Nitrogen Addition Did Not Alter the Relationships Between the Leaf and Root Traits of Machilus Pauhoi seedlings

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Research

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Abstract

Leaves and roots are important resource acquisition organs of seedlings and both are sensitive to the environment. However, it is currently unclear whether leaf and root traits have a similar response model to nitrogen (N) deposition. Furthermore, the relationships between the responses of leaf and root traits to N deposition are still unknown.

Exogenous nitrogen input experiments were conducted to simulate the effects of nitrogen deposition in Shunchang County, south of China. We measured the biomass, morphological characteristics, and nutrient concentrations (total of 12 functional traits of leaves and roots) of *Machilus pauhoi* seedlings. The responses of leaf and root traits to N addition were analyzed. In addition, the relationships between paired leaf and root traits were analyzed.

We found that the responses of the leaves and roots to short-term nitrogen deposition were not consistent. The specific leaf area (SLA) (specific root length, SRL), tissue density (TD), carbon (C) content, N content, C/N, and N/phosphorus (P) of the leaf and root did not appear to respond to N addition. However, the biomass, P content, and C/P of the leaf and root markedly responded to N addition. The nutrient concentrations of the leaf and root were correlated, while the phenotypic traits were not. Furthermore, short-term N addition did not alter the relationship between the leaves and roots.

Our results show that, in the context of global change of nitrogen deposition, the correlation between the leaves and roots of a plant has a certain tolerance for nitrogen deposition, which is of great significance for the efficient cultivation of quality seedlings and understanding how terrestrial forest ecosystems respond to nitrogen deposition.

Introduction

Leaves and roots are important resource acquisition organs of seedlings. Leaves produce carbohydrates by photosynthesizing (Niinemets et al. 2010), while the root's main role is to absorb nutrients (Laliberte 2017). Both of them are plastic to environmental change and can, therefore, be used as indicators (Kou et al. 2018). Previous studies have been conducted on leaves and roots, such as leaf nutrients (Mayor et al. 2014), leaf microbial diversity (Laforest-Lapointe et al. 2017), root morphology (Zhou et al. 2019), root functional traits (Ma et al. 2018), and root decomposition (See et al. 2019). Furthermore, the theories of leaf (Osnas et al. 2013; Wright et al. 2004) and root economic spectra (Prieto et al. 2015; Roumet et al. 2016) have gained interest in recent years. As the vital components of the plant, a strategy was established between the leaves and roots, where the water and nutrients required for leaf photosynthesis are absorbed by the root and the carbohydrates that sustain root turnover are produced by the leaf (Chapin III 1980; Fortunel et al. 2012). While the monitoring of roots is inconvenient, the observations of the above-ground organs may be extrapolated to the below-ground organs (Fort et al. 2012; Holdaway et al. 2011; Liu et al. 2010). A significant correlation was found between pairs of analogous leaf and root traits, such as the specific leaf area (SLA) and specific root length (SRL), leaf Nmass (leaf nitrogen
content per unit mass) and root Nmass (root nitrogen content per unit mass), and leaf Narea (leaf nitrogen content per unit area) and root Nlength (leaf nitrogen content per unit area) (Holdaway et al. 2011; Liu et al. 2010; Reich 2014). While a relationship between the tissue density of the root and stem exists, this is not the case for the leaf (Fortunel et al. 2012). However, there are still few studies on the paired relationship between plant leaves and roots. Therefore, further research is required concerning the relationships between traits (Holdaway et al. 2011).

Nitrogen (N) deposition is an important part of the global nitrogen cycle (Stevens 2019). Due to anthropogenic influences, the amount of nitrogen entering a terrestrial ecosystem gradually increases (Liu et al. 2011). This is especially true for China's subtropical region in recent years, which has become an area that is severely affected by nitrogen deposition, with a deposition rate higher than 50 kg N ha\(^{-1}\) yr\(^{-1}\) (Zhu et al. 2015). Nitrogen is an essential nutrient that regulates plant growth and development (Vitousek 2004). An excess of nitrogen in an ecosystem will inevitably affect plant growth (Li et al. 2015; Oldroyd and Leyser 2020; Schleuss et al. 2020). However, current studies mainly focus on certain plant organs, such as the leaf (Laforest-Lapointe et al. 2017; Mayor et al. 2014; Osnas et al. 2013) or roots (Laliberte 2017; Ma et al. 2018; Zhou et al. 2019). Although some studies have reported on the effects of nitrogen deposition on plant leaves and roots (Lu et al. 2018; Talhelm et al. 2017; Xia et al. 2018), there is still a lack of research on whether nitrogen deposition alters the correlation between these organs. Therefore, it is necessary to acquire more information concerning the variation in leaf and root trait relationships in the context of global N deposition.

*Machilus pauhoi*, the dominant forest species in the south of China, is a rare evergreen broad-leaved species with rapid growth in subtropical areas. So far, studies have been carried out concerning the biomass (Zhong et al. 2001), roots (Wang et al. 2018b; Zou et al. 2018), leaves (Li et al. 2016a; Wu et al. 2016; Yu et al. 2019), and different provenances (Yu et al. 2018) of *M. pauhoi*. However, in the context of a global increase in nitrogen deposition, whether the leaves and roots of *M. pauhoi* seedlings express the same response model and the relationship between the two is not understood. We conducted a nitrogen deposition simulation experiment to investigate the response of *M. pauhoi* seedlings to N deposition. We hypothesized that: 1) The leaves and roots have different response strategies for the addition of exogenous nitrogen; and 2) Plant leaf and root traits may differ after N addition and the correlation between the leaf and root will change. Our research will provide important guidance for the efficient cultivation of quality seedlings in the context of nitrogen deposition.

**Materials And Methods**

**Site Description and Experimental Design**

This study was conducted in Shunchang County (117°48′E, 26°48′N), Nanping City, Fujian Province, south of China. The area is characterized by a subtropical monsoon climate with a mean annual temperature of 18.9 °C. The annual rainfall ranges from 1600 to 1900 mm, with most rainfall occurring from February to September. For the exogenous nitrogen input experiment, two-year-old *M. pauhoi* seedlings were selected.
The N treatment received an input of 100 kg·hm$^{-2}$·a$^{-1}$, which was double the local N deposition rate of 50 kg·hm$^{-2}$·a$^{-1}$. In addition, a control treatment was established for comparison (CT, 0 kg·hm$^{-2}$·a$^{-1}$). Each treatment was completed in triplicate for statistical significance.

The exogenous nitrogen input experiment was conducted in a 70% shade frame at the Forestry Technology Center of Shunchang County. Polyvinyl chloride pots (inner diameter of 30 cm and height of 30 cm) containing red soil from Shunchang County were used for seedling transplants. Two-year-old seedlings, which were planted in July 2014 and cultivated until the experiment began, were transplanted on March 1st, 2016. To allow for acclimatization, N addition commenced after two months and was conducted from May to November. Ammonium nitrate ($\text{NH}_4\text{NO}_3$) was used for the addition of N, and the soil surface area was calculated based on the inner diameter of the pot. Then, the amount of N added per pot was calculated using the soil surface area ($N_{100}$ treatment: 2.02 g of $\text{NH}_4\text{NO}_3$). $\text{NH}_4\text{NO}_3$ was dissolved in 10 ml of distilled water and uniformly applied with a pipette to the treatment and control pots. Each treatment had 16 seedlings and three replicates, totaling 96 $M. \text{pauhoi}$ seedlings.

Leaf/Root Sampling and Chemical Analysis

At the end of the experiment, the seedlings from each treatment were harvested. The leaves, stems, and roots were collected separately. Root samples were cleaned using distilled water, and then scanned with an Epson V370 scanner. Each organ from one seedling was one sample. All 288 samples were transported to the laboratory as soon as possible.

A Vernier caliper (0.01 mm) was used to measure the thickness of the leaves (top, middle, and bottom; avoiding important veins), and the average of these values was used as the leaf thickness (LT; mm). The leaf area (LA) was measured using a LI-3000C portable leaf-area meter (Li-Cor, Lincoln, NE, USA). The saturated weight (Lsmass) was determined after submerging a leaf in deionized water for 24 h in the dark. The SLA and leaf tissue density (TD$_L$) were calculated for selected leaf samples. Root samples were analyzed using WinRHIZO root-scanning software (Regent Instruments Inc., Ottawa, ON Canada). The SRL and root tissue density (TD$_R$) were calculated for selected root samples.

The dry weights of the leaf and root (Lmass and Rmass, respectively) samples were determined after oven-drying at 65 °C until a constant weight was obtained. For both the N and P analyses, the dried samples were ground into a fine powder using a sample grinder and screened with a 1-mm sieve. The N content was determined using a CHNS/O Elemental Analyzer (Vario EL III, Elementar, Langenselbold, Germany). The P content was determined using a Continuous Flow Analytical System (SAN ++, Skalar, Breda, Holland) after digestion and boiling in a solution of H$_2$SO$_4$-HC$_2$O$_4$. In addition, we investigated the total nutrient content of the leaves and roots (total leaf carbon content (TC$_L$), total leaf nitrogen content (TN$_L$), total leaf phosphorus content (TP$_L$), total root carbon content (TC$_R$), total root nitrogen content (TN$_R$), and total root phosphorus content (TP$_R$)). The formulae used for these calculations can be found in additional materials S1.
**Statistical Analysis**

A comparison between the different treatments was completed using an independent sample *t*-test. We calculated the relative effects (RE) of N addition \([(\text{means from N addition plots} - \text{means from ambient plots})/(\text{means from ambient plots})\times100]\) on the leaf and root traits of *M. pauhoi* seedlings (Duval et al. 2011). The independent sample *t*-test was also performed to determine the differences between the relative effects of the leaf and root.

The relationships between all leaf and root functional traits were best fit by the mathematical equation \(\log(y) = \log(\beta) + \alpha \log(x)\), where \(\beta\) is the normalization constant and \(\alpha\) is the scaling exponent. Model Type II regression was used to determine the numerical values of \(\beta\) and \(\alpha\) using the (Standardized) Major Axis Estimation package ‘smatr’ version 3.4-3 in R software (R Core Development Team 2014; Taskinen 2012). Prior to the analysis, all data were log\(_{10}\) transformed to fit a normal distribution pattern. All statistical analyses were performed in R (version 3.1.0) using the packages “ggplot2 (Wickham 2016)” and “ggpuber (Kassambara 2020)”. OriginPro 2020 software (Origin Lab Corp., Northampton, MA, USA) was used to create the figures.

**Results**

**Responses of the Leaf and Root Traits to Nitrogen Addition**

The different leaf and root traits have different responses to nitrogen addition (Table 2). On the one hand, the SLA (SRL), TD, C, N, carbon/nitrogen (C/N), and nitrogen/phosphorus (N/P) of the leaf and root did not appear to respond to N addition. On the other hand, the biomass, P, and carbon/phosphorus (C/P) of the leaf and root markedly responded to N addition (Table 2). Overall, except for the TP\(_R\), the total nutrient traits were significantly altered by the N treatment (Table 2).

Notably, despite the biomass of both organs increasing after nitrogen application, their proportions in the whole seedling varied (S2a). Overall, the proportion of leaves increased, the proportion of roots decreased, and the proportion of the biomass of these two organs showed a significant negative correlation (S2b).

**RE of the Leaf and Root Traits**

The response of the leaves and roots exhibited the same patterns for the following traits: 1) both the \(P_L\) and \(P_R\), and \(TD_L\) and \(TD_R\) were induced by N addition (Fig. 1); and 2) the C/P, TC, TN, and biomass increased significantly after N addition. Furthermore, we found that the responses of the following root and leaf traits differed: 1) after N addition, the SLA decreased, while the SRL increased, and the RE *t*-test results of the two were significant; and 2) the N, C, and C/N had different responses, even though their *t*-test results were not significant.
Standardized major axis (SMA) of the Leaf and Root Traits

The majority of the paired leaf and root traits exhibited a linear positive correlation, except for the C\textsubscript{R} and C\textsubscript{L}, SRL and SLA, NP\textsubscript{R} and NP\textsubscript{L}, and TD\textsubscript{R} and TD\textsubscript{L} (Fig. 2, Table 3). Notably, the relationships between the Bio\textsubscript{R} and Bio\textsubscript{L}, TN\textsubscript{R} and TN\textsubscript{L}, and TC\textsubscript{R} and TC\textsubscript{L} were not altered after the addition N, due to their common slope and intercept (Fig. 2, Table 3).

Specifically, the common slopes were 0.99 (95% CI = 0.89 – 1.23, P = 0.67) for N\textsubscript{R} and N\textsubscript{L}, 1.01 (95% CI = 0.92 – 1.27, P = 0.85) for C/N\textsubscript{R} and C/N\textsubscript{L}, and 1.03 (95% CI = 0.85 – 1.18, P = 0.06) for TP\textsubscript{R} and TP\textsubscript{L}.

Across the two treatments, the scaling exponents of N\textsubscript{R} and N\textsubscript{L}, C/N\textsubscript{R} and C/N\textsubscript{L}, and TP\textsubscript{R} and TP\textsubscript{L} were 1.02, 1.01, and 0.99, respectively (Table 3), each of which were statistically indistinguishable from 1.0 (all P\textsubscript{1.0} > 0.05) (Table 3).

Responses of the Leaf and Root Traits to Nitrogen Addition

The different leaf and root traits have different responses to nitrogen addition (Table 2). On the one hand, the SLA (SRL), TD, C, N, carbon/nitrogen (C/N), and nitrogen/phosphorus (N/P) of the leaf and root did not appear to respond to N addition. On the other hand, the biomass, P, and carbon/phosphorus (C/P) of the leaf and root markedly responded to N addition (Table 2). Overall, except for the TP\textsubscript{R}, the total nutrient traits were significantly altered by the N treatment (Table 2).

Notably, despite the biomass of both organs increasing after nitrogen application, their proportions in the whole seedling varied (S2a). Overall, the proportion of leaves increased, the proportion of roots decreased, and the proportion of the biomass of these two organs showed a significant negative correlation (S2b).

RE of the Leaf and Root Traits

The response of the leaves and roots exhibited the same patterns for the following traits: 1) both the P\textsubscript{L} and P\textsubscript{R}, and TD\textsubscript{L} and TD\textsubscript{R} were induced by N addition (Fig. 1); and 2) the C/P, TC, TN, and biomass increased significantly after N addition. Furthermore, we found that the responses of the following root and leaf traits differed: 1) after N addition, the SLA decreased, while the SRL increased, and the RE t-test results of the two were significant; and 2) the N, C, and C/N had different responses, even though their t-test results were not significant.

Standardized major axis (SMA) of the Leaf and Root Traits

The majority of the paired leaf and root traits exhibited a linear positive correlation, except for the C\textsubscript{R} and C\textsubscript{L}, SRL and SLA, NP\textsubscript{R} and NP\textsubscript{L}, and TD\textsubscript{R} and TD\textsubscript{L} (Fig. 2, Table 3). Notably, the relationships between the
Bio$_R$ and Bio$_L$, TN$_R$ and TN$_L$, and TC$_R$ and TC$_L$ were not altered after the addition N, due to their common slope and intercept (Fig. 2, Table 3).

Specifically, the common slopes were 0.99 (95% CI = 0.89 – 1.23, P = 0.67) for N$_R$ and N$_L$. 1.01 (95% CI = 0.92 – 1.27, P = 0.85) for C/N$_R$ and C/N$_L$, and 1.03 (95% CI = 0.85 – 1.18, P = 0.06) for TP$_R$ and TP$_L$. Across the two treatments, the scaling exponents of N$_R$ and N$_L$, C/N$_R$ and C/N$_L$, and TP$_R$ and TP$_L$ were 1.02, 1.01, and 0.99, respectively (Table 3), each of which were statistically indistinguishable from 1.0 (all $P_{1.0} > 0.05$) (Table 3).

Discussion

Leaf and Root Responses of Seedlings to N Addition

Nitrogen is one of the main limiting elements that plants need to absorb from soil to mature (Güsewell 2004). Plants optimize the uptake of nitrogen and phosphorus by changing their functional traits and interacting with microorganisms to facilitate nutrient capture (Oldroyd and Leyser 2020). Nitrogen addition improves the soil available N content, alters the processes of microbe nitrogen conversion (mineralization, nitrification, and denitrification), and affects the N absorption of plants (Matson et al. 1999). The effect of N on the plant P content is influenced by many factors. Plant P uptake may benefit from a higher N availability because plants might use N for up-regulating P uptake transport systems (Zeng et al. 2012). Similarly, elevated N and P inputs may alter root traits in a way that promotes plant nutrient uptake (Schleuss et al. 2020). Our first hypothesis, concerning the similarities between the responses of leaves and roots to exogenous nitrogen addition, was partially supported. According to the results, the N$_L$, P$_L$, and P$_R$ of *M. pauhoi* seedlings decreased after N addition, which may have resulted from the “dilution effect” (Jarrell and Beverly 1981) because the leaf and root dry mass significantly increased. On the other hand, N addition caused soil acidification and bound the phosphorus with a metal ion to reduce the available P in the soil, which affected the plants owing to the decrease in the amount of P that could be absorbed (Bünemann et al. 2010; Kou et al. 2018). The decrease in the soil available P may have reduced the leaf P content, which, on account of the P availability, was insufficient to balance the increasing P requirements under N addition (Huang et al. 2016; Jonard et al. 2015; Li et al. 2016b). The mycorrhizal activity may have been inhibited by the N addition (Nilsson and Wallander 2003), even though nitrogen enrichment has been known to increase the phosphatase activity and soil P availability (Deng et al. 2017; Olander and Vitousek 2000). Consequently, the availability of the phosphorous required for the seedlings would be limited (Wallander et al. 2001). One exception is that the root N concentrations increase with increasing N availability (Hendricks et al. 2000). However, in this study, the N$_L$ and N$_R$ did not significantly respond to N addition. When nutrient concentrations are limited, the homeostasis of the leaf may be higher than that of the other plant organs (Jiang et al. 2014; Wang et al. 2018a; Wang et al. 2019). Roots absorb water and nutrients from the soil, which are transported to the leaf, potentially resulting in a lower homeostasis (Jin et al. 2017; Paez-Garcia et al. 2015; Wang et al. 2018a). This may partially explain why the N$_R$ significantly responds to N addition, while the N$_L$ does not.
The responses of phenotypic traits to N addition were various. N addition significantly suppressed the SLA, which is consistent with numerous previous findings (Peng et al. 2018; Xiao et al. 2016; Zhang et al. 2006). However, some other studies have found that N addition promoted (Valliere et al. 2017; Wang et al. 2016; Wu et al. 2008) or did not affect (Zhang et al. 2018) the SLA. The SLA (reciprocal of leaf mass area (LMA)) represents the light resources captured on the LA per unit of leaf dry matter, which is closely related to the light interception efficiency of plants (Reich 2014). A decrease in the SLA may represent a deterioration in the environmental conditions, as plants begin to adopt conservative growth strategies (Rose et al. 2013). Li et al. (2015) constructed a multivariate analysis of the effects of nitrogen deposition on fine-root traits and found that N deposition had no visible effects on the root morphology, but significantly increased the total root biomass. Similar results were obtained in this study, with no visible change in the SRL or TD\textsubscript{R} after N addition, while the Bio\textsubscript{R} increased markedly. Other studies found similar results (Davidson et al. 2004; Högborg 2007; Zhang et al. 2006). This may illustrate that roots do not enhance the exchange rate of resources in the plant-soil interface by changing the root surface or length (Eissenstat 1992; Li et al. 2015). Furthermore, N addition may simulate carbon accumulation in the root biomass (Li et al. 2015). After nitrogen addition, the increase in the leaf biomass was greater than that of the root biomass and the plant growth strategy tended to allocate more nutrients to the above-ground parts. This helps to predict how plants distribute C between the above- and below-ground biomass in response to global change of nitrogen deposition.

At present, most studies on plant nutrient contents are based on unit mass, but lack the overall nutrient contents of the above-ground or below-ground parts, which may be why it is not conducive to measure the below-ground parts in the field. In this paper, the total amount of nutrients in the leaves and roots of seedlings increased obviously (except for the TP\textsubscript{R}) after N addition. This means that the exogenous nitrogen addition facilitated the absorption of nutrients by seedlings. However, due to the complex nutrient uptake, utilization, and exchange mechanisms of plants and microorganisms, the effects of N addition on plant organs are still unclear and further research is required.

Paired Relationships of the Leaf and Root

Leaf traits are widely used to describe plant growth and resource use strategies because they are easy to measure. However, roots play an important role for plant organs, as they are located underground and form the interface between the plants and soil (Hajek et al. 2013). Both the leaf and root are important organs for plants to obtain resources, and the strategy of observing above-ground organs can be extrapolated to below-ground organs (Fort et al. 2012; Holdaway et al. 2011; Kembel et al. 2008; Liu et al. 2010).

Previous studies have found that the relationship between the N and P contents in plant organs can be expressed by an allometric relationship, and its slope is significantly smaller than 1 (Elser et al. 2010; Reich et al. 2010; Zhao et al. 2016). However, there are few studies on the exponential relationships between the same traits in different organs.
Our results found that the phenotypic traits of the leaves and roots may be independent of each other. The SLA and SRL, which represent the most important plant resource acquisition strategies, are irrelevant in this article. The lack of a relationship between the SLA and SRL is consistent with the results of Tjoelker et al. (2005) and Hajek et al. (2013), but differs from other studies that found a positive relationship (Liu et al. 2010; Withington et al. 2006). Tissue structure is an inherent constraint that prevents the simultaneous maximization of nutrient acquisition and conservation (Ryser 1996). No link between the $TD_L$ and $TD_R$ has been found, which may indicate that the leaves and roots of plants have different nutrient and growth strategies.

The relationship between the leaves and roots was not altered after N addition. Leaves and roots coordinate due to nutrient, water, and carbohydrate exchange (Chapin III 1980; Fortunel et al. 2012). For the nutrient traits, the positive correlations between the N (Freschet et al. 2010; Liu et al. 2010; Tjoelker et al. 2005) and P (Holdaway et al. 2011; Kerkhoff et al. 2006) contents of the leaves and roots have been previously reported. The significant positive relationship between the N concentrations of the leaves and roots was also present in our research, which differs from the findings of Hajek et al. (2013), but is consistent with Liu's research in Chinese semi-arid and arid ecosystems (Liu et al. 2010). The addition of N decreased the intercept for the $N_L$ and $N_R$ but increased the intercept for the $C/N_L$ and $C/N_R$. This may be because the seedlings tended to allocate more N to the roots, which would result in a higher $C/N_R$.

Plants and microbes may invest an element that is in excess into the acquisition of a limiting element until their growth is equally limited by both elements (Bloom et al. 1985). In this paper, the relationship between the $P_L$ and $P_R$ was not altered by the N addition, even though the P contents in the leaves and roots were significantly affected. This indicated that the P content in the plant leaves and roots was more stable than the N content. The addition of nitrogen led to the rapid growth of plants, but the unit mass P content did not visibly change. Interestingly, differing from the common slope and intercept of the $P_L$ and $P_R$, the $TP_L$ and $TP_R$ had different intercepts. In addition, the intercepts of the $TP_L$ and $TP_R$ were highest after fertilization, which indicated that, overall, the plants tended to allocate more P to the roots after nitrogen application. In general, plants tend to allocate more P and C to roots after the addition of nitrogen. Although the addition of nitrogen did not change the above-/below-ground distribution of N, we can still deduce that the N:P of the above-ground part of the plant was higher than that of the below-ground part. This proves that an increase in the P contents of the roots reduces the N:P, providing that the overall N contents is not altered.

Conclusion

The effects of N addition on *M. pauhoi* seedlings were tested. After investigating the leaf and root traits and their relationships, we found that N addition had no significant effects on the correlations between leaf and root traits. Further research into other species is required to clarify the relationship between the leaf and root for understanding ecosystem functions and services. Our research provides an important reference for the efficient cultivation of quality seedlings in the context of nitrogen deposition.
Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from Mr. Yuxing Zou (looe_x@126.com) on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author Contributions

Conceptualization, Y.-X.Z. and Q.-L.Z.; methodology, Y.-X.Z. and B.-Y.L.; software, Y.-X.Z. and X.-P.C.; validation, Y.-X.Z. and H.Y.; formal analysis, Y.-X.Z.; investigation, X.-P.C., X.-Y.D. and H.Y.; resources, Y.-X.Z.; data curation, Y.-X.Z.; writing—original draft preparation, Y.-X.Z.; writing—review and editing, Y.-X.Z., Q.-L.Z. and D.-L.C.; supervision, B.-Y.L.; funding acquisition, Q.-L.Z., H.Y. and D.-L.C.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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### Tables

**Table 1.** Information concerning the seedlings and soil traits (Avg ± SE)
| Seedling Trait | Soil Trait |
|---------------|------------|
| TH (cm)       | GD (cm)    | STN (mg/g) | STC (mg/g) | STP (mg/g) | pH  |
| 61.3±15.76    | 5.58±1.35  | 0.716±0.01  | 4.629±0.05 | 1.142±0.07 | 4.97±0.18 |

Note: TH: tree height; GD: ground diameter; STN: soil total nitrogen content; STC: soil total carbon content; and STP: soil total phosphorus content

Table 2. Response of the leaf and root traits to nitrogen addition (Avg ± SE, n = 96)

| Trait        | Leaf | Root |
|--------------|------|------|
| SLA(SRL)     |      |      |
| 137.5±2.1    |      |      |
| 4             |      |      |
| 2             |      |      |
| 0.006*       |      |      |
| TD           |      |      |
| 0.04±0       |      |      |
| 0.88         |      |      |
| 5.98±0.15    |      |      |
| 5.68±0.17    |      |      |
| 0.194        |      |      |
| Bio          |      |      |
| 4.75±0.4     |      |      |
| 0.04±0       |      |      |
| 0.88         |      |      |
| 8.48±0.42    |      |      |
| 11.89±0.54   |      |      |
| 0.006**      |      |      |
| C            |      |      |
| 492.6±1.3    |      |      |
| 5            |      |      |
| 0.006**      |      |      |
| 443.17±4.1   |      |      |
| 445.85±4.8   |      |      |
| 0.676        |      |      |
| N            |      |      |
| 15.51±0.44   |      |      |
| 14.97±0.36   |      |      |
| 0.343        |      |      |
| 10.62±0.3    |      |      |
| 11.35±0.27   |      |      |
| 0.071        |      |      |
| P            |      |      |
| 1.19±0.04    |      |      |
| 1.07±0.03    |      |      |
| 0.016*       |      |      |
| 2.34±0.12    |      |      |
| 1.69±0.09    |      |      |
| 0.676        |      |      |
| C/N          |      |      |
| 33.18±1.12   |      |      |
| 34.57±0.96   |      |      |
| 0.348        |      |      |
| 42.71±1.21   |      |      |
| 39.78±1.01   |      |      |
| 0.066        |      |      |
| N/P          |      |      |
| 13.16±0.4    |      |      |
| 14±0.29      |      |      |
| 0.093        |      |      |
| 5.02±0.27    |      |      |
| 7.48±0.35    |      |      |
| 0.006**      |      |      |
| C/P          |      |      |
| 429.7±16.4   |      |      |
| 497.5±17.4   |      |      |
| 0.006**      |      |      |
| 216.74±12.45 |      |      |
| 302.98±16.82 |      |      |
| 0.006**      |      |      |
| TC           |      |      |
| 2343.14±19   |      |      |
| 3781.48±22   |      |      |
| 8.63         |      |      |
| 6.79         |      |      |
| 0.006**      |      |      |
| 3723.6±173.51|      |      |
| 5281.12±24   |      |      |
| 3.99         |      |      |
| TN           |      |      |
| 74.72±7.13   |      |      |
| 110.9±7.0    |      |      |
| 0.006**      |      |      |
| 90.22±5.62   |      |      |
| 136.36±6.2   |      |      |
| 0.006**      |      |      |
| TP           |      |      |
| 5.76±0.61    |      |      |
| 7.92±0.54    |      |      |
| 0.01**       |      |      |
| 19.83±1.56   |      |      |
| 21.02±1.62   |      |      |
| 0.597        |      |      |

Note: "*" and "**" indicate a significant correlation at < 0.05 and 0.01, respectively. The RP and RNP were not analyzed. The abbreviations of the indicators are shown in table 2.

Table 3 Summary of the regression parameters (slopes and y-intercepts, α and log β, respectively) for the relationships between paired leaf and root traits in the two treatments (n = 96)
### Supplementary Information

The following additional information is available in the online version of this article:

**Table S1** Computational formula for each trait

Note: LA: leaf area; Lmass: leaf mass; Lfmass: leaf fresh mass; Lsmass: leaf saturated mass; LT: leaf thickness; RL: root length; Rmass: root mass; Rvolume: root volume; and Rarea: root area

**Figure S2** Proportions of the leaf and root biomass and their correlation

Note: A) Proportion of leaf and root biomass in the total seedling biomass under different treatments. The inner and outer rings are the control and N addition treatments, respectively. B) The relationship between the proportions of leaf and root biomass. Effects of N addition on the scaling relationship between BioR/Bio_total (BioR/T) and BioL/Bio_total proportion (BioL/T) in *M. pauhoi* seedlings. The lines are significant standardized major axis (SMA) regressions (P < 0.05). The scaling slopes of BioR/T and BioL/T
in *M. pauhoi* seedlings under different N treatments did not significantly differ, with a common slope of -1.2 (95% CI = -1.4 - -1.0, *P* = 0.85) and common constant of -1.12 (95% CI = -1.12 - -1.14, *P* = 0.36).

**Figures**

**Figure 1**

Standardized major axis (SMA) regression of leaf and root paired-trait correlation under N addition. Note: (A) The relationship between the leaf and root nitrogen content, (B) The relationship between the leaf and root total nitrogen content, (C) The relationship between the leaf and root biomass, (D) The relationship between the leaf and root phosphorus content, (E) The relationship between the leaf and root total phosphorus content, (F) The relationship between the leaf and root total nitrogen/total phosphorus, (G) The relationship between the leaf and root carbon/nitrogen, (H) The relationship between the leaf and root total carbon/total nitrogen, and (I) The relationship between the leaf and root total carbon content. The insignificant correlation is shown in Table 3. The abbreviations of the indicators are shown in S1.
Figure 2

Relative effects [(means from N addition plots – means from ambient plots)/(means from ambient plots)×100] of nitrogen addition on the leaf and root traits of seedlings Note: “*” indicates that the relative effects of nitrogen addition on the leaf and root are significantly different. The abbreviations of the indicators are shown in S1.

Supplementary Files

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- APPENDIXS2.tif
- APPENDIXS1.xlsx