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Above- and below-ground biomass distribution and morphological characteristics respond to nitrogen addition in *Pinus tabuliformis*

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Abstract

Background: With the continuing deposition of atmospheric nitrogen (N), N has become a major factor affecting ecosystem carbon (C) balance and N cycles. However, it is still unclear how increasing N deposition alters above- and below-ground biomass allocation, especially among different order roots. *Pinus tabuliformis* Carrière is an important native species in the Loess Plateau of China, as well as the preferred species for artificial afforestation in this area.

Methods: One-year-old *P. tabuliformis* seedlings were treated with six different N concentrations (0, 0.02, 0.04, 0.08, 0.16, and 0.32 g N kg^{-1} soil year^{-1}) for 3 years. Individual *P. tabuliformis* seedlings were separated into leaves, shoots, and roots in May 2011. Variations in leaf morphology, root characteristics, and their biomasses were investigated.

Results: (1) The highest N fertilizer level (0.32 g N kg^{-1} soil year^{-1}) significantly decreased leaf area, root length, and specific root area. (2) Lower levels of N significantly reduced root length, root diameter, and specific root area in the first- and second-order roots. (3) Leaf biomass increased significantly in the higher N treatments (0.02–0.08 g N kg^{-1} soil year^{-1}). However, total seedling biomass under the highest treatment decreased significantly due to excessive N. Biomass of the roots and leaves in the 0.04 g N kg^{-1} soil year^{-1} treatment increased compared with that in the control.

Conclusions: (1) The lower-order roots (and leaves) were more sensitive than the higher-order roots (and stems) to N application. (2) The specific root area to specific leaf area ratio might be more suitable than area or biomass indices in explaining the utilisation of N by plants. (3) The functional balance hypothesis did not adequately explain the distribution of photosynthetic products among *P. tabuliformis* organs, but the source-sink relationship hypothesis did. This indicates that roots were the main sink and received a greater distribution of photosynthetic products.

Keywords: Soil available nitrogen, Root morphology, Leaf morphology, Root order, Biomass

Background

Global nitrogen (N) deposition profoundly affects temperate ecosystems where plant growth is commonly limited by N availability (LeBauer and Treseder 2008). In many regions, N deposition has significantly increased due to anthropogenic activity, leading to increases in the availability of this often growth-limiting nutrient (Vitousek et al. 1997; Galloway et al. 2008). Several studies have suggested a variety of plant responses to N deposition (Driscoll et al. 2003; Fenn et al. 2003; Bobbink et al. 1998, 2010) and illustrated that anthropogenic N deposition in temperate ecosystems is expected to affect the carbon (C) assimilation capacity of plants (Gastal and Lemaire 2002; Yang et al. 2012; Wang and Liu 2014). These biomass changes in plant organs will have an important influence on C distribution and might also have a profound impact on C distribution of whole forest ecosystems.

Plants change their morphological characteristics in response to N addition. Above ground, N availability can affect plant C distribution by influencing leaf area parameters (leaf area and specific leaf area) (Pregitzer et al. 1995, 1998). In contrast, variations in the morphological

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traits of roots, including average diameter, root length, root area, specific root length (length/mass ratio), and specific root area (area/mass ratio), are considered to result from adaptation to soil resource capture (water and mineral nutrients) (Wang et al. 2013). These traits have important effects on root production and turnover (Pregitzer et al. 1997; Borken et al. 2007), which reflect the ratio of photosynthetic C in roots, and ultimately affect the overall plant C allocation balance.

The biomass of plant organs is determined by the allocation of photosynthetic products when adapted to N deposition. Nitrogen deposition influences the allocation of photosynthetic products between ephemeral and long-lived tissues, respiration and biomass production, and above- and below-ground components (Litton et al. 2007), as well as the allocation and amount of photosynthates to plant organs (Yang et al. 2012). Analysis of the above- to below-ground biomass ratio has led many researchers to conclude that the effects of N on C distribution in different plant organs generally support the functional equilibrium hypothesis (that is, when plant growth is restricted by a certain resource, plants will preferentially allocate photosynthetic products to the organ that absorbs the resource) (Gower et al. 1992; Albaugh et al. 1998; Tateno et al. 2004). However, other researchers believe that the source-sink relationship hypothesis better describes the effect of N on C distribution in various plant organs (Dewar 1993). This hypothesis holds that the plant is a system that consists of interactions between sources and leaves, sinks and stems, and roots and fruit. The sources provide the required assimilates through phloem transport, and the sinks obtain the assimilates by competition with other sink organs. However, how changes in biomass among different organs—such as between leaves and branches, roots and leaves, and thick and fine roots—conform to the various hypotheses is unclear.

Bloom et al. (1985) proposed a general theory in which fine-root systems are assumed to be well adapted to maximize soil-resource capture (water and mineral nutrients), while minimising the energetic costs of construction, maintenance, and resource uptake. The indices of specific leaf area (leaf area/mass ratio) and specific root area (root area/mass ratio) combine plant growth and C allocation and might be more suitable than area or biomass indices to explain the utilisation of N by plants.

Pinus tabuliformis Carrière is an important native species in the Loess Plateau of China, as well as the preferred species for artificial afforestation in this area. Based on the concept of ephemeral root modules of perennial plants (Pregitzer et al. 2002; Xia et al. 2010), we hypothesised that the morphology (root length, root diameter, specific root length, specific root area, and specific leaf area) of lower-order roots (or leaves) would be more sensitive than that of higher-order roots (or branches) to N addition. We

also expected that C allocation to different organs would conform to the functional equilibrium hypothesis.

Methods
Study site
This research was carried out in an experimental field at the Institute of Soil and Water Conservation in Yangling, Shaanxi Province, China (108° 4′ E, 34° 16′ N). The site has a mainland monsoon-type climate with average annual precipitation of 674 mm and a mean annual temperature of 13.2 °C. The soil is classified as Calcic Cambisol (FAO-UNESCO, 1977) and is mainly a wind-deposited loess soil that is highly erosive. The soil was collected from a planted *P. tabuliformis* forest site. The clay, silt, and sand composition in the top 0–20 cm soil layer was 36.8, 24.3, and 38.9%, respectively. The organic matter content was 13.6 ± 1.3 g kg^{-1} (mean ± standard deviation, $n = 8$); total soil N content was 1.54 ± 0.24 g kg^{-1}; total soil P content was 1.42 ± 0.38 g kg^{-1}; and soil pH was 8.6 ± 0.3. Previous studies have indicated that low soil N availability has limited the growth of *P. tabuliformis* at this site (Wang et al. 2013). The local atmospheric N deposition rate is 2.06 g m^{-2} $year^{-1}$, which is lower than most polluted regions in the south of China (7.3 g m^{-2} $year^{-1}$; Mo et al. 2008). However, the N deposition rate at this site is increasing rapidly (Galloway et al. 2004; Wei et al. 2010).

Experimental design
The seeds of *P. tabuliformis* were germinated on 23 March 2007, before sowing. The germinated seedlings, which ranged in height from 2.33 to 3.25 cm, were sown in a 5 m × 10 m seedling bed on 28 March 2007. In March 2008, 408 PVC pots of 35-cm diameter and 40-cm depth were filled with forest soil from the research site. Each pot was packed to a bulk density of approximately 1.14 g cm^{-3} (18 kg dry soil per pot). The pots were arranged into six blocks based on a randomised block design, and different amounts of urea (Fu Min Agriculture Product Company, Xian, China), as specified below, were dissolved into 10 mL of distilled water and added to the pots evenly when it rained. This took place at the end of March or in early April of each year from 2008 to 2011. This annual application of N over 3 years was provided to ensure the full effects of N application on seedling growth. The maximum precipitation at this site did not cause any leaching of urea from pots (distance from the soil surface to the top of the pot was 7 cm, allowing sufficient space for the pots to hold precipitation). Nitrogen treatment levels in each of the six blocks were as follows: 0 (control group, CK), 0.02 (N1), 0.04 (N2), 0.08 (N3), 0.16 (N4), and 0.32 (N5) g N kg^{-1} soil $year^{-1}$ (0, 1.77, 3.54, 7.08, 14.16, and 28.32 g m^{-2} $year^{-1}$, respectively, dissolved in 10 mL of distilled water). There were 68 pots per N treatment, i.e., 408

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pots in total. These seedlings were used not only for our research in this paper but also for root respiration, biomass, and N concentration of seedling foliage (data not shown). The 68 pots per N treatment ensured that root sampling met the requirements of measurement.

Sampling

Plant sampling was conducted in May 2011. Individual plants were separated into leaves, shoots, and roots. Samples were gently tapped to remove loose organic matter and mineral soil, then immediately placed in a plastic box with ice, transported within several hours to the laboratory, and frozen for dissection at a later date. In the laboratory, fine roots contained in each soil block were sorted according to Pregitzer et al. (2002). Before dissection, root segments were cleaned of residual soil particles with forceps in deionized water (1 °C). Individual roots were dissected by order following the same classification method as Valenzuela-Estrada et al. (2008). Only live roots were measured; dead roots were removed and discarded. Live and dead roots were distinguished according to Vogt and Perrson (1991). The leaf and shoot samples were freeze-dried and weighed individually. All samples were placed in a cool room within 1 h from the start of sampling.

Seedling roots were sorted and classified by root order (first–sixth order roots). As the first-order roots of each seedling were sparse, the first-order roots of five seedlings were combined into one sample in each N treatments, to meet the requirements of instrument measurement. All pots were sampled in May 2011, with no samples retained for subsequent sampling.

Root imaging and statistics

All roots were dried on absorbent filter paper and then spread over a rectangular, transparent, plastic sheet so that no two segments touched. A scanner was used to scan the roots on the plastic sheet at a resolution of 300 dpi, and root images were recorded in TIFF format. Root length, area, and diameter were measured using CIAS 2.0 image analysis software (CID, Inc., WA, USA). Before roots were scanned, the scanner and software were calibrated with CIAS image standards for length and area. Root diameter classes were set at 0.1-mm intervals. After scanning, the leaves, shoots, and roots were oven-dried at 70 °C for 48 h before being weighed. Root length and root biomass were obtained for individual seedlings. The specific root length and specific root area were obtained by dividing the root length and root area by the root biomass, respectively. The specific leaf area was obtained by dividing leaf area by leaf biomass.

Data analysis

Differences between any two treatments in leaf and root morphological characteristics (i.e., root diameter, root length, root area, specific root length, specific root area, leaf area, specific leaf area, and organ biomass) were determined with SPSS 21.0 statistical software using one-way blocked analysis of variance (ANOVA) (IBM, NY, USA), with each treatment representing a block. Homogeneity of variances were tested by the Levene test. Post hoc separation of means was performed using Tukey's HSD test.

Results

Effects of N addition on leaf morphology

The leaf areas per plant for application treatments N1–N3 did not differ significantly from each other or from the CK but were significantly higher than N4 and N5 (Fig. 1). In contrast, specific leaf areas of the five N application treatments and CK showed no significant differences (Fig. 1).

Effects of N addition on root morphology

The morphological characteristics of *P. tabuliformis* seedling roots following N application treatment varied among root orders, but not all were significant (Fig. 2). The cumulative root length decreased sharply with increasing root order (Fig. 2a). The cumulative root length was longest for the first-order roots (ranging from 259.62 cm for N5 to 852.84 cm for CK) and shortest for the sixth-order roots (ranging from 15.14 for N5 to 31.29 cm for N1). The cumulative root length of the first two root orders comprised over 60% of the total length of fine roots. The cumulative root lengths of the first-order roots were not significantly different for N1, N2, or CK, whereas the cumulative root lengths of the second-order roots were not significantly different for N2, N3, N4, or CK. The only significant difference in total cumulative root length was for N5 compared with N1–N4 and CK (Fig. 2a). The specific root length of the first-order roots was the greatest, almost 1.5–24 times than that of the sixth-order roots (Fig. 2d). In most cases, however, there were no significant differences between N application treatments within each root order (Fig. 2d) and there were no significant differences in mean specific root length with N application treatment.

As expected, mean root diameter increased with increasing root order (Fig. 2b). All levels of N application significantly reduced the average diameter of the first- and fourth-order roots compared with those of CK. A significant reduction in average diameter also occurred for the second-order roots with N application, except for N3. However, there were no significant differences in mean root diameter with N application treatment.

No consistent differences in specific root area were observed among N application treatments for the various orders of roots tested or between N application treatments

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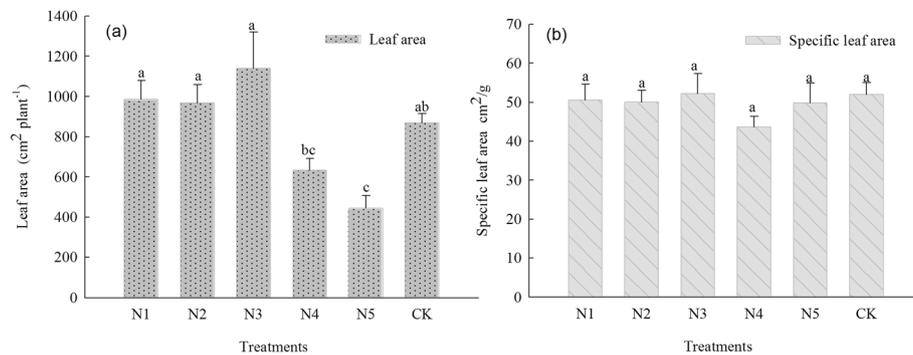
Fig. 1 Effect of N fertiliser application on **a** cumulative leaf area and **b** specific leaf area for each plant ($n = 5$ pots). CK, N1, N2, N3, N4, and N5 are 0, 0.02, 0.04, 0.08, 0.16, and 0.32 g N kg^{-1}, respectively. Different letters indicate significant differences for a given index among the different N additions ($p < 0.05$)

and CK. Overall, treatments N2–N5 resulted in significantly lower specific root areas than those of N1 and CK.

Effects of N addition on plant biomass

Treatments N2 and N3 resulted in plants with significantly higher leaf biomass than that of CK plants, whereas leaf biomass was significantly lower for plants in N5 (Table 1) and stem biomass was significantly lower for plants in N4 and N5 (Table 1). For root biomass, only the fourth-order roots in treatment N3 resulted in significantly higher values than those in CK, and only the second- and sixth-order roots in treatment N5 had significantly lower values. There were no consistent differences in biomass for the various orders of roots with N treatment. The sum biomass of the first-order roots, second-order roots, and leaves increased significantly in N3 compared with CK. Overall, only total biomass under N5 treatment was reduced significantly compared with that under CK (Table 1).

The effect of N addition on the biomass ratios of different organs is shown in Table 2. The only significant difference observed was in the leaf to stem biomass ratio for the N4-treated plants relative to that of CK.

The specific root area to specific leaf area ratio for different N levels is shown in Table 3, with N application significantly reducing the ratio in the N2, N4, and N5 treatments. For the first- and second-order roots, the specific root area and leaf area ratio were significantly lower in the N2 and N5 treatments compared with CK, whereas in the third- to sixth-order roots, the ratio was reduced only in N5, with N2 showing no significant difference to CK.

Discussion

Sensitive responses of lower-order roots and leaves to N addition

In this study, we found that lower-order roots (or leaves) were more sensitive than higher-order roots (or stems) to N application. There was also a combined response to

N addition in the lower-order roots, leaf biomass, and morphological data (Tables 1 and 3). This result supported our hypothesis that the morphology of lower-order roots (or leaves) would be more sensitive than that of higher-order roots (or branches) to N addition.

Compared to Wang et al. (2013), addition of N resulted in slightly reduced fine-root biomass and significant changes in root morphology (including root number, cumulative root length, individual root length, and specific root length), responses that were associated primarily with the first- and second-order roots. The specific root-leaf area decrease observed under N2 treatment was mainly caused by the decrease in lower-order (first and second) roots. For example, the sum of the biomass of the first- and second-order roots and leaves under low N treatment was significantly higher than that of the CK but was significantly lower than that of the highest N treatment. However, the sum of the biomass of the third- to sixth-order roots and stems under low N treatment showed no corresponding significant differences since these roots were woody and had only water- and nutrient-transport functions. Similarly, the specific root area of the first- and second-order roots and specific leaf area ratios were also significantly different.

Baguall et al. (1988) proposed that soil nutrients and moisture affect leaf expansion and discourage assimilate transport out of leaves, leading to thickening of the leaves and decreased specific leaf area. In our study, however, the specific leaf area under all N treatments showed no corresponding significant differences. It is generally believed that N addition increased the leaf area to improve plant photosynthesis but decreased the specific leaf area to reduce C input to the leaves.

Most previous studies have analysed plant mechanisms of distribution of photosynthetic products using the root to shoot ratio. However, some researchers believe that this indicator only represents net C accumulation and does not adequately show the adaptability of plant communities in response to environmental change (Weiner 1990). In

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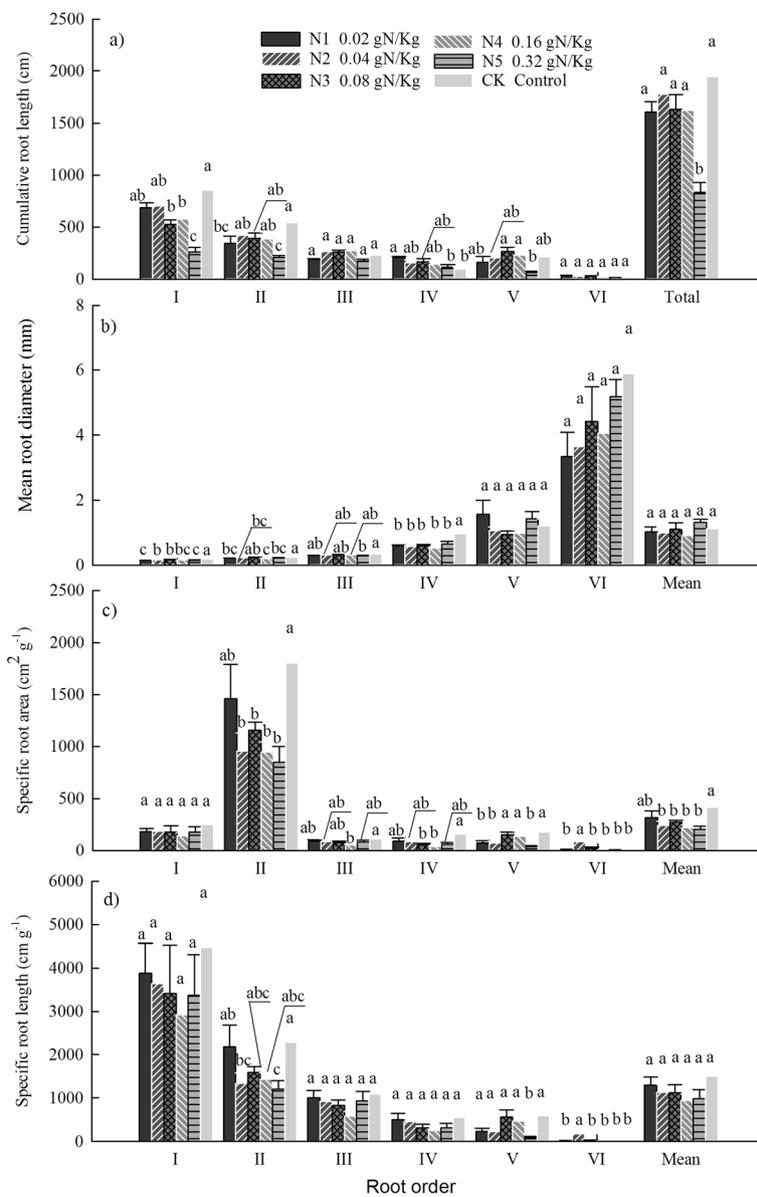
Fig. 2 Effect of N fertilization on **a** cumulative root length, **b** mean root diameter, **c** specific root area, and **d** specific root length for each root order ($n = 5$ pots). CK, N1, N2, N3, N4, and N5 are 0, 0.02, 0.04, 0.08, 0.16, and 0.32 g N kg^{-1}, respectively. I–VI indicate the first- to sixth-order roots. Different letters indicate significant differences for a given index among the different N additions ($p < 0.05$)

addition, it does not correspond well with biomass and does not reflect the loss caused by respiration and senescence, root production, root turnover rate, and other indicators (Dai et al. 2012; Jaana et al. 2014). These indicators are very important for nutrient uptake and photosynthesis of plants. Therefore, the response of photosynthate allocation to environmental change has certain limitations if the root to shoot ratio is the only index used. In fact, plant competition for above- and below-ground resources depends not only on biomass distribution characteristics but also on plant morphological characteristics, especially specific leaf and root areas (Aerts et al. 1991).

Specific root area represents root area per unit root mass and is an important indicator to describe fine-root morphology and physiological function. Studies have shown that specific root area is associated with the effective use of soil resources (Burton 2000; Gordon and Jackson 2000). Specific leaf area represents leaf area per unit mass and usually reflects plant photosynthetic capacity. In the present study, N addition significantly reduced the specific root area to specific leaf area ratio, which suggests that the proportion of assimilates in the root system was less than that in the leaves. Consequently, we consider that this ratio might be more

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Table 1 Effect of N addition on leaf, stem, and root biomass of *P. tabuliformis* (mean ± SE, $n = 5$)

Biomass (g plant^{-1})	N treatment (g N kg^{-1})					
	N1 (0.02)	N2 (0.04)	N3 (0.08)	N4 (0.16)	N5 (0.32)	CK (control)
Leaf biomass	19.4 ± 0.6 ab	19.9 ± 2.7 a	21.8 ± 2.1 a	14.5 ± 1.3 b	8.8 ± 0.6 c	17.0 ± 1.5 b
Stem biomass	9.8 ± 1.2 a	11.6 ± 1.9 a	10.0 ± 1.5 a	5.9 ± 0.6 b	4.0 ± 0.5 b	10.5 ± 1.4 a
Root biomass						
First-order roots	1.3 ± 0.3 ab	1.2 ± 0.4 ab	1.7 ± 0.4 a	1.5 ± 0.4 a	0.5 ± 0.1 b	1.2 ± 0.3 ab
Second-order roots	1.8 ± 0.2 a	2.5 ± 0.1 a	2.2 ± 0.2 a	1.9 ± 0.4 a	1.1 ± 0.1 b	2.2 ± 0.3 a
Third-order roots	2.4 ± 0.3 ab	3.1 ± 0.4 b	2.6 ± 0.2 ab	2.3 ± 0.3 ab	1.8 ± 0.3 a	2.4 ± 0.2 ab
Fourth-order roots	2.6 ± 0.3 ab	2.9 ± 0.2 ab	3.0 ± 0.2 a	2.0 ± 0.3 bc	1.6 ± 0.1 c	2.1 ± 0.1 bc
Fifth-order roots	1.7 ± 0.2 ab	2.4 ± 0.4 a	1.5 ± 0.4 ab	2.1 ± 0.5 ab	1.2 ± 0.2 b	1.3 ± 0.2 ab
Sixth- and above order roots	3.1 ± 0.7 a	3.2 ± 0.5 a	2.8 ± 0.3 a	2.8 ± 0.1 ab	1.2 ± 0.2 b	2.8 ± 0.5 a
First- and second-order roots	3.1 ± 0.5 a	3.7 ± 0.4 a	3.9 ± 0.5 a	3.3 ± 0.7 a	1.5 ± 0.1 b	3.4 ± 0.6 a
Third- and above order roots	9.3 ± 0.6 ab	11.0 ± 1.2a	9.3 ± 0.7 ab	8.1 ± 0.9 b	5.8 ± 0.3 c	8.0 ± 0.2 b
Total root biomass	13.0 ± 0.7 ab	15.2 ± 1.1 a	13.8 ± 0.8 ab	11.9 ± 1.6 b	7.3 ± 0.4 c	12.0 ± 1.0 b
First- to second-order roots and leaves	22.6 ± 0.9 a	23.6 ± 2.5 a	25.7 ± 1.7 a	17.8 ± 0.8 c	10.3 ± 0.5 d	20.5 ± 1.2 b
Third- and above order roots and stems	19.6 ± 1.1 a	23.1 ± 1.4 a	19.9 ± 1.9 a	14.6 ± 1.4 b	9.8 ± 0.3 c	19.1 ± 1.4 a
Total plant biomass	42.2 ± 1.6 a	46.7 ± 3.6 a	45.6 ± 3.4 a	32.4 ± 1.9 b	20.1 ± 0.8 c	39.6 ± 2.3 ab

Values in a row followed by the same letter are not significantly different among the N treatments ($p < 0.05$), and CK, N1, N2, N3, N4, and N5 are 0, 0.02, 0.04, 0.08, 0.16, and 0.32 g N kg^{-1}, respectively

suitable than the area or biomass indices to explain the utilization of N by plants.

The cost-benefit theory, in which root N absorption is dependent on photosynthetic products (C input) (Eissenstat and Yanai 1997; Nadelhoffer 2000; Norby and Jackson 2000), can explain the mechanism of our hypothesis. According to this theory, N concentrations decrease, but C concentrations increase, with the increase in root order (Pregitzer et al. 1997; Majdi 2001; Wells et al. 2002). Jackson et al. (1997) found that fine roots need to consume 41 units of C in order to absorb one unit of N, so that soil N availability affects the growth of fine roots by influencing C allocation to the root system (Hendricks et al. 2006). Pregitzer et al. (1997, 2002) and Wells (2002) stated that the first-order roots have a shorter life than the second- and third-order roots. Thus, the order in which roots die would have the opposite trend to their development order: that is, the first-order roots die first, then the second-order roots, and so on.

Based on the cost-benefit theory, the benefit (increasing absorption of N) of accelerating turnover of lower-order roots was greater than the cost of maintaining fine roots (C consumption). Thus, when soil nutrients were low, a greater specific root area for lower-order roots compared with other orders indicated that roots choose the minimum cost to obtain the maximum benefit. However, when soil nutrient supply was adequate, plants reduced C input to fine roots and increased C into leaves, increasing photosynthesis by increasing leaf area to obtain greater growth and efficiency.

Effect of N addition on biomass allocation to different plant organs

The distribution of photosynthetic products by plants is affected by physiological processes such as photosynthesis, assimilate transport, respiration, and storage of assimilates (Grechi et al. 2007). The environmental effects on the distribution of photosynthetic products in

Table 2 Effect of N addition on the biomass ratios of leaf/stem, root/leaf, root/stem, and root/above ground of *P. tabuliformis* (mean ± SE, $n = 5$)

Biomass ratio	N treatment (g N kg^{-1})					
	N1 (0.02)	N2 (0.04)	N3 (0.08)	N4 (0.16)	N5 (0.32)	CK (control)
Leaf/stem	2.1 ± 0.2 ab	1.8 ± 0.2 b	2.3 ± 0.3 ab	2.5 ± 0.3 a	2.2 ± 0.2 ab	1.7 ± 0.2 b
Root/leaf	0.7 ± 0.10 a	0.8 ± 0.1 a	0.7 ± 0.1 a	0.9 ± 0.2 a	0.9 ± 0.1 a	0.8 ± 0.1 a
Root/stem	1.4 ± 0.2 a	1.5 ± 0.3 a	1.6 ± 0.3 a	2.0 ± 0.3 a	2.0 ± 0.3 a	1.2 ± 0.2 a
Root/above ground	0.5 ± 0.0 a	0.5 ± 0.1 a	0.5 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a	0.5 ± 0.1 a

Values in a row followed by the same letter are not significantly different among the N treatments ($p < 0.05$), and CK, N1, N2, N3, N4, and N5 are 0, 0.02, 0.04, 0.08, 0.16, and 0.32 g N kg^{-1}, respectively

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Table 3 Effect of N addition on the specific root area (SRA) and specific leaf area (SLA) ratios of root/leaf, the first- and second-order roots/leaves, and third- and above order roots/leaves of *P. tabuliformis* (mean ± SE, $n = 5$)

Biomass ratio	N treatment (g N kg^{-1})					
	N1 (0.02)	N2 (0.04)	N3 (0.08)	N4 (0.16)	N5 (0.32)	CK (control)
SRA/SLA	37.5 ± 6.8 ab	29.6 ± 4.5 b	33.5 ± 5.0 ab	30.6 ± 3.5 b	26.3 ± 5.7 b	49.3 ± 6.6 a
First- and second-order SRA/SLA	32.3 ± 6.4 ab	23.2 ± 4.6 b	27.2 ± 4.0 ab	25.2 ± 2.7 ab	22.2 ± 4.9 b	40.7 ± 6.7 a
Third to sixth-order SRA/SLA	5.3 ± 0.6 ab	6.4 ± 2.2 ab	6.3 ± 1.1 ab	5.5 ± 1.3 ab	4.1 ± 0.9 b	8.6 ± 1.5 a

Values in a row followed by the same letter are not significantly different among the N treatments ($p < 0.05$); CK, N1, N2, N3, N4, and N5 are 0, 0.02, 0.04, 0.08, 0.16, and 0.32 g N kg^{-1}, respectively; and SRA and SLA are the specific root area and specific leaf area

plants are more complicated. The functional balance hypothesis is based on environmental factors affecting the distribution mechanism of plant photosynthetic products and also indicates a balanced and coordinated relationship between C supply by plant leaves and root uptake of water and nutrients. Plants obtain light, nutrients, water, carbon dioxide, and other resources to achieve maximum growth by distribution of photosynthetic products (Bloom et al. 1985; Reynolds and Chen 1996; Chen and Reynolds 1997).

We previously studied the carbon allocation of *P. tabuliformis* seedlings along a nitrogen addition gradient using stable carbon isotope technology (Wang and Liu 2014). We found that the photosynthetic rate, root respiration, biomass, and N concentration of seedling foliage exhibited a modal response, peaking at 11.2 g N m^{-2} year^{-1} (N levels used were 0, 2.8, 5.6, 11.2, and 22.4 g N m^{-2} year^{-1}). The highest N treatment (22.4 g N m^{-2} year^{-1}) had detrimental effects on seedling physiological function (photosynthesis, root respiration) and was strongly decoupled from N concentrations in plant tissues. In the present study, we used different N levels, which included either limited N treatment or excessive N treatment. We found that only moderate N treatment promoted *P. tabuliformis* biomass accumulation, while higher N levels led to reduced biomass. The proportion of photosynthetic products in the root system increased when the soil N supply was low (i.e., CK treatment). In higher nutrient treatments (N1–N3 with N concentrations of 1.77, 3.54, and 7.08 g m^{-2} year^{-1}, respectively), more photosynthetic products were allocated to leaves, which led to significant increases in leaf biomass. This phenomenon supported the functional balance hypothesis. However, we also found that root and leaf biomass increased under moderate N application (N2) and decreased under excessive N application (N5) compared with CK. This indicated that the functional balance hypothesis did not adequately explain the distribution of photosynthetic products among *P. tabuliformis* organs as it does not consider plant ontogeny or genetic characteristics that affect the distribution of such products.

According to another hypothesis of photosynthetic product distribution, Marcelis et al. (1998) found that the intensity of plant sinks differed at different growth stages, leading to changes in the distribution of photosynthetic products. In our experiment, only the seedling stage was considered. During this period, roots are the main sink and obtain more photosynthetic products than the leaves or stem. Thus, the biomass of roots and leaves both increased under N2 treatment, suggesting that 0.04 g kg^{-1} was the most suitable N level for biomass accumulation in *P. tabuliformis* seedlings. Furthermore, the disadvantages of the functional equilibrium hypothesis could be avoided if plant morphology and the distribution of photosynthates can be combined.

Conclusions

1) The highest N fertiliser level of N5 significantly decreased leaf area, root length, and specific root area.
2) Lower levels of N significantly reduced root length, root diameter, and specific root area for the first- and second-order roots.
3) N2 was the most suitable N level for biomass accumulation in 2-year-old *P. tabuliformis* seedlings. Leaf biomass increased significantly under the abundant nutrient treatments (N1–N3). The biomass of higher-order roots reached a maximum under N2.
4) Lower-order roots (or leaves) were more sensitive than higher-order roots (or stems) to N application.
5) The specific root area to specific leaf area ratio might be more suitable than the area or biomass indices in explaining the utilisation of N by plants.
6) The functional balance hypothesis did not adequately explain the distribution of photosynthetic products among *P. tabuliformis* organs, but the source-sink relationship hypothesis did. This indicates that roots were the main sink and received a greater distribution of photosynthetic products.

Authors' contributions
All authors contributed substantially to the work reported here. LY and WG participated in the design of the study. LY, LP, and WG analyzed the data and wrote the manuscript. LG and LZ reviewed the manuscript. All authors read and approved the final manuscript.

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