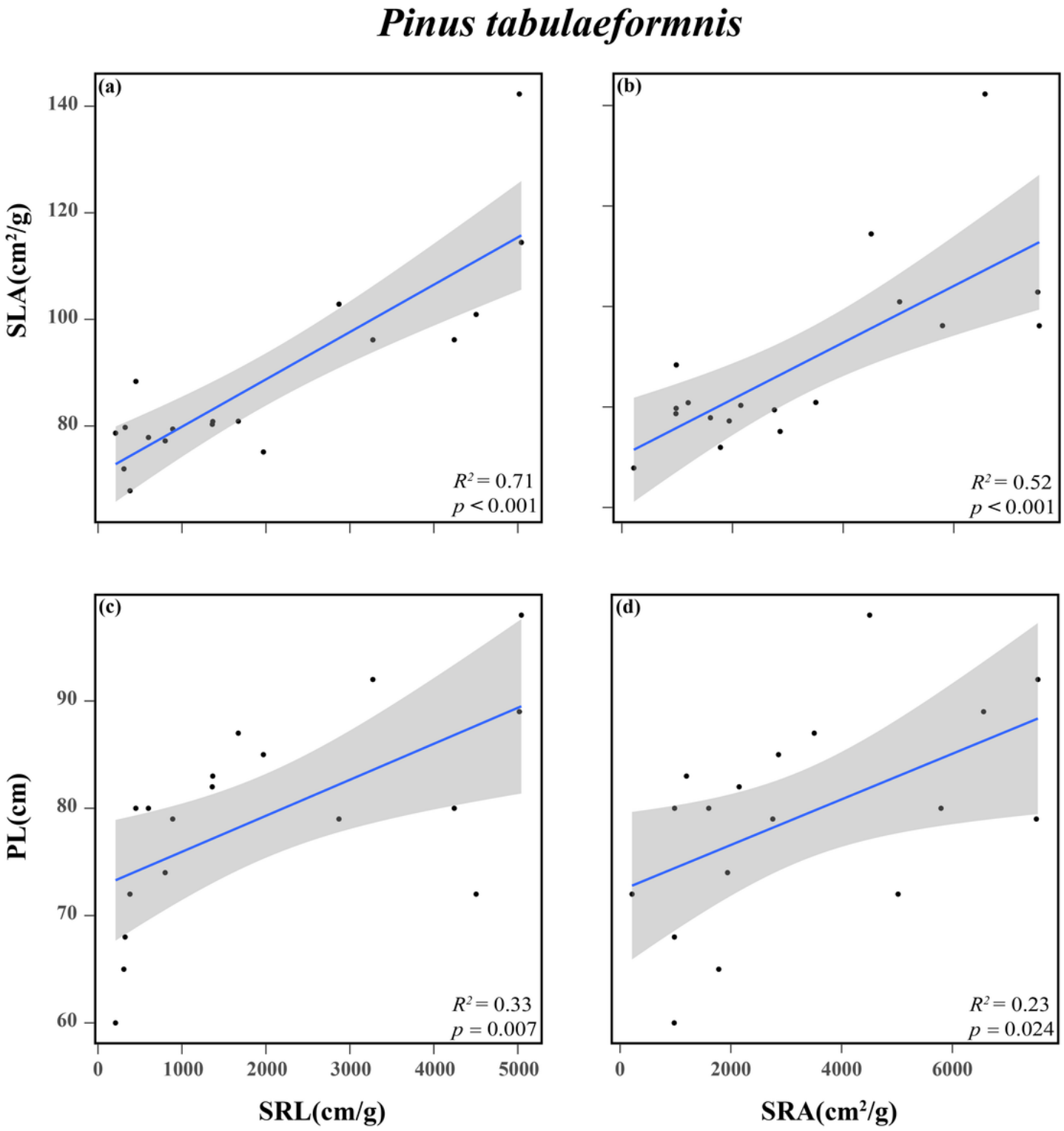

Figure 5

Relationships between SLA and SRL (a), SLA and SRA (b), PL and SRL (c), PL and SRA (d) of *Pinus tabuliformis*. Trait acronyms: SLA = specific leaf area, PL = plant height, SRL = specific root length, SRA = specific root area. The R^2 (coefficient of determination), and *p*-values are obtained from the linear regression analyses. Shaded areas show a 95% confidence interval of the fit test.

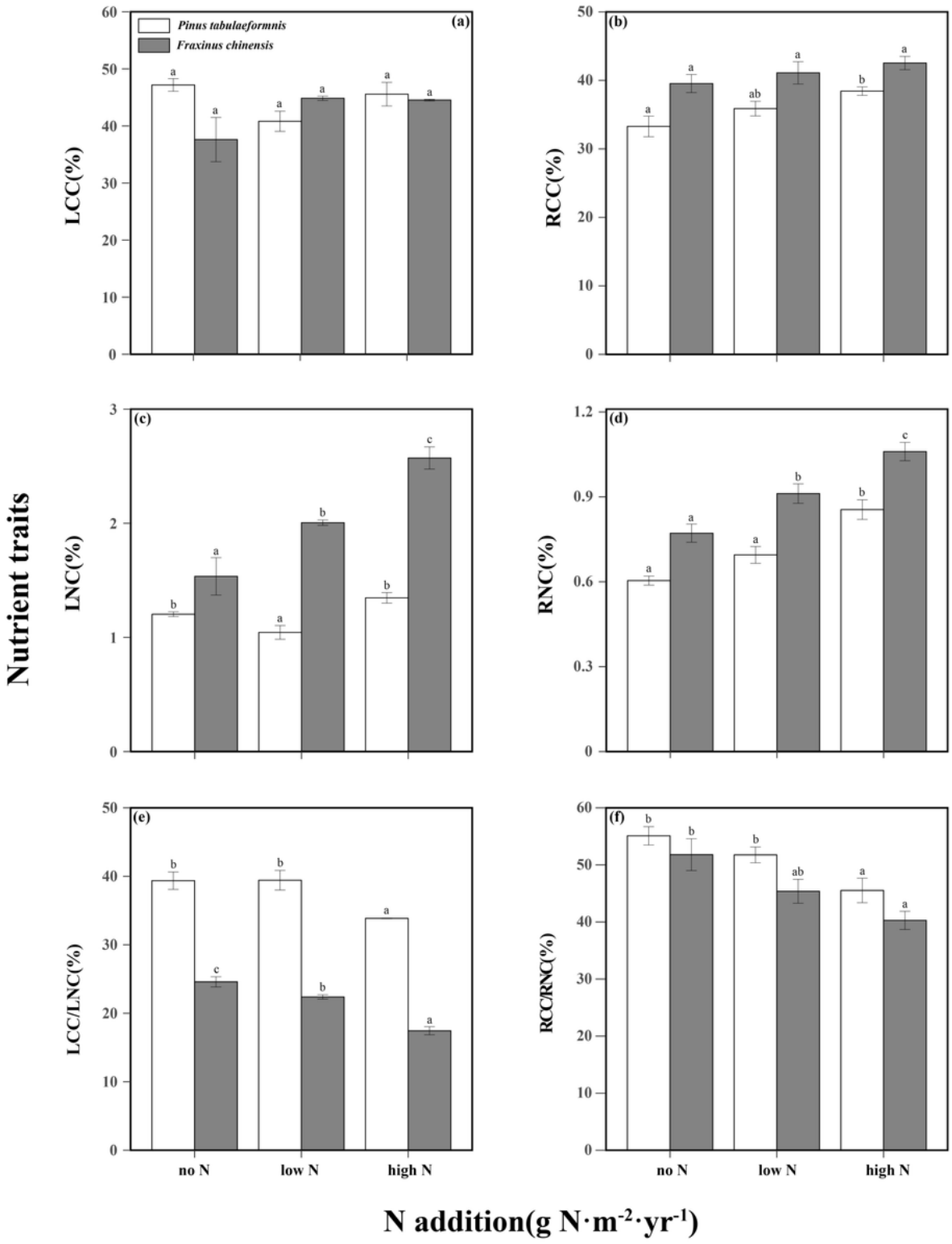

Figure 6

Nutrient traits including leaf carbon content (LCC), leaf nitrogen content (LNC), root carbon content (RCC), root nitrogen content (RNC), LCC/LNC, and RCC/RNC of *Pinus tabuliformis* (a, c, e) and *Fraxinus chinensis* (b, d, f) under different N addition levels. Bars and error bars show means ± SE. Different lowercase letters indicate significant differences among N treatments at the same time at a significance level of *p*-value < 0.05.

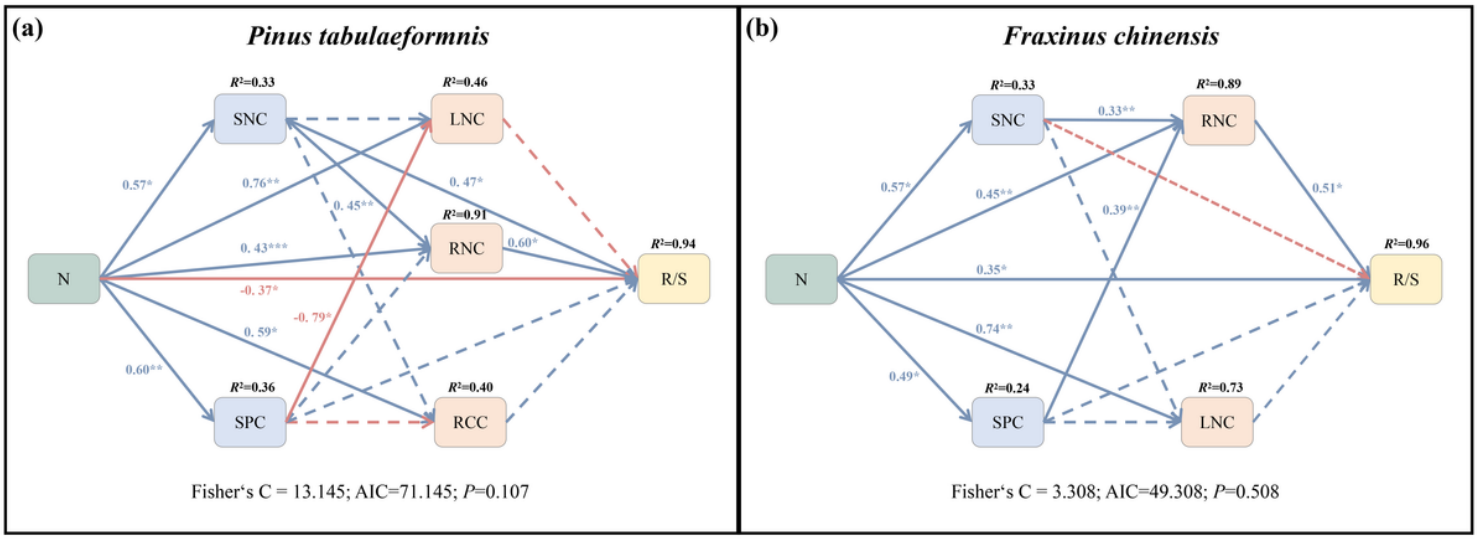

Figure 7

Structural equation model (SEM) showing the direct and indirect effects of N addition on root-shoot ratio (R/S) and soil N, P content (SNC, SPC) and root C, N content (RCC, RNC) and leaf N content (LNC) of *Pinus tabuliformis* (a) and *Fraxinus chinensis* (b). The single-headed arrows represent paths in this conceptual model. The blue and red arrows separately indicate positive and negative pathways. The solid and dotted lines separately indicate significant ($p < 0.05$) and insignificant pathways. Numbers at arrows are standardized path coefficients and the proportion of variance explained (R^2) appears at the top of response variable in the model. *, ** and *** indicate statistically significant paths at $0.01 < p < 0.05$, $0.001 < p < 0.01$ and $p < 0.001$, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AppendixA.Supplementarydata.docx