

Effects of Nitrogen Concentration on Root Respiration Rate and Nonstructural Carbohydrates of Walnut Seedlings

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Abstract

Nitrogen is a critical determinant of forest growth and productivity, and root respiration rate and nonstructural carbohydrates are important parameters for evaluating the effects of supplied nutrients. Here, we assessed the effects of nitrogen on walnut seedlings (*Juglans regia* Linn.) that grew consistently for a year under favorable conditions. Seedlings were potted in seven concentrations of N availability, 0, 1, 2, 4, 8, 12, and 16 mmol·L⁻¹, and the specific root respiration rate and nonstructural carbohydrate content of the root system were determined via the off-body root system and the ketone color ratio methods, respectively. Nitrogen concentration was positively correlated with specific root respiration, and root diameter was the main factor related to specific root respiration and nonstructural carbohydrate content of walnut seedlings. Under the same nitrogen concentrations, the soluble total sugar and starch content of roots increased with increasing root diameter, while the specific root respiration increased with decreasing root diameter. Compared to the 0 mmol·L⁻¹ nitrogen concentration, the content of nonstructural carbohydrates in the root system and the specific root respiration were higher when nitrogen concentration was N16. Our comprehensive analysis showed that a nitrogen concentration of N16 improved the respiration rate of the seedling root system, significantly increased the content of nonstructural carbohydrates in the seedling root system, and effectively promoted the growth and development of the seedling root system of walnut seedlings.

Additional key words: correlation, regulatory mechanism, root diameter, soluble total sugar, starch

Introduction

The root system is the most sensitive plant organ to soil conditions, and changes to the soil first affect the respiration rate and carbon-nitrogen metabolism of the root system (Zhang et al., 2014). Root respiration drives root growth, root maintenance, ion absorption, and ion transport into the xylem (Lambers et al., 2008), and it is also a sensitive indicator of root function that indicates root matter and energy changes (Wang et al., 2002). Root system nonstructural carbohydrates (NSCs) are

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the main product of carbon-nitrogen metabolism, and NSCs play an important role in plant growth and development (Philipson, 1988), carbon-nitrogen metabolism (Mei et al., 2015), and resistance (Duque and Setter, 2019). Therefore, root respiration rate and carbon-nitrogen metabolism have a significant effect on the growth of plant parts belowground (Rewald et al., 2016), and the primary abiotic factor driving this effect is nitrogen. Recent studies have found that nitrogen increases the storage of NSCs in the larch [*Larix gmelinii* (Rupr.) Kuzen.] root system (Mei et al., 2015). The application of nitrogen fertilizer helps transform organic carbon in the *Pinus tabulaeformis* Carr. root system, which is achieved by inhibiting root respiration (Zhang et al., 2019) since plant root respiration determines the total amount of carbon allocated to the root (Haynes and Gower, 1995). In woody plants, roots can be differentiated into levels based on root diameter, with corresponding differences in root morphology and physiology. Fine roots have higher respiration rates, but coarse roots have higher NSC content (Yu et al., 2011; Abramoff and Finzi, 2016). Recent studies have shown that in larch and ash (*Fraxinus mandshurica* Rupr.), root NSC content increases as root diameter increases (Jia et al., 2013). For example, in red oak (*Quercus rubra* Linn.) and white ash (*Fraxinus americana* Linn.), coarse roots have higher NSC content than fine roots (Abramoff and Finzi, 2016). However, studies of the effect of nitrogen concentration on root respiration and NSC content have focused on tree species that are economically important for their wood, while the effects on other tree species have rarely been reported.

To further explore the complex relationships between nitrogen and root respiration and nonstructural carbohydrate content of walnut seedlings, we measured the root respiration rate and NSC content of walnut (*Juglans regia* Linn.) seedlings under favorable growth conditions with the off-body root system and the ketone color ratio methods, respectively. We aimed to evaluate the regulation mechanism of nitrogen on the root respiration rate and nonstructural carbohydrates content of walnut seedlings.

Materials and Methods

Experimental Site and Plant Material

The experiment was conducted at the Key Laboratory of Forestry Ecology and Industry Technology in Arid Region, Education Department of Xinjiang in Xinjiang Province from March to August 2019. One-year-old walnut seedlings (*Juglans regia* Linn.) were used and were grown for a year under favorable conditions; they experienced consistent growth.

Experimental Design

Seedlings were planted during the walnut leaf budding period (March 27). The seedlings were removed from the field; soil was then washed off the walnut seedlings, and one walnut seedling was planted in each flower pot. Unfertilized pearl stone and zircon were used as a planting matrix and mixed at a 2:1 ratio. The pots had a bottom diameter of 22 cm, upper diameter of 30 cm, and height of 50 cm and contained 6 kg of planting matrix.

After 2 weeks (11 April), we began adding whole nutrient solution, with 200 mL of nutrition solution added to each pot once every 7 days. To ensure adequate moisture for walnut seedlings, 2,000 mL of water was added to each pot every day between 8:00 and 9:00 am. Thirty days after planting (25 April), all walnut seedlings were treated with different nitrogen concentrations, while the whole nutrient and moisture supply schedules did not change, and we applied nitrogen concentration

Table 1. Nutrient solution formulation for the nitrogen concentration treatment

Chemical	Concentration (mmol·L ⁻¹)	Chemical	Concentration (μmol·L ⁻¹)
NH ₄ NO ₃	4	FeSO ₄ ·7H ₂ O	50
KH ₂ PO ₄	1	MnSO ₄ ·6H ₂ O	5
K ₂ SO ₄	1	CuSO ₄ ·2H ₂ O	0.5
CaSO ₄ ·6H ₂ O	1	KI	0.5
MgSO ₄ ·7H ₂ O	1	ZnSO ₄	5
H ₃ BO ₃ ·6H ₂ O	0.02	NaMoO ₄ ·2H ₂ O	0.5

treatment once every 20 days three times. The nutrient solution formation followed Jarkko and Toini (2001) and Ren (2009). Due to the large amount of measured data in this experiment, according to the absorption characteristics of walnut seedlings and the needs of our experiment, we appropriately adjusted and formulated the nutrient solution based on previous research methodology (Table 1). The pH of the nutrient solution was adjusted to between 5.8 and 6.0 with Ca(OH)₂ of 1 mmol·L⁻¹. We applied nutrient solutions at seven concentrations of N availability (0, 1, 2, 4, 8, 12, and 16 mmol·L⁻¹) by changing the concentration of NH₄NO₃ in the nutrient solution. The control concentration in the experiment was 0 mmol·L⁻¹, and the concentration of other nutrients did not change. Each nutrient concentration treatment was replicated and applied to six pots.

We applied seven different nitrogen concentration treatments to six pots of walnut seedlings with the same growth conditions at each concentration. Forty-two pots of walnut seedlings were used in this study. In addition, to increase the reliability and accuracy of the data, we repeated the experiments three times for each walnut seedling treated, further increasing the data volume.

Root Respiration Measurements

Root respiration rate was determined with the off-body root method (Clark et al., 2010). We selected three walnut seedlings from each nitrogen concentration treatment, removed each walnut seedling root system, washed it quickly with clean water, and then used the cursor caliper to divide the root system into three stages according to root diameter (d): $d \leq 1$ mm, $1 \text{ mm} \leq d \leq 2$ mm, and $d > 2$ mm. We quickly weighed the root of each diameter stage in segments ($d \leq 1$ mm cut into 8 cm segments, $1 \text{ mm} \leq d \leq 2$ mm cut into 5-cm segments, $d > 2$ mm cut into 2-cm segments). We immersed the selected root samples in a constant temperature circulating water bath at 18°C and allowed them to equilibrate for 30 min. Root respiration was determined at 18°C by measuring O₂ consumption using gas-phase O₂ electrodes (Model Chlorolab-2, Hansatech Instruments Ltd, King's Lynn, UK) connected to the circulating water baths. Oxygen consumption measurements started when the respiratory response reached a stable state; the oxygen consumption of the living root at each diameter level in each cell was measured three times, and the respiration measurements were completed within a 2-h period. Following respiration measurements, the same samples from each root order were scanned using an Expression v700 photo scanner (Epson Telford Ltd, Telford, UK). Root images were analyzed by WinRHIZO (Pro2009a) software (Regent Instruments Company, Canada) for the diameter and length of each individual root. The same root samples were then oven-dried at 80°C for 24 h and weighed. The specific root respiration rate (SRR) was calculated using the following formulae (Eq. 1):

$$\text{SRR (nmol O}_2\cdot\text{g}^{-1}\cdot\text{s}^{-1}) = \frac{(V_S \times V_T) \times 60^{-1}}{m} \quad (1)$$

where, V_S indicates total respiration rate of unit length root ($\text{nmol}\cdot\text{min}^{-1}$), V_T is the volume of unit length root (cm^3), and m is the weight of the unit length root system (g).

Nonstructural Carbohydrate Content Measurements

Nonstructural carbohydrates (NSCs)—soluble total sugar (C) and starch content (X)—were measured using the ketone color ratio. The content of soluble total sugar and starch was calculated using the following formulae (Eq. 2 and Eq. 3):

$$C (\%) = \frac{c_1 \times V_T}{V_1 \times W \times 10^6} \times 100\% \quad (2)$$

$$X (\%) = \frac{c_1 \times V_T}{V_1 \times W \times 10^6} \times 0.9 \times 100\% \quad (3)$$

where, C_1 indicates the amount of grapes found from the standard curve (μg), V_T indicates the total volume of the sample extract (mL), V_1 represents the volume of the sample extracted when color rendering (mL), W indicates the determination of sample fresh weight (g), and 0.9 is the coefficient of conversion from glucose to starch.

Statistical Analysis

The data were analyzed using Microsoft Excel 2010, and correlation analysis and significant statistical analysis of variables were conducted using SPSS 20. Differences in the measured properties across treatments were tested at the level of $\alpha = 0.05$ using one-way analysis of variance (ANOVA) with tests for normal distribution and homogeneity of variance. A linear regression analysis was conducted to measure the relationships between study parameters.

Results

Correlation between Respiration Rate and Nonstructural Carbohydrates

Nitrogen addition can increase the respiration rate of walnut roots and have little effect on NSC content in the root system. Both the root diameter and the specific root respiration rate were significantly correlated with the root content of soluble total sugar and starch ($p < 0.01$) (Table 2). The specific root respiration rate was significantly correlated with the root diameter level ($p < 0.01$), and there was a significant correlation between soluble total sugar content and starch content ($p < 0.01$). However, the nitrogen concentration was not significantly related to total sugar content and soluble starch ($p > 0.05$). Root diameter was the major factor affecting the specific root respiration rate of walnut and NSC content.

Table 2. Correlation between root respiration rate and nonstructural carbohydrate content of walnut at different nitrogen concentrations

Observation index	Root diameter	Nitrogen concentration	Specific root respiration rate	Soluble total sugar	Starch
Root diameter	1	0	-0.741**	0.815**	0.808**
Nitrogen concentration		1	0.280*	0.096	0.026
Specific root respiration rate			1	-0.473**	-0.493**
Soluble total sugar				1	0.970**
Starch					1

*, ** Correlation is highly significant at the 0.05 or 0.01 level, respectively.

Table 3. Linear relationship between specific root respiration rate and nonstructural carbohydrate content of walnut at different nitrogen concentrations

Dependent variables	Nitrogen concentration (mmol·L ⁻¹)	Fitted equation	R ²	p
Soluble total sugar	N0	y = -0.63x + 26.768	0.982	< 0.05
	N1	y = -0.454x + 35.216	0.712	< 0.05
	N2	y = -0.604x + 41.136	0.997	< 0.05
	N4	y = -1.013x + 79.282	0.672	< 0.05
	N8	y = -0.634x + 47.76	0.867	< 0.05
	N12	y = -1.107x + 65.412	0.817	< 0.05
	N16	y = -2.278x + 179.021	0.900	< 0.05
Starch	N0	y = -0.846x + 20.977	0.908	< 0.05
	N1	y = -0.321x + 21.562	0.732	< 0.05
	N2	y = -0.497x + 21.63	0.947	< 0.05
	N4	y = -0.892x + 50.389	0.661	< 0.05
	N8	y = -0.469x + 27.692	0.67	< 0.05
	N12	y = -1.346x + 40.285	0.865	< 0.05
	N16	y = -1.46x + 90.756	0.890	< 0.05

Linear Relationship between Respiration Rate and Nonstructural Carbohydrates

The specific root respiration rate and the NSC content had a negative linear correlation at different nitrogen concentrations (Table 3). The R² values between the specific root respiration rate and the soluble total sugar content and the starch content were between 0.672 - 0.997 and 0.661 - 0.947, respectively, and all the correlations were significant. The correlations between specific root respiration rate and the content of soluble total sugar and starch were the highest when the nitrogen concentration was N2. This shows that NSC content has the greatest effect on specific root respiration rate at N2 levels and explains the specific root respiration rate of 94.7% to 99.7% at different nitrogen concentrations.

Effects of Nitrogen on Respiration Rate and Nonstructural Carbohydrates

As illustrated in Table 4, in roots with diameters of $d \leq 1$ mm, $1 \text{ mm} < d \leq 2$ mm, and $d > 2$ mm, soluble total sugar and starch contents and the specific root respiration rate were significantly lower in N0 than other nitrogen concentrations

Table 4. Effects of nitrogen concentration on specific root respiration rate and nonstructural carbohydrate content of walnut at each diameter level

Root diameter	Nitrogen concentration (mmol·L ⁻¹)	Specific root respiration rate (nmol O ₂ ·g ⁻¹ ·s ⁻¹)	Soluble total sugar (mg·g ⁻¹)	Starch (mg·g ⁻¹)
1 mm ≤ d	N0	13.16 ± 0.06 f ^z	25.01 ± 0.25 g	12.12 ± 0.17 e
	N1	16.45 ± 0.76 d	50.32 ± 0.38 c	25.64 ± 0.64 a
	N2	15.35 ± 0.49 e	46.69 ± 0.26 d	15.62 ± 0.85 d
	N4	39.33 ± 0.83 b	53.48 ± 0.30 b	25.09 ± 1.30 b
	N8	24.76 ± 0.80 c	43.86 ± 0.39 e	19.05 ± 0.70 c
	N12	27.06 ± 0.25 c	43.05 ± 0.33 f	13.42 ± 0.92 e
	N16	58.09 ± 0.44 a	57.28 ± 0.83 a	26.91 ± 1.63 a
1 mm < d ≤ 2 mm	N0	5.50 ± 0.15 e	33.29 ± 0.25 f	15.93 ± 0.29 g
	N1	6.78 ± 0.05 c	58.42 ± 1.32 d	37.19 ± 0.26 c
	N2	5.02 ± 0.17 f	64.53 ± 1.18 b	33.84 ± 1.96 d
	N4	6.49 ± 0.07 d	58.53 ± 2.86 bc	37.78 ± 0.53 b
	N8	7.65 ± 0.12 b	61.43 ± 3.01 c	28.29 ± 0.76 e
	N12	7.69 ± 0.09 b	46.59 ± 0.83 e	22.19 ± 0.24 f
	N16	7.80 ± 0.02 a	73.72 ± 1.86 a	54.46 ± 0.46 a
d > 2 mm	N0	1.72 ± 0.15 f	41.85 ± 1.22 e	26.94 ± 0.98 e
	N1	3.31 ± 0.09 d	76.74 ± 4.65 b	64.37 ± 1.42 a
	N2	2.67 ± 0.05 e	65.93 ± 0.94 c	41.16 ± 0.54 c
	N4	4.65 ± 0.02 b	81.22 ± 4.31 a	57.22 ± 0.38 b
	N8	3.86 ± 0.02 c	75.49 ± 6.11 b	60.76 ± 4.11 b
	N12	3.53 ± 0.15 d	56.62 ± 0.23 d	31.94 ± 0.18 d
	N16	6.09 ± 0.05 a	81.51 ± 0.75 a	67.29 ± 0.36 a

^zDifferent lowercase letters after the same diameter and column data represent a significant difference between different nitrogen concentration ($p < 0.05$).

($p < 0.05$), while they were significantly higher than other nitrogen concentrations, such as N16 ($p < 0.05$). Total soluble sugar and starch contents in roots increased with increased root diameter under the same nitrogen concentration, but specific root respiration rate decreased with increased root diameter, and the specific root respiration rate decreased with the increase of soluble total sugar and starch content.

Discussion

Previous studies of the effects of nitrogen on plant growth have shown that nitrogen fertilizer has a growth-promoting effect and that increasing nitrogen fertilizer increases the number of plant cytokines (Bloom et al., 2005), separates tissue cells, and promotes cell growth (Lawlor, 2002). Therefore, respiration provides energy for growing trees to form new tree structures (Ramos, 1985). The effect of nitrogen addition on the respiration rate of forest roots has been reported for *Acer negundo* Linn. (Burton et al., 2012), *Pinus tabulaeformis* Carr. (Zhang et al., 2019), *Populus tremula* Linn. (Ceccon et al., 2016), and *Cunninghamia lanceolata* (Lamb.) Hook. (Fan et al., 2017). These prior studies found that higher nitrogen

concentrations in the root soil significantly contributed to the respiration rate of forest fine roots while also significantly increasing the biomass of the coarse roots. Jia et al. (2011) reported that nitrogen concentration of the root system has a highly significant linear relationship with root respiration rate. We observed a significant positive correlation between the specific root respiration rate and nitrogen application levels of walnut seedlings, and the specific root respiration rate of walnut seedlings increased as root diameter decreased at the same nitrogen level, which is consistent with previous studies (Chen et al., 2010; Bravo et al., 2017). Fine roots play a vital role in root systems because they have high physiological activity during nutrient and water uptake from soil. This characteristic of fine roots enables them to be used as an indicator for plant physiological status during environmental changes (Razaq et al., 2017).

Chen et al. (2017) found that the diameter of the forest root system was related to root growth and carbohydrate distribution and that the NSC concentration of poplar fine roots was lower than that in coarse roots. Poplar may have a higher turnover rate for fine roots than other tree species and therefore does not store large amounts of reserves in fine roots (Regier et al., 2010). Additionally, some studies have reported that NSC content increases with increasing root diameter, but root respiration rate also monotonically decreases with increasing root diameter in the roots of *Larix gmelinii* Rupr. and *Fraxinus mandshurica* Rupr. (Jia et al., 2013). This has also been reported in *Pinus palustris* Mill. (Pregitzer et al., 2002; Aubrey and Teskey, 2018), where root respiration rates increased with increasing nitrogen concentration and the fine roots contained high concentrations of nitrogen when N was fully effective in the soil. Overall, numerous studies have reported that fine roots have higher rates of carbon-nitrogen metabolism than other root sizes (Hishi, 2007). This is because fine roots have a fast metabolism to provide energy for the growth, and development of the root system requires the consumption of large amounts of NSCs, while coarse roots need to store sufficient nutrients for complex organic compounds such as synthetic proteins when forests enter hibernation (Bazot et al., 2013; Martinez-Vilalta et al., 2016). The same results were obtained in this study, where the NSC content of the roots of walnut seedlings increased with the increasing root diameter, which is inversely proportional to the specific root respiration rate, which decreases with increasing root diameter.

Studies have found that low nitrogen levels can significantly increase the NSC content in the roots of seven northern hardwood tree species in the United States (Kobe et al., 2010), and studies have shown that increasing nitrogen application levels has a tendency to reduce the respiration rate of *Pinus taeda* Linn. fine roots (Drake et al., 2008). Here, we found that the respiration rate and NSC content of the roots of walnut seedlings were higher at N16 levels, and the effect is not significant at lower nitrogen levels. This is inconsistent with previous studies, which may be due in part to the fact that our experimental materials were one-year-old walnut seedlings. At this stage, the walnut seedlings are in a stage of prolonged vertical root growth, with the majority of total root weight of 87.82% in the coarse root and less in the side roots (Yang et al., 1980). In this early stage, the root system is strongly influenced by the external environment and nutrient demand is high. Under high nitrogen concentrations, the physiological activity of roots was stimulated to absorb and store a large amount of nitrogen nutrients, which are then converted into organic substances to meet the needs of their own root growth (Fan et al., 2013). Some studies have shown that an appropriate amount of nitrogen application helps increase the underground biomass of walnut trees in adult stages and promotes the growth of the root system (Liu et al., 2007). However, when the nitrogen concentration increases, the walnut trees will suffer from excessive nutrition, which inhibits the growth of underground biomass (Liu et al., 2007). The response of walnut seedling roots to higher nitrogen concentration is different from that of adult walnut roots to nitrogen nutrition supply, which may be due to the different absorption

effects of walnut on nitrogen supply during seedling growth and adult fruiting. Walnut root metabolism is fast in the seedling stage and can promote root growth under the condition of higher nitrogen. In addition, transforming nitrogen absorbed by the root from soil into carbohydrates is highly efficient, and the nonstructural carbohydrate content in the roots increases accordingly (Liu, 2005; Li et al., 2006). However, in adult walnut trees, a higher concentration of nitrogen not only provides nutrients for root growth, but also provides energy for nutrients accumulated in fruits, which adds more processes to the distribution of nitrogen in adult walnut trees, and the metabolism speed is different in each stage. Therefore, a higher concentration of nitrogen in adult trees cannot be distributed to various organs in time, resulting in nitrogen accumulation (Xi et al., 1992). In addition, fertilization depth affects the growth of the walnut root system. Previous research shows that growth of the walnut root system at a depth of 30 cm is higher than that at a depth of 50 cm (Li, 2015). This is because the root tip region of walnut seedlings is the main part for absorbing nitrogen. As the organ with more root tips, the fibrous root system is mainly distributed in a shallow soil layer, while the straight root system plays a supporting role in forest growth and grows in a deeper soil layer (Yang et al., 1980). This is the main reason why walnut seedlings with different fertilization depths have different nitrogen absorption effects. In this study, walnut seedlings were planted in 50-cm-high flowerpots, and the depth of fertilization was relatively shallow, thus making the root system more sensitive to nitrogen concentration. Therefore, a higher nitrogen concentration had obvious effects on the respiration rate of the seedling root system and the content of nonstructural carbohydrates.

In conclusion, a nitrogen concentration was $16 \text{ mmol}\cdot\text{L}^{-1}$ promotes root respiration and increases the content of NSCs in walnut seedlings.

Literature Cited

- Abramoff RZ, Finzi AC (2016) Seasonality and partitioning of root allocation to rhizosphere soils in a midlatitude forest. *Ecosphere* 7:e01547. doi:10.1002/ecs2.1547
- Aubrey DP, Teskey RO (2018) Stored root carbohydrates can maintain root respiration for extended periods. *New Phytol* 218:142-152. doi:10.1111/nph.14972
- Bazot S, Barthes L, Blanot D, Fresneau C (2013) Distribution of non-structural nitrogen and carbohydrate compounds in mature oak trees in a temperate forest at four key phenological stages. *Trees* 27:1023-1034. doi:10.1007/s00468-013-0853-5
- Bloom AJ, Frensch J, Taylor AR (2005) Influence of inorganic nitrogen and pH on the elongation of maize seminal roots. *Ann Bot* 97:867-873. doi:10.1093/aob/mcj605
- Bravo K, Marcolini G, Sorrenti G, Baldi E, Quartieri M, Toselli M (2017) Effect of time of application on nitrogen uptake, partitioning, and remobilization in walnut trees. *J Plant Nutr* 40:719-725. doi:10.1080/01904167.2016.1262402
- Burton AJ, Jarvey JC, Jarvi MP, Zak DR, Pregitzer KS (2012) Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Global Change Biol* 18:258-266. doi:10.1111/j.1365-2486.2011.02527.x
- Ceccon C, Tagliavini M, Schmitt AO, Eissenstat DM (2016) Untangling the effects of root age and tissue nitrogen on root respiration in *Populus tremuloides* at different nitrogen supply. *Tree Physiol* 36:618-627. doi:10.1093/treephys/tpw022
- Chen D, Zhou L, Rao XQ, Lin YB, Fu SL (2010) Effects of root diameter and root nitrogen concentration on in situ root respiration among different seasons and tree species. *Ecol Res* 25:983-993. doi:10.1007/s11284-010-0722-2
- Chen HY, Dong YF, Xu T, Wang YP, Wang HT, Duan BL (2017) Root order-dependent seasonal dynamics in the carbon and nitrogen chemistry of poplar fine roots. *New Forests* 48:587-607. doi:10.1007/s11056-017-9587-3
- Clark NM, Apple ME, Nowak RS (2010) The effects of elevated CO₂ on root respiration rates of two Mojave Desert shrubs. *Global Change Biol* 16:1566-1575. doi:10.1111/j.1365-2486.2009.02075.x
- Drake JE, Stoy PC, Jackson RB, Delucia EH (2008) Fine-root respiration in a loblolly pine (*Pinus taeda* L.) forest exposed to elevated CO₂ and N fertilization. *Plant Cell Environ* 31:1663-1672. doi:10.1111/j.1365-3040.2008.01869.x
- Duque LO, Setter TL (2019) Partitioning index and non-structural carbohydrate dynamics among contrasting cassava genotypes under early terminal water stress. *Environ Exp Bot* 163:24-35. doi:10.1016/j.envexpbot.2019.03.023
- Fan R, Sun J, Yang FC, Li M, Zheng Y, Zhong QL, Cheng DL (2017) Divergent scaling of respiration rates to nitrogen and phosphorus

- across four woody seedlings between different growing seasons. *Ecol Evol* 7:8761-8769. doi:10.1002/ece3.3419
- Fan WG, Ge HM, Wu SF, Yang TT, Luo Y (2013) Effect of nitrogen forms and the ratios on growth and nutrient absorption of *Juglans sigillata* seedling. *Sci Silvae Sinicae* 49:77-84. doi:1001-7488(2013)05-0077-08
- Haynes BE, Gower ST (1995) Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiol* 15:317-325. doi:10.1093/treephys/15.5.317
- Hishi T (2007) Heterogeneity of individual roots within the fine root architecture: causal links between physiological and ecosystem functions. *J Forest Res-Jpn* 12:126-133. doi:10.1007/s10310-006-0260-5
- Jarkko U, Toini H (2001) Influence of nitrogen and phosphorus availability and ozone stress on Norway spruce seedlings. *Tree Physiol* 21:447-456. doi:10.1093/treephys/21.7.447
- Jia SX, McLaughlin NB, Gu JC, Li XP, Wang ZQ (2013) Relationships between root respiration rate and root morphology, chemistry and anatomy in *Larix gmelinii* and *Fraxinus mandshurica*. *Tree Physiol* 33:579-589. doi:10.1093/treephys/tp040
- Jia SX, Wang ZQ, Li XP, Zhang XP, McLaughlin NB (2011) Effect of nitrogen fertilizer, root branch order and temperature on respiration and tissue N concentration of fine roots in *Larix gmelinii* and *Fraxinus mandshurica*. *Tree Physiol* 31:718-726. doi:10.1093/treephys/tp057
- Kobe R K, Iyer M, Walters M B (2010) Optimal partitioning theory revisited: nonstructural carbohydrates dominate root mass responses to nitrogen. *Ecology* 91:166-179. doi:10.1890/09-0027.1
- Lambers H, Chapin III FS, Pons TL (2008) Plant physiological ecology. Springer Science & Business Media, NY, USA, pp 23-24. doi:10.1007/978-0-387-78341-3
- Lawlor DW (2002) Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J Exp Bot* 53:773-787. doi:10.1093/jexbot/53.370.773
- Li M (2015) Effects of fertilization on phenology, growth and yield of walnut. Northwest A&F University, China
- Li YH, Li BG, Guo SP, Yang L (2006) Studies on the effects of fertilizer application in young precocious walnut. *J Hebei Agric Univ* 1:9-11
- Liu QZ, Zhang LS, Ai CX (2007) Specification for walnut germplasm resources description and data standard. China Agriculture Press, China, pp 49-50
- Liu W (2005) The effect of N,P,K level on the growth of young walnut and its physiology index. Baoding: Agricultural University of Hebei, China
- Martinez-Vilalta J, Sala A, Asensio D, Galiano L, Hoch G, Palacio S, Piper FI, Lloret F (2016) Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecol Monogr* 86:495-516. doi:10.1002/ecm.1231
- Mei L, Xiong YM, Gu JC, Wang ZQ, Guo DL (2015) Whole-tree dynamics of non-structural carbohydrate and nitrogen pools across different seasons and in response to girdling in two temperate trees. *Oecologia* 177:333-344. doi:10.1007/s00442-014-3186-1
- Philipson JJ (1988) Root growth in Sitka spruce and Douglas-fir transplants: dependence on the shoot and stored carbohydrates. *Tree Physiol* 4:101-108. doi:10.1093/treephys/4.2.101
- Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root architecture of nine North American trees. *Ecol Monogr* 72:293-309. doi:10.1890/0012-9615(2002)072[0293:FRAONN]2.0.CO;2
- Ramos DE (1985) Walnut orchard management. Cooperative Extension, University of California, Division of Agriculture and Natural Resources, CA, USA, pp 121-122
- Razaq M, Shen H, Sher H, Zhang P (2017) Influence of biochar and nitrogen on fine root morphology, physiology, and chemistry of *Acer mono*. *Sci Rep-UK* 7:5367. doi:10.1038/s41598-017-05721-2
- Regier N, Streb S, Zeeman SC, Frey B (2010) Seasonal changes in starch and sugar content of poplar (*Populus deltoides* × *nigra* cv. Dorskamp) and the impact of stem girdling on carbohydrate allocation to roots. *Tree Physiol* 30:979-987. doi:10.1093/treephys/tpq047
- Ren J (2009) Characteristics and mechanisms of root respiration of *Fraxinus mandshurica* Rupr. to soil nitrogen. Beijing: Beijing Forestry University, China
- Rewald B, Kunze ME, Godbold DL (2016) NH₄:NO₃ nutrition influence on biomass productivity and root respiration of poplar and willow clones. *GCB Bioenergy* 8:51-58. doi:10.1111/gcbb.12224
- Wang X, Curtis P (2002) A meta-analytical test of elevated CO₂ effects on plant respiration. *Plant Ecol* 161:251-261. doi:10.1023/A:1020305006949
- Xi RT, Zhang YP (1992) Chinese walnut. Beijing: China Forestry Press, CN, pp 28
- Yang WH, Wei CC, Chi SK, Liu RZ (1980) Studies on the distribution of the walnut roots and its correlation with the top. *Acta Hort Sinica* 2:11-20
- Yu ML, Wang CK, Wang XC (2011) Allocation of nonstructural carbohydrates for three temperate tree species in Northeast China. *Chin J Plant Ecol* 35:1245-1255. doi:10.3724/SP.J.1258.2011.01245
- Zhang H, Liu YH, Zhou ZY, Zhang YY (2019) Inorganic nitrogen addition affects soil respiration and belowground organic carbon fraction for a *Pinus tabulaeformis* forest. *Forests* 10:369. doi:10.3390/f10050369
- Zhang P, Qin SJ, Zhou WJ, LV DG, Ma HY (2014) Effects of *Pseudomonas fluorescens* inoculation on root respiration and seedling growth of cherry. *Sci Agric Sinica* 47:3857-3865. doi:10.3864/j.issn.0578-1752.2014.19.014