Fine root turnover and longevity and their responses to nitrogen fertilization of nitrogen-fixing and non-nitrogen-fixing tree species

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Abstract

Background and aims

Fine root longevity and turnover respond to changes in soil nitrogen (N) determine plants’ performance at elevated N-level under global N-deposition. This study was conducted to understand the fine root dynamics of N-fixing and non-N-fixing tree species at elevated N-level by N fertilization.

Methods

To determine fine root dynamics (biomass, length density, longevity, and turnover rate) under N-fertilization, a three-year fertilization experiment at 150 g CO(NH₂)₂ m⁻² year⁻¹ was conducted for two N-fixing species and two non-N-fixing tree species.

Results

Fine root biomass of the two N-fixing tree species was lower than that of non-N-fixing tree species. N-fertilization decreased root length density, root surface area, and root volume of all tree species. The fine root turnover rate of N-fixing trees was higher but root longevity was lower than that of non-N-fixing species. For all four species, N fertilization decreased fine root turnover rate but increased fine root longevity. Moreover, both the root turnover rate and root longevity of the two N-fixing tree species were more sensitive than the two non-N-fixing tree species to N fertilization. The proportion of long-lived roots of non-N-fixing tree species was higher than that of N-fixing tree species.

Conclusion

Fine roots of N-fixing tree species have shorter longevity and faster turnover rate than that of non-N-fixing tree species. The fine root longevity and turnover of N-fixing tree species were more sensitive to elevated N-level than that of non-N-fixing species at elevated N-level, they have different response strategies to the increase of soil N.

Introduction

Fine roots produce, die, and transfer carbon (C) and nutrients into the soil (Jackson et al. 1997; McCormack et al. 2015), which represents one of the major pathways of soil C fluxes in forests and the key processes that govern forest productivity and biogeochemical cycles (Kubisch et al. 2016). Fine root biomass (FRB), age structure, absorption efficiency, nutrient content and fine root turnover rate determined the carbon and nutrients flux from plants to soil. Despite considerable progress in recent times, the fine root longevity, functioning, and variation among the type of tree species were still know
less when compared with aboveground organs (Fuchs et al. 2020; McCormack et al. 2015; Meier et al. 2019).

Fine root turnover (FTR) and longevity were affected by soil nutrients, soil temperature, root chemical composition, root diameter, tree species and mycorrhizal functional type. (Adams and Eissenstat 2015; Kubisch et al. 2016; Leppälammi-Kujansuu et al. 2012). Tissue N (N) content has been proven an important factor affecting fine root longevity, the higher the fine root N content, the shorter the fine root longevity and the faster the turnover rate (Guo et al. 2008; Nadelhoffer 2000). For example, coarser roots or higher branching order roots have lower N contents and higher C/N ratios, which typically live longer than that of finer diameter or lower-order roots (Chen and Brassard 2012; Guo et al. 2008; Majdi 2001; Valverde-Barrantes et al. 2009; Wang et al. 2013). The response of fine root biomass and turnover to environmental change appears to be species-specific, generalization across species is difficult (Finér et al. 2011; Fuchs et al. 2020; Weemstra et al. 2016). Studying the responses of fine root system size and dynamics of different tree species to soil fertilization could help to compare different species with respect to root response strategies to global N deposition.

Fine root longevity of different tree species varies greatly. Fine root production and biomass of conifer species are greater than those of broadleaved species, root longevity of broadleaved species may be shorter than that of conifer species, while fine root turnover of conifer species is lower than that of broadleaved species in boreal forests (Yuan and Chen 2010). Research results showed that the faster-growing species tend to have shorter-lived roots than slower-growing species (McCormack et al. 2012; Ryser 1996). Many studies have shown that the relationship between root structure and function also includes the symbiosis of rhizobia of root and mycorrhizal fungi, which together play a role in the process of nutrient acquisition (Chen et al. 2016). Symbiotic N-fixation means that plants cooperate with rhizobia to form nodules, which fix N in the atmosphere for plant utilization and increase the content of N in root tissues (Batterman et al. 2013). It is a fact that the average value of N content in fine root tissues of N-fixing legumes species is higher than that of non-legumes species (Fyllas et al. 2009). Based on these research results, we speculated that the fine root longevity of N-fixing tree species should be shorter than that of non-N-fixing tree species due to the higher tissue N content, however, the research evidences are not enough to support this viewpoint now.

Fine roots are the most sensitive organs to environmental changes in forest trees. Many environmental conditions, such as soil nutrients, temperature, moisture, and atmospheric CO₂ content, will greatly affect the longevity of fine roots (Hertel and Scholing 2011; Majdi 2001; Yuan and Chen 2010). In the past 40 years, many scientists have carried out research on the fine root longevity of trees, basically revealing the general pattern of fine root longevity and turnover in different forest ecosystems, and identifying the main environmental factors affecting fine root longevity. Fine root production and turnover were sensitive to changes in soil N availability (Yan et al. 2017), however, the results were not consistent regarding the effect of soil environmental N availability on fine root longevity and turnover rate. Some studies’ results showed that fine root production and turnover increased with soil N availability (Pregitzer et al. 2002). Some studies, however, reported that increasing soil N availability decreased fine root production and
turnover (Burton et al. 2002; Noguchi et al. 2012; Pregitzer et al. 1993). These contrasting results may reflect that the fine root dynamics have different responses to N availability of different tree species (Guo and Fan 2007; Nadelhoffer 2000).

Forest intensive management has developed gradually, especially in fast-growing and high-yield timber forests, N fertilizer application has become an important practice to improve forest productivity, which will lead to the increase of soil N content in forest ecosystems (Andersson et al. 2001). In addition, some researchers predict that global atmospheric N deposition will increase 2.5 times by the end of the 21st century, which will lead to the increase of soil N content in woodland inevitably. For predicting changes in the amount of C returned to the soil from forest fine roots and the pattern of forest and global C cycling under the background of N deposition and N application, understanding how fine root turnover and longevity respond to soil N increase of different tree species were needed. The most important characteristic of N-fixation species is that roots can fix N in the atmosphere by symbiotic N-fixing bacteria, thus less reliance on soil N content. However, whether the effect of N on fine root longevity depends on root N content or soil environmental N, whether the fine root longevity and turnover of N-fixing species were less responsive to soil N increase than non-N-fixing tree species for their less dependent on soil N, are still unclear (Keel et al. 2008; Noguchi et al. 2012).

The objective of this study was to evaluate the influence of soil N content on the fine root biomass, root length density, longevity, and turnover rate of N-fixing and non-N-fixing tree species. We hypothesized that: (1) The fine root longevity of N-fixing species should be shorter than that of non-N-fixing species for their higher fine root N contents. (2) The fine root longevity and turnover of N-fixing tree species are less responsive to soil N application than that of non-N-fixing tree species because of their lower dependence on soil N condition. Aims to increase our understanding of how elevated N-level under global N-deposition influences the fine root biomass, root length density, longevity, and turnover rate, predicting fine root longevity and its contribution to C cycle of N-fixing and non-N-fixing tree species under the background of forest N-fertilization management and N-deposition.

Materials And Methods

Study site description

Two legume N-fixing (*Robinia pseudoacacia, Albizia julibrissin*) and two non-N-fixing tree species (*Pinus massoniana, Cunninghamia lanceolata*) pure plantations were located in Xiangyang City (112°1′~112°3′E, 31°59′~32°1′N), Hubei Province, China. This region has a temperate continental and monsoon-influenced climate and acidic yellow-brown soil. The mean annual precipitation was 1051.9 mm, the highest was 124.6 mm in April, and the lowest was 26.2 mm in February. The mean temperature was the highest (28°C) in July and the lowest in January (5.2°C).

Three N application plots and three control plots (15 m × 20 m for each plot) were performed for each species. Twenty-four experimental plots in total were established under similar climate and terrain
conditions. For the N application plots, urea (CO(NH$_2$)$_2$) was applied by spread evenly in the ring area 25 cm from the tree trunk in April (375 kg/ha), June (750 kg/ha), and August (375 kg/ha) of each year from 2014 to 2017 according to the growth rate of trees and seasonal changes (Tingey et al. 1997).

**Fine root biomass, soil sampling, and analysis**

Four duplicated soil core samples (8.2 cm inner diameter, 40 cm in depth) were taken randomly from each plot in February, April, July, and October each year. The samples were brought back to the laboratory and then separated root and soil immediately by passed through a 5 mm mesh sieve and manually discriminate. Part of each soil sample was naturally air-dried for pH and N determination, while the remaining was temporarily stored at 4°C for extraction. The roots were then cleaned and washed carefully using distilled water to remove the residual soil particles and debris. The fine roots (diameter < 2 mm) were selected using a vernier caliper and oven-dried at 65 °C until constant weight. The root total N content was determined using the Kjeldahl method.

Soil pH values were measured in a soil-water suspension (1:2.5 soil:water) by using an automatic acid-base titrator. Soil organic C content was determined by K$_2$Cr$_2$O$_7$ oxidation and phoroline indicator - FeSO$_4$ solution titration. Total N was determined by H$_2$SO$_4$ digestion and H$_2$SO$_4$ titration (Autochem1100&1200).

**Minirhizotron installation, image collection, and analysis**

Five minirhizotron tubes were randomly installed at an Angle of 45° with a vertical depth of 40 cm in each plot on December 2013. Wrap the pipe exposed to the ground with black tape to block light, and seal the tubes to prevent rain from entering. The minirhizotron tubes were left in place for 6 months before the first image collection to allow the root system to recover from the disturbance caused by their installation and to improve contact between the tube surfaces and soil.

Nondestructive minirhizotron root image data were collected and processed using a root scanner system (CI-600 Root Growth Monitoring System, Fa. CID, Camas, WA, USA) every month from June 2014 to July 2017. The roots visible around the minirhizotron (21.59×19.56 ≈ 0.04223 m$^2$) tubes in two soil depth layers (0–20 cm, 20–40 cm) were traced to estimate their respective length, surface area, and root volumes using CI e400 root image analysis system (CID Bio-Science, Inc. Camas, WA, USA). The total monthly root length was divided into the area covered by the image of the camera to which the roots were visible to determine the fine root length density (FRLD). The monthly FRLD data were grouped based on traditional root classification: fine roots (d < 2 mm) and coarse root (d > 2 mm).

**Fine root length density turnover rate and longevity**

The total length of new roots and the increase in the length of existing roots during each observation interval were calculated as fine root production in 3 years. Fine root turnover rates (year$^{-1}$) were estimated in two ways: (1) as the ratio of annual root length production to average live root length observed, (2) as the ratio of annual root length mortality to average live root length observed (Majdi et al.
2005). We calculated the turnover of FRP and fine root mortality (FRM) following the annual length-based method as below (Gill et al. 2002).

Fine root length density (cm/m$^2$) = $\sum RLD_{Ti} / 36$

Fine root length production turnover rate (%) = $\sum (RLD_{Ti+1} - RLD_{Ti}) / 3RLD_{T} \times 100$

Fine root length mortality turnover rate (%) = $\sum (RLD_{Ti} - RLD_{Ti+1}) / 3RLD_{T} \times 100$

$RLD_{T+1}$ represent the root length density of the next month (cm/m$^2$), $RLD_{T}$ present quantity of root length density in the present month (cm/m$^2$).

Fine root longevity was defined as the reciprocal value of fine root length production turnover rate and fine root length mortality turnover rate respectively.

**Fine root longevity distribution**

The scanning imagines were arranged according to the order of observation time, and the growth and change of fine roots were observed one by one. The length and diameter of new roots shown on the minirhizotron screen (0–20 cm, 20–40 cm depth) were recorded and monitored until they completely disappeared from 2014 to 2017 (Johnson et al. 2001). The first-time-observed white roots were recorded as new, those remaining white or changing to brownish in subsequent viewings were recorded as living, and roots when they turned black or wrinkled and later produced no new roots in subsequent viewings were defined as dead (marked gone) (Hendrick and Pregitzer 1992). The mean existence time of each fine root is the fine root longevity.

The time and number of new fine roots in different treatments of different tree species were observed and recorded, and the number and percentage of new fine roots in different months were analyzed. The survival time of new fine roots was recorded, and the number and percentage of different fine root longevity were analyzed.

**Statistical analysis**

Statistical analyses were performed with SPSS version 26 (SPSS, IBM, NY). ANOVA and post hoc Tukey’s HSD were applied to compare soil characters, fine root morphology parameters, biomass, fine root production, and turnover rates among treatments. T-test was applied to compare mean values between the species and treatments. Plotted with Origin pro10.0. In all cases, a P value < 0.05 was considered to be significant.

**Results**

**Effects of N fertilization on soil characteristics**
The results of ANOVA analysis showed that the soil pH, total N, and total C contents have significant differences between the four species. Soil pH values of two N-fixing species (*R. pseudoacacia*, *A. julibrissin*) were higher than that of two non-N-fixing tree species (*P. massoniana*, *C. lanceolata*) significantly, but the total N content of N-fixing tree species was lower than that of non-N-fixing tree species significantly (Table 1). There was no significant difference in soil total C content between N-fixing and non-N-fixing tree species. Fertilization increased the N content in the soil of *A. julibrissin* and *P. massoniana* plantations significantly and decreased the soil pH value of four tree species significantly. The contents of total N and total C in different soil layers were different significantly. The contents of total C and total N in the top layer (0–20 cm) were higher than those in the bottom layer (20–40 cm) significantly (*P* < 0.05). There was no significant difference in soil pH value among different soil layers.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soil pH values</th>
<th>Total N (%)</th>
<th>Total C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rp-C</td>
<td>5.72 ± 0.03 b</td>
<td>1.37 ± 0.04 de</td>
<td>16.82 ± 1.17 ab</td>
</tr>
<tr>
<td>Rp-F</td>
<td>4.80 ± 0.05 d</td>
<td>1.53 ± 0.08 d</td>
<td>14.93 ± 0.98 b</td>
</tr>
<tr>
<td>Aj-C</td>
<td>6.11 ± 0.04 a</td>
<td>1.20 ± 0.04 e</td>
<td>14.03 ± 0.97 bc</td>
</tr>
<tr>
<td>Aj-F</td>
<td>5.13 ± 0.05 c</td>
<td>1.78 ± 0.06 c</td>
<td>14.68 ± 1.14 b</td>
</tr>
<tr>
<td>Pm-C</td>
<td>4.91 ± 0.06 d</td>
<td>1.81 ± 0.06 b</td>
<td>14.20 ± 0.86 bc</td>
</tr>
<tr>
<td>Pm-F</td>
<td>4.64 ± 0.06 e</td>
<td>2.05 ± 0.11 a</td>
<td>11.07 ± 0.76 c</td>
</tr>
<tr>
<td>Cl-C</td>
<td>4.55 ± 0.06 e</td>
<td>2.06 ± 0.10 a</td>
<td>17.94 ± 1.94 a</td>
</tr>
<tr>
<td>Cl-F</td>
<td>4.14 ± 0.06 f</td>
<td>2.09 ± 0.12 a</td>
<td>15.72 ± 1.76 b</td>
</tr>
</tbody>
</table>

**Note:** Rp for Robinia pseudoacacia, Aj for Albizia julibrissin, Pm for Pinus massoniana, Cl for Cunninghamia lanceolata. -C for control, -F for N fertilization. The same in the below tables.

**Effects of N fertilization on fine root biomass, morphology and N content**

The fine root biomass of *C. lanceolata* was the highest, followed by *P. massoniana* and *R. pseudoacacia*, and the lowest is *A. julibrissin*. ANOVA analysis showed that the fine root biomass of N-fixing tree species was lower than that of non-N-fixing tree species significantly. Fertilization tended to decrease the fine root biomass of four species, among which, the fine root biomass of non-N-fixing tree species decreased significantly, but the fine root biomass of N-fixing tree species did not decrease significantly (Table 2).

The fine root length density, root surface area, and root volume of fine roots of four species were the highest in *A. julibrissin*, followed by *P. massoniana*, and the root length density, root surface area, and root volume of *C. lanceolata* were the lowest among four species. N fertilization decreased root length density, root surface area, and root volume of four species significantly. *R. pseudoacacia* has the highest root N content among the four species, followed by *A. julibrissin*, *P. massoniana* and *C. lanceolata*. N fertilization
increased the root N content of *R. pseudoacacia* significantly, but no significant effects on the N content in the fine roots of the other 3 species (Table 2).

<table>
<thead>
<tr>
<th>Species treatments</th>
<th>Fine root biomass (g.m$^{-2}$)</th>
<th>Root length density (m.m$^{-2}$)</th>
<th>Root surface area (cm$^2$.m$^{-2}$)</th>
<th>Root volume (cm$^3$.m$^{-2}$)</th>
<th>Root N content (g.kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rp-C</td>
<td>9.70 ± 0.65 c</td>
<td>1012.96 ± 57.25 c</td>
<td>340.49 ± 17.16 c</td>
<td>9.81 ± 0.42 d</td>
<td>6.53 ± 1.26 b</td>
</tr>
<tr>
<td>Rp-F</td>
<td>10.58 ± 0.96 c</td>
<td>887.34 ± 17.59 d</td>
<td>291.36 ± 5.12 d</td>
<td>8.16 ± 0.12 e</td>
<td>11.98 ± 1.37 a</td>
</tr>
<tr>
<td>Aj-C</td>
<td>11.01 ± 1.17 c</td>
<td>1818.20 ± 14.55 a</td>
<td>661.87 ± 6.04 a</td>
<td>21.99 ± 0.30 a</td>
<td>4.92 ± 0.94 bc</td>
</tr>
<tr>
<td>Aj-F</td>
<td>8.83 ± 1.88 c</td>
<td>1143.29 ± 34.28 b</td>
<td>430.19 ± 10.33 b</td>
<td>15.74 ± 0.29 b</td>
<td>5.26 ± 0.65 bc</td>
</tr>
<tr>
<td>Pm-C</td>
<td>16.44 ± 2.48 b</td>
<td>1143.26 ± 22.43 b</td>
<td>432.48 ± 6.69 b</td>
<td>15.19 ± 0.22 b</td>
<td>4.21 ± 0.43 bc</td>
</tr>
<tr>
<td>Pm-F</td>
<td>8.54 ± 0.57 c</td>
<td>1011.08 ± 15.43 c</td>
<td>356.34 ± 5.92 c</td>
<td>10.69 ± 0.21 c</td>
<td>3.95 ± 0.35 c</td>
</tr>
<tr>
<td>Cl-C</td>
<td>28.80 ± 1.74 a</td>
<td>702.14 ± 8.13 e</td>
<td>226.89 ± 2.70 e</td>
<td>6.01 ± 0.07 f</td>
<td>2.92 ± 0.31 c</td>
</tr>
<tr>
<td>Cl-F</td>
<td>20.52 ± 1.23 b</td>
<td>574.93 ± 6.84 f</td>
<td>194.41 ± 2.51 f</td>
<td>5.50 ± 0.08 f</td>
<td>2.93 ± 0.33 c</td>
</tr>
</tbody>
</table>

**Effect of N fertilization on fine root length turnover rates and longevity**

When calculating the turnover rate and longevity of fine root length density, there was a significant difference between the results of the two methods. The mean turnover rate calculated by the fine root length density production (range from 0.76 to 1.63) was higher than the turnover rate calculated by fine root length density mortality (range from 0.41 to 1.49). The turnover rate of fine roots in the upper layer was higher than that in the lower layer significantly, and the longevity of fine roots in the upper layer was shorter than that in the lower layer. There were significant differences in turnover rates and longevity of fine root length density among four tree species. Fine roots average turnover rate of N-fixing trees was higher, and the longevity of fine roots was lower than that of non-N-fixing trees significantly regardless of the calculation methods.

N fertilization decreased the fine root turnover rate and increased fine root longevity significantly of four tree species, among which the fine root turnover rate and longevity of N fixing tree species were more
sensitive to N fertilization, no significant difference was found between fertilized and non-fertilized treatments of non-N-fixing tree species ($p > 0.05$).

**Distribution patterns of fine root longevity**

Some of the traced newly-born fine roots were still alive at the end of the 3 years’ observation period. The number of fine roots in different life spans fluctuated seasonally in the normal distribution each year. Fine roots with whole year (12 and 24 months) longevity have the biggest proportion in each year of four species (Fig. 2).

The average fine root longevity cannot be determined exactly according to the changes in fine root number because some of the long-lived fine roots were still alive at the end of the observation period in this research. Fine root longevity was divided into: short-lived (< 1 year), middle-lived (1–2 yrs), long-lived (2–3 yrs) and ultra-long-lived ($\geq$ 3 yrs) in this research (Fig. 3). The proportion of short-lived and middle-lived root (< 2 yrs) of *R. pseudoacacia* control treatment is 53.13%, *A. julibrissin* 60.81%, *P. massoniana* 37.08%, and *C. lanceolata* 25.85%. The proportion of ultra-long-lived root ($\geq$ 3 yrs) was 49.44% and 69.39% for *P. massoniana* and *C. lanceolata*, and 20.83% and 27.03% for *R. pseudoacacia* and *A. julibrissin* respectively. The proportion of short-lived roots (< 1 year) and middle-lived roots (1–2 yrs) decreased after N fertilization treatment, while the proportion of ultra-long-lived roots ($\geq$ 3 yrs) increased.

These results showed that both N-fixing and non-N-fixing tree species could prolong the root longevity in response to N fertilization. When comparing the fine root longevity of four tree species, the proportion of long-lived roots of two non-N-fixing tree species was higher than that of two N-fixing tree species.

**Distribution pattern of fine root production and mortality in different months**

The peak values of fine root birth rate of four tree species were ranged from April to August, and the peak values of the fine root birth rate of *P. massoniana* and *C. lanceolata* were sharply in April-May and May-June, accounting for 44.50% and 62.89% of the total new birth roots respectively during the three-years observation period (Fig. 4). The peak period of fine root birth of *R. pseudoacacia* and *A. julibrissin* lasted for 4 months (March-July), during which the number of fine root birth accounted for 81.74% and 73.85% of the total birth rate respectively.

Similar to the times of the birth rate, the peak of the death rate also appeared from April to June at the turn of spring to summer. Different from the peak of the birth rate, there were two peaks of death within a year for *P. massoniana* and *C. lanceolata*, and the peak of death was from April to May, there was a small second death peak between September and October. Compared with the seasonal dynamics of birth and death, the peak of root death times in spring (April-May) was earlier than that of birth (May-June).

**Discussion**
Fine root biomass, morphological, and their responses to N application

Many researchers have given evidence that the fine root biomass and morphological characteristics were different between tree species types. For example, fine roots diameter, quantity, and biomass of the five types of boreal forests in the same geographical area were different significantly, and the naturally regenerated broad-leaved forest had more fine roots and larger fine root biomass than that of the coniferous forest (Wang et al. 2020). Gymnosperms have higher fine root biomass than angiosperms (Jackson et al. 1997), boreal conifers have larger average fine root diameter than broadleaved tree species (Bauhus and Messier 1999), and lower root length. Differences in root characteristics among these different types of tree species may be related to the genetic variances and their adaptation to environmental changes (Comas and Eissenstat 2004; Eissenstat et al. 2015; Zadworny et al. 2016).

We found that the fine root biomass of two N-fixing tree species (angiosperms) was lower than that of two non-N-fixing tree species (gymnosperms). It was in contrast to the global trend of angiosperm fine root biomass exceeding gymnosperms biomass summarized by Wang et al. (2022), however, consistent with the results of Yuan and Chen (2010) that gymnosperms have larger fine root biomass than angiosperms. This result may be related to the differences in water and nutrient uptake and utilization strategies of different tree species under different environmental conditions. Studies have also found that angiosperms produce more root tips for resource acquisition than gymnosperms in warmer climates and more fertile soils (Wang et al. 2019), while gymnosperms adapt to low nutrient utilization through higher nutrient absorption and utilization efficiency (Yuan and Chen 2010). These strategies were consistent with differences in resource uptake characteristics determined by anatomical, morphological, and structural features of fine roots between angiosperms and gymnosperms (Guo et al. 2008; Kong et al. 2014). That's to say, the angiosperm resource uptake strategy in tropical forests is to acquire resources by generating finer roots and faster turnover, whereas gymnosperms acquire resources by establishing larger roots and longer longevity (Guo et al. 2008; Kong et al. 2014; Wang et al. 2022).

The fine root biomass in different tree species may also be related to their symbiotic microorganisms, such as conifers colonized mainly by ectomycorrhizal fungi and broadleaved plants colonized by endomycorrhizal fungi (Pregitzer et al. 2002). AM fungi-colonized trees are more dependent on fine roots, whereas EM fungi-colonized trees are more dependent on mycorrhiza (Chen et al. 2016; Eissenstat et al. 2015). The two angiosperm species in this study are leguminous N-fixing trees, which differ from non-N-fixing broad-leaved angiosperms in their root tip strategies by producing thinner roots and more N resources, it does not require more C to build more fine roots, which may be the main reason that the fine root biomass of the two N-fixing angiosperm species is not significantly greater than that of the two coniferous gymnosperms, but more research data are needed to support this conclusion.

As we hypothesized, there was no significant change in fine root biomass of N-fixing tree species after N application, which can be explained by the weak dependence on soil N availability of N-fixing tree species. Plants reduce C-related maintenance costs through a reduction in biomass and root length.
density to obtain N with the soil N availability increasing (Ma et al. 2021). Both absorption fine roots and their associated mycorrhizal fungi are major organs for nutrient acquisition (van der Heijden et al. 2015), however, there is competition and trade-offs between these two nutrient access mechanisms, as both fine roots and their associated mycorrhizal fungi require the allocation of underground C for growth and maintenance (Lynch 2005). Mycorrhiza can obtain nutrients more efficiently than roots, but mycorrhiza requires more C because hyphae turnover is very fast (Jakobsen and Rosendahl 1990). Studies have shown that trees rely more on fine roots than mycorrhizal fungi for nutrients acquired during the long-term addition of large amounts of N (Ma et al. 2021). Our study results showed that the fine root length and biomass reduced after N addition, which may be reduced not only the C consumption of fine root but also the C consumption of symbiotic root mycorrhizal fungi.

**Fine root turnover rate, longevity, and their responses to N application**

As we hypothesized, the fine roots average longevity of N-fixing tree species was shorter than that of non-N-fixing tree species in this study, which was also consistent with other studies showing that the higher the root N content, the shorter the fine root longevity and the faster the root turnover (Guo et al. 2008; Nadelhoffer 2000). Because of the reduced carbon used in the production of structural compounds and defense materials, fine roots with higher nitrogen content and more metabolic activity have a relatively short life (Nadelhoffer 2000; Tateno et al. 2004). N content in the fine root of N-fixing legumes was higher than that of non-N-fixing species (Fyllas et al. 2009), which may be the direct reason that the fine root longevity of N-fixing trees was shorter than that of non-N-fixing trees.

Nitrogen fertilization increased the fine root longevity and reduced fine root turnover rate of both N-fixing and non-N-fixing tree species in this study, whether based on the fine root length density production and mortality dynamics or based on tracking the number of fine root lifespan from the date of birth to death. Among them, fine root length density turnover rate and longevity response to the N application of N-fixing tree species significantly, while no significant response of non-N-fixing tree species, which was contrary to our hypothesis that fine root turnover with high N content was less responsive to the increase in soil N. The results of increasing root longevity and decreasing turnover rate of four tree species were consistent with those results of Burton et al. (2002), Pregitzer et al. (1993) and Noguchi et al. (2012). Fine root longevity increasing can be explained as a response to the maximum root efficiency to obtain N from the fertile soil of the trees (Noguchi et al. 2012; Yanai and Eissenstat 2002). That’s consistent with the theory that favorable conditions increase fine root longevity and decrease the turnover rate of a given tree species, while stress decreases fine root longevity and increases turnover rate (Eissenstat et al. 2000; McCormack and Guo 2014). In this study, the fine root biomass decreased and the turnover rate decreased, which indicated that fine root mortality was greater than production in a short period after fertilization. This supported meta-analysis results that when N deposition increased, in the short-term, trees regulate their resource acquisition strategy by producing highly active and short-lived fine roots, while in the long-term, the fine roots will maintain a lower biomass and a slower turnover, this is the adaptive strategy for reducing C consumption (Ma et al. 2021).
In this study, fine root N contents of two N-fixing tree species were significantly higher than that of non-N-fixing tree species, and the fine root longevity of N-fixing tree species was shorter than that of non-N-fixing tree species. These results provide basic data for estimating the difference in fine root longevity and their roles in the C and N cycle of two types of tree species with different root N content (N-fixing and non-N-fixing). The responses of fine roots to N deposition and other environmental factors vary with tree species evolutionary positions and forest communities, suggesting that climate change in the future may alter the carbon flux function and competition relationships among coexisting species (Wang et al. 2022). In this research, the responses of fine root turnover and longevity to N fertilization were different between the two types of tree species, which provided a reference for predicting the fine root function responses of different species and forest types under the background of global N deposition.

The results showed that the fine root turnover rate and longevity calculated by the methods of root length density production and mortality were different significantly. The turnover rate based on root length production was higher significantly than that based on root length mortality. However, no matter which one method was used, the trend of increasing fine root longevity and decreasing turnover rate after N fertilization was the same among the four tree species basically. When the distribution pattern of single fine roots longevity was further calculated by root tracking method, the results showed that the proportion of fine roots in shorter longevity of N-fixing tree species was higher than that of non-N-fixing tree species, after fertilization, the fine root longevity of every tree species were prolonged. This result was also consistent with the trend of applying fertilizer to prolong root longevity and decrease turnover rate based on the change of fine root length density of this and other studies (Wu et al. 2023).

**Seasonal patterns of fine root birth and mortality**

During the 3-year observation period, the new-born-roots of four tree species were tracked until the death dates. The fine roots’ birth and death rates of four tree species fluctuated significantly between different months, and the peak values of root death (April-May) were earlier than the peak values of birth (May-June). This indicates that a large proportion of the fine roots were dead before a great number of new roots were produced, which may be an adaptation strategy of the root system.

The fine roots longevity of the four species varied greatly, with the longevity of short-lived roots being only one month, and the longevity of a considerable proportion of fine roots being more than 3 years (Fig. 3). The maximum distribution frequency of fine root longevity of the four tree species was around 12 and 24 months, indicating that a large proportion of fine root longevity was an annual cycle, which was similar to the annual leaf changing in the aboveground part of evergreen tree species, and may be related to the seasonal phenological changes. But more research data are needed to support this conclusion.

**Conclusion**
The fine root turnover rate and longevity of N-fixing tree species were more sensitive to N fertilization than those of non-N-fixing tree species. The two types of tree species had different response strategies to the increase of soil N, the non-N-fixing tree species reduced the C consumption of root redundancy by reducing the fine root standing biomass, while the N-fixing tree species mainly by reducing fine root turnover rate and increasing longevity. In both N-fixing and non-N-fixing tree species, there was a tendency that more proportion of the root longevity approaches the whole year.

**Declarations**

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**Data availability** The complete data file is available from the corresponding author on reasonable request.

**Code availability** Not applicable

**Ethical approval** Not applicable.

**Informed consent** Not applicable.

**Conflict of interest** The authors of this manuscript have no competing interests to declare.

**References**


**Figures**
Figure 1

Fine root length density turnover rates and longevity.
Figure 2

Quantity distribution patterns of different longevity’s fine roots.
Figure 3

The number proportion of different longevity's fine roots.

Figure 4
Fine root birth rate in different months.