



Article

Remobilization of Storage Nitrogen in Young Pear Trees Grafted onto Vigorous Rootstocks (*Pyrus betulifolia*)

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Abstract: The remobilization of storage nitrogen (N) is affected by growth characteristics of young pear trees. Aboveground parts of young pear trees grafted on *P. betulifolia* grew more vigorously than that on dwarfing rootstocks. Therefore, the remobilization of storage N within young pear trees on vigorous rootstocks may be different from that on dwarfing rootstocks. A ¹⁵N tracing experiment, including six groups of one-year-old pear trees grafted on vigorous rootstocks in 2016, was conducted to investigate the mobilization of storage N from 2016 to 2018. Results indicated that about 44%, 31.4% and 24.6% of storage N remobilized in new growth was derived from the trunk, shoots and roots, respectively. Most of storage N remobilized in new organs were supplied by trunks and shoots. About 82.2% of storage N withdrawn from senescent leaves were recovered in the trunk and shoots during autumn. The aboveground parts played a more important role than roots in the cycling process of storage N in young pear trees. However, as compared with young pear trees on dwarfing rootstocks, more storage N recovered in new organs were supplied by roots of that on vigorous rootstocks, due to vigorous growth and more nutrient requirement of aboveground parts.

Keywords: labelled nitrogen; nitrogen derived from fertilizer; nitrogen concentration; new organs; perennial structures



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1. Introduction

The remobilization of stored nitrogen (N) is a key factor influencing N uptake, growth, development, and longevity of deciduous fruit trees [1–4]. The remobilization of N mainly occurs during two periods, including the early spring [5–7] and defoliation stage [8–11].

In early spring, the low soil temperatures result in minimal low activity in root systems, and the N to sustain new organ growth is mainly remobilized from the perennial tissues [9,12–15]. Studies on apple [16,17], walnut [18] and persimmon trees [19] show that approximately half of the total tree N amount is remobilized to sustain new organ growth. The researches on the fruit trees indicated the decline in N content of root system is far higher than that found in stems during the spring, and the root system contributes greatly to N storage during winter [20,21]. Similar results were concluded by the studies on the sycamore [22], nectarine [6], apple [23], walnut [24], persimmon [19], cherry [25,26] and peach trees [27]. However, the studies on the apple [28], pear [10] and walnut trees [29] concluded that the remobilized N of new growth in early spring is mainly supplied by the aboveground structures.

Studies on the remobilization of N stored in the perennial structures of pear trees during the early spring have been conducted [5,8,10,30–34]. The pear trees mentioned in these studies were *P. communis* L. grafted on the quince rootstocks (dwarfing rootstocks) or mature pear trees grafted on vigorous rootstocks. However, as compared with that on the quince rootstocks, the aboveground growth of young pear trees grafted on the

P. betulifolia (a type of vigorous rootstocks) are more vigorous [35]. The obvious differences in root length density between the quince and *P. betulifolia* are also observed by Zhao et al. [35]. *P. betulifolia* is the major rootstock and has been widely used in pear orchards in North China [35]. N is one of the key nutrients affecting the growth of young pear trees. The N uptake of perennial structures is used to meet their own growth and support the new growth in next spring. The absorption and remobilization of N is affected by the characteristics of various structures' growth. Therefore, the remobilization of storage N in young pear trees on *P. betulifolia* may be different from that on the dwarfing rootstocks. Tagliavini et al. [32] also concluded that storage capacity is related to tree size and age, internal N cycling within trees grafted on vigorous rootstocks might be more essential in the overall N economy than on dwarfing rootstocks. Although the remobilization of storage N of mature pear trees grafted on vigorous rootstocks was reported by Jiang et al. [36], there were obviously differences in growth trends between mature and young pear trees. The studies on the remobilization of the reserve N stored in perennial structures of young pear trees (*P. bretschneideri* Rehd) grafted on the *P. betulifolia* rootstocks are scarce.

During leaf senescence, more than half of the leaf N is mobilized to the perennial parts of trees [8,9,37], which sustains the growth of new organs in the next spring [10,16,38–40]. To date, researches mainly focus on the total amount of N withdrawn from senescent leaves [8,34]. The studies on the redistribution of stored N, withdrawn from leaves, in various perennial structures of young pear trees are scarce.

More than 60% chemical fertilizer is applied once before bud break stage by farmers in North China, which can save labor on the pear orchards in the following periods. Therefore, the investigation on the internal cycling of N taken up in early spring were necessary for improving nutrient use efficiency of young pear grafted on vigorous rootstocks. The study aimed to evaluate (1) the remobilization of stored N of young pear trees (*P. bretschneideri* Rehd) grafted on the *P. betulifolia* rootstocks during spring, (2) redistribution of stored N withdrawn from senescent leaves during autumn.

2. Materials and Methods

2.1. Experimental Site and Materials

The experiment was conducted in an experimental pear orchard in Haidian District (Beijing, China, 39°95' N, 116°03' E) from March 2016 to June 2018. The experimental site has a continental monsoon climate with a mean annual precipitation amount and temperature of 550 mm and 12.8 °C, respectively. One-year-old pear trees (*P. bretschneideri* Rehd 'Huangguan') grafted on the rootstock of *P. betulifolia* were planted in spring 2016. Spacing was 1 m within rows and 3.5 m between rows of trees (2857 trees·ha⁻¹). The soil of the experimental orchard was clay loam with an average bulk density of 1.4 g·cm⁻³, and the soil N content at depth of 0–60 cm was 0.5 g·kg⁻¹.

2.2. Experimental Design

The young pear trees were fertilized with the labelled N once in early spring, to evaluate the remobilization of labelled N in the next years. The experimental trees were divided into groups A, B, C, D, E and F. The groups A, B, D and E were fertilized with 10 g unlabelled and 5 g labelled N (enriched to 10.19 atom% ¹⁵N) per tree, the groups C and F were fertilized with 15 g unlabelled N per tree in 2016. The groups B and F were fertilized with 20 g unlabelled N per tree, the groups C, D and E were fertilized with 15 g unlabelled and 5 g labelled N per tree in 2017. The group E was fertilized with 30 g unlabelled N per tree, the group F was fertilized with 25 g unlabelled and 5 g labelled N per tree in 2018 (Table 1). The urea-N fertilizer was dissolved in water and sprinkled evenly onto the soil before bud break stage (March 20) each year. The treated ground was immediately covered with soil to prevent ammonia volatilization [30]. The experiment had a randomized design and was repeated three times. Each repetition in groups B, C and D consisted of 4 trees, and each repeat in the other groups consisted of 2 trees.

Table 1. The timing and rate of fertilization of pear trees divided according to their groups.

| Group | 2016 | 2017 | 2018 |
|-------|--------------------------|--------------------------|--------------------------|
| A | 10 g/tree ○ + 5 g/tree ● | - | - |
| B | 10 g/tree ○ + 5 g/tree ● | 20 g/tree ○ | - |
| C | 15 g/tree ○ | 15 g/tree ○ + 5 g/tree ● | - |
| D | 10 g/tree ○ + 5 g/tree ● | 15 g/tree ○ + 5 g/tree ● | - |
| E | 10 g/tree ○ + 5 g/tree ● | 15 g/tree ○ + 5 g/tree ● | 30 g/tree ○ |
| F | 15 g/tree ○ | 20 g/tree ○ | 25 g/tree ○ + 5 g/tree ● |

Note: ○ means unlabelled N; ● means labelled N (enriched to 10.19 atom% ¹⁵N); - means no fertilization.

2.3. Measurements

The trees in group A were harvested on June 10 and November 20, 2016. The trees in groups B, C and D were harvested on June 10 and November 20, 2017, and those in groups E and F were harvested on June 10, 2018 (Table 2). Six trees per group were harvested at each sampling time. Six more of the same trees were harvested in March 2016 to evaluate the initial dry weight and N content of each organs. The trees were removed from the soil and separated into trunk, leaves, shoots, 2 and 3 year old branches, fruits and roots. All plant structures were washed, dried to constant weight at 70 °C, weighed to measure the dry matter, ground (<0.25 mm), and then analyzed for total N and % ¹⁵N enrichment. Total N was determined using a Kjeldahl azotometer (QSY-II, Beijing Qiangsheng Analysis Instrument Manufacturing Centre, China). The %¹⁵N enrichment was determined using an isotope ratio mass spectrometer (MAT251, Thermo Finnigan, San Francisco, CA, USA). The dry mass weight and N content in each organ of the pear trees at the beginning of the experiments are shown in Table 3.

Table 2. The harvest sampling time and quantity of pear trees of each group.

| Group | 2016 | | 2017 | | 2018 |
|-------|---------|---------|------|---------|---------|
| | Nov. | Jun. | Nov. | Jun. | Jun. |
| A | 6 trees | - | - | - | - |
| B | - | 6 trees | - | 6 trees | - |
| C | - | 6 trees | - | 6 trees | - |
| D | - | 6 trees | - | 6 trees | - |
| E | - | - | - | - | 6 trees |
| F | - | - | - | - | 6 trees |

Table 3. The dry mass weight, N content and concentration of each pear tree structure.

| Years | Organs | N Content (mg) | Dry Mass Weight (g) | N Concentration (%) |
|----------------------|----------------------|--------------------|---------------------|---------------------|
| Mar. 2016 Nursery | Trunk | 278.7 ± 14.8 c E | 43.2 ± 1.4 b D | 0.64 ± 0.02 b C |
| | Roots | 485.0 ± 15.8 b D | 42.3 ± 1.1 b D | 1.14 ± 0.06 a B |
| | Total | 763.7 ± 11.7 a E | 85.6 ± 2.3 a E | |
| Nov. 2016 | Leaves | 89.6 ± 10.4 d D | 6.9 ± 0.8 d D | 1.30 ± 0.01 a D |
| | Shoots | 168.4 ± 20.0 d D | 13.2 ± 1.3 c E | 1.27 ± 0.04 a A |
| | Trunk | 668.8 ± 39.0 c D | 65.8 ± 4.6 b D | 1.01 ± 0.03 c A |
| | Roots | 789.6 ± 45.6 b C | 64.6 ± 3.8 b CD | 1.22 ± 0.03 b A |
| | Total | 1716.4 ± 86.2 a D | 150.5 ± 8.2 a D | |
| Jun. 2017 | Leaves | 2286.3 ± 178.2 b B | 86.8 ± 6.7 c B | 2.64 ± 0.01 a B |
| | Shoots | 345.3 ± 27.2 d C | 38.1 ± 3.2 d D | 0.91 ± 0.08 c D |
| | 2 years old branches | 129.3 ± 4.6 d D | 16.3 ± 0.5 d E | 0.79 ± 0.01 d E |
| | Trunk | 1035.3 ± 80.9 c C | 168.7 ± 12.7 b C | 0.61 ± 0.01 e C |
| | Root | 1042.5 ± 67.0 c C | 84.6 ± 5.0 c C | 1.23 ± 0.01 b A |
| | Total | 4838.7 ± 332.6 a C | 394.5 ± 26.2 a C | |

Table 3. Cont.

| Years | Organs | N Content (mg) | Dry Mass Weight (g) | N Concentration (%) |
|-----------|----------------------|----------------------|---------------------|---------------------|
| Nov. 2017 | Leaves | 738.3 ± 66.4 e C | 50.6 ± 5.2 e C | 1.46 ± 0.02 a C |
| | Shoots | 1554.6 ± 149.6 d A | 140.8 ± 15.8 d B | 1.11 ± 0.03 c B |
| | 2 years old branches | 180.2 ± 13.6 f D | 17.7 ± 1.6 e E | 1.02 ± 0.05 d C |
| | Trunk | 3832.6 ± 203.1 b B | 455.6 ± 34.0 b B | 0.84 ± 0.02 e B |
| | Roots | 3211.6 ± 174.3 c B | 253.2 ± 20.6 c B | 1.27 ± 0.07 b A |
| | Total | 9517.3 ± 584.4 a B | 917.9 ± 74.5 a B | |
| Jun. 2018 | Leaves | 8099.7 ± 541.4 b A | 295.0 ± 24.7 d A | 2.75 ± 0.07 a A |
| | Shoots | 1150.5 ± 124.5 ef B | 117.7 ± 12.3 f C | 0.98 ± 0.02 d C |
| | 2 years old branches | 1456.3 ± 108.5 e A | 208.7 ± 16.3 e A | 0.70 ± 0.01 f F |
| | 3 years old branches | 248.9 ± 22.9 g CD | 23.0 ± 2.1 g D E | 1.08 ± 0.01 c B |
| | Trunk | 3263.5 ± 194.1 d A | 632.1 ± 32.0 b A | 0.52 ± 0.02 g D |
| | Roots | 4914.6 ± 311.8 c A | 406.7 ± 27.7 c A | 1.21 ± 0.01 b AB |
| | Fruits | 465.5 ± 41.9 fg | 51.1 ± 4.8 fg | 0.91 ± 0.01 e |
| | Total | 19599.0 ± 1177.7 a A | 1734.3 ± 108.9 a A | |

Note: The numbers are mean ± standard deviation (n = 6). Different small letters in the same column and for each sampling time indicate significant differences ($p < 0.05$) among organs; different capital letters in the same column, for the same organ, indicate significant differences ($p < 0.05$) among sampling times. Shoots and branches were regarded as the same organ for variance analysis among sampling times.

2.4. Calculations and Data Analysis

Nitrogen derived from the ^{15}N -fertilizer in each plant structure (%Ndff) represented the contribution percentage of N uptake from the ^{15}N -fertilizer and was determined in accordance with the formula described by Carranca et al. [41]:

$$\% \text{Ndff} = \frac{\text{at. } ^{15}\text{N excess of sample}}{\text{at. } ^{15}\text{N excess of labelled fertilizer}} \times 100 \pm \quad (1)$$

where natural atom% ^{15}N is equal to 0.366.

The amount of N derived from the fertilizer in different pear tree structures (NF) was determined by

$$\text{NF} = \text{total N in structure} \times \% \text{Ndff} \times \frac{\text{amount of total fertilizer}}{\text{amount of labelled fertilizer}} \quad (2)$$

$$\text{TNF} = \sum_{\text{all structures}} \text{NF} \quad (3)$$

where TNF represents the sum of NF in all structures of whole pear trees.

The trees in groups A and C were destructively sampled in 2016 and 2017, respectively. According to Equation (2), the NFA and NFC was defined as the amount of N derived from the fertilizer, which were applied in 2016 and 2017, in different pear tree structures of group A and C trees, respectively.

The trees in group B were destructively sampled in 2017, according to Equation (2), and the NFB was defined as the amount of N derived from the fertilizer, which was applied in 2016, in different pear tree structures of group B trees. The NFB consists of two parts: a. the amount of fertilizer-N taken up in 2016 (NFB₂₀₁₆); b. The amount of fertilizer-N taken up in 2017 (NFB₂₀₁₇). The NFB was calculated as follows:

$$\text{NFB} = \text{NFB}_{2016} + \text{NFB}_{2017} \quad (4)$$

The sum of NFB in all organs of a whole tree (TNFB) were calculated as follows:

$$\text{TNFB} = \text{TNFB}_{2016} + \text{TNFB}_{2017} \quad (5)$$

where TNFB₂₀₁₆ and TNFB₂₀₁₇ represents the sum of NFB₂₀₁₆ and NFB₂₀₁₇ in all organs of a whole tree, respectively.

The sum of NFA in all organs of a whole tree was equal to the sum of NFB₂₀₁₆ in all organs of a whole tree, thus

$$TNFB_{2016} = TNFA \tag{6}$$

where TNFA represents the sum of NFA in all organs of a whole tree.

According to Equations (5) and (6), TNFB₂₀₁₇ were calculated as follows:

$$TNFB_{2017} = TNFB - TNFA \tag{7}$$

We hypothesized that the ratio of NFC in some structures to TNFC was equal to the ratio of NFB₂₀₁₇ in the same structure to TNFB₂₀₁₇, so Equation (8) was calculated as follows:

$$\frac{NFC \text{ in some structure}}{TNFC} = \frac{NFB_{2017} \text{ in some structure}}{TNFB_{2017}} \tag{8}$$

Therefore, NFB₂₀₁₇ was calculated as follows:

$$NFB_{2017} \text{ in some structure} = TNFB_{2017} \times \frac{NFC \text{ in some structure}}{TNFC} \tag{9}$$

$$NFB_{2016} = NFB - NFB_{2017} \tag{10}$$

NFB₂₀₁₆ also represents the amount of the fertilizer-N, which was derived in 2016, in different pear tree structures in 2017. The calculation and data analysis was shown in Figure 1. In the following parts, the NFB₂₀₁₆ was abbreviated to N₂₀₁₆.

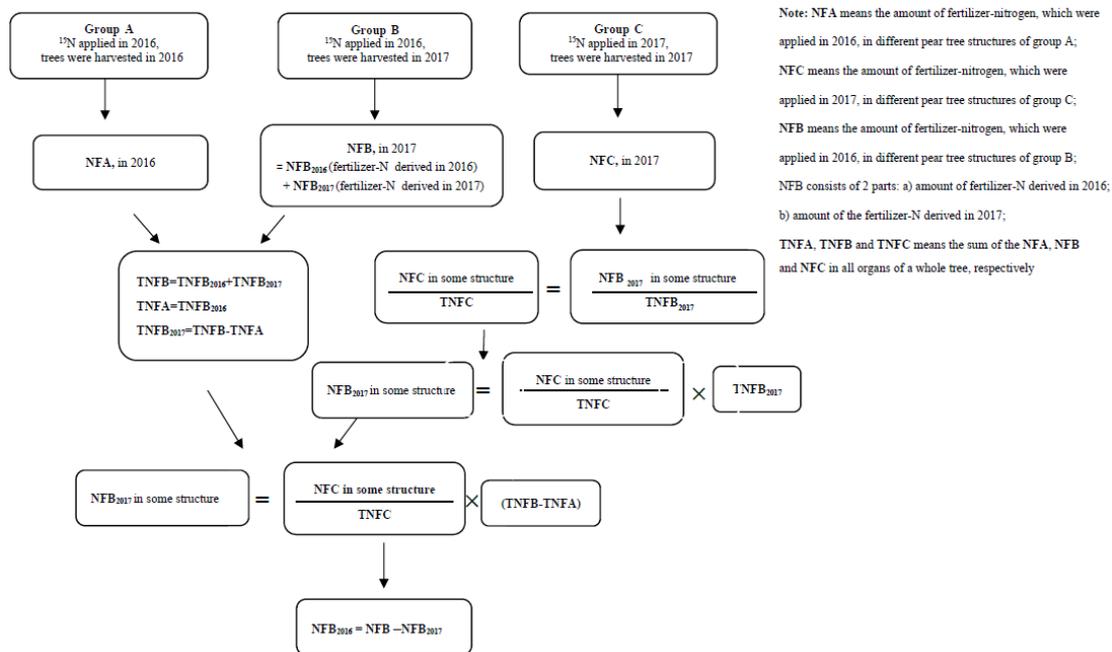


Figure 1. The calculation method of the amount of fertilizer-N, which was taken up in 2016, in each structure in 2017.

The amount of fertilizer-N, derived in 2016 and 2017, in the different structures of group E trees in 2018 was determined with the same method, and was abbreviated to N_{2016&2017}.

2.5. Statistical Analysis

Statistical analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Means and standard deviations were calculated to show the measuring accuracy of each biological parameter. The measurements were analyzed with analyses of variance. Tukey

tests were used to assess the differences between means and significant differences were based on $p < 0.05$. All measurements were repeated six times.

3. Results

3.1. The N Content and Concentration in Each Structure of Young Pear Trees

From spring 2016 to June 2018, the total N content in whole pear trees increased from 0.76 g to 19.6 g (Table 3), and the N uptake from the soil was significantly higher than that from the fertilizer (Table 4). The N content was highest in the leaves, followed by the trunk and roots, and the lowest was in the branches and fruits in June 2017 and 2018 (Table 3). The highest N content were observed in trunk and roots in November 2016 and 2017. From June to November 2017, the N content in the leaves sharply declined, and accounted for less than 8% of that in whole pear trees (Table 3).

Table 4. The increment of total N of pear trees, N uptake from the fertilizer and the soil from March 2016 to June 2018.

| Time | Increment of Total N (mg) | Increment of N Uptake from Fertilizer (mg) | Increment of N Uptake from Soil (mg) |
|------------------------|---------------------------|--|--------------------------------------|
| Mar. 2016 to Nov.2016 | 953 ± 93 d | 285 ± 11 c | 668 ± 88 d |
| Nov.2016 to Jun. 2017 | 3122 ± 333 c | 743 ± 57 b | 2379 ± 287 c |
| Jun. 2017 to Nov. 2017 | 4679 ± 584 b | 855 ± 39 b | 3823 ± 550 b |
| Nov. 2017 to Jun. 2018 | 10082 ± 1178 a | 2128 ± 160 a | 7954 ± 1030 a |

Note: The numbers are mean ± standard deviation (n = 6). Different small letters in the same column. indicate significantly differences ($p < 0.05$).

The highest N concentrations were observed in leaves throughout the whole experimental periods. The N concentrations in the roots kept constant (1.2–1.3%) in three consecutive experimental years, except in March 2016. As compared with that in November, the N concentration in the trunk was significantly lower in the next June. The lower N concentration was observed in 2 year old branches in June, as compared with that in shoots in the previous November (Table 3).

3.2. Stored N Remobilized in Spring and Withdrawn from Senescent Leaves in Autumn

About 28.1% of the fertilizer-N derived in 2016 (N_{2016}) within a whole tree was recovered in the leaves in June 2017 (Figure 2), which was far higher than that in the shoots, and similar results were observed in June 2018 (Figure 3). From November 2016 to the next spring, the N_{2016} stored in the trunk and 2 years old branches decreased 37.1 and 26.5 mg, respectively (Figure 2), which accounted for about 44.0% and 31.4% of the total N_{2016} remobilized in new organs (Figure 4). The decrease in N_{2016} stored in the trunk and 2 year old branches was higher than that in the roots (Figure 4), in particular the differences between the trunk and roots were significant, and similar results were observed in spring of 2018 (Figure 5). These results indicate that the N remobilized in new tissues was mainly supplied by the trunks and 2 year old branches of young pear trees. The $N_{2016\&2017}$ derived from the 3 year old branches was 11.2 mg in the spring of 2018, which was the lowest among all perennial tissues.

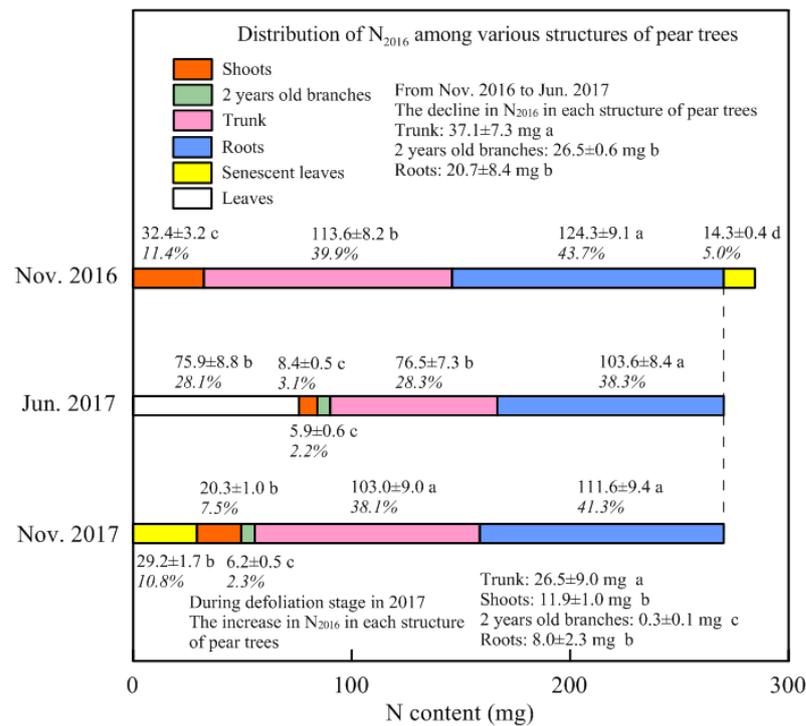


Figure 2. The distribution of N_{2016} among various structures of pear trees. N_{2016} means amount of fertilizer-N derived in 2016. The numbers means the N_{2016} amount in each structure. Different small letters for each sampling time indicate significant differences ($p < 0.05$) among organs. The italic numbers mean the ratio of N_{2016} in each structure to total N_{2016} in a whole tree.

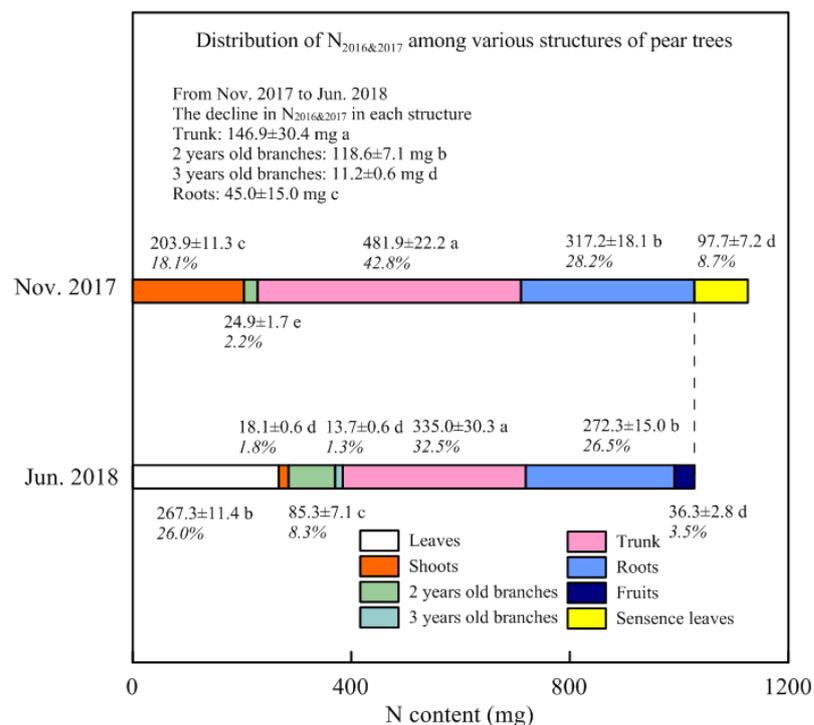


Figure 3. The distribution of $N_{2016\&2017}$ among various structures of pear trees. $N_{2016\&2017}$ means amount of fertilizer-N derived in 2016 and 2017. The numbers mean the $N_{2016\&2017}$ amount in each structure. Different small letters for each sampling time indicate significant differences ($p < 0.05$) among organs. The italic numbers means the ratio of $N_{2016\&2017}$ in each structure to total $N_{2016\&2017}$ in a whole tree.

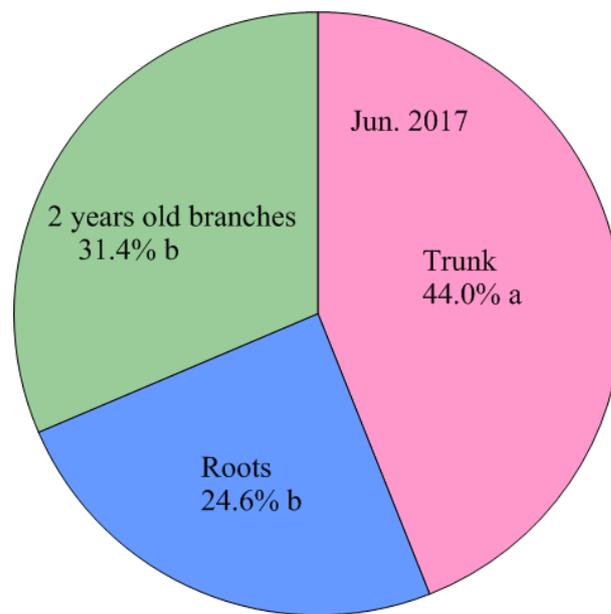


Figure 4. The ratio of N_{2016} supplied by each perennial structure of pear trees to total remobilized N_{2016} in spring of 2017. N_{2016} means amount of fertilizer-N derived in 2016. Different small letters indicate significant differences ($p < 0.05$) among organs.

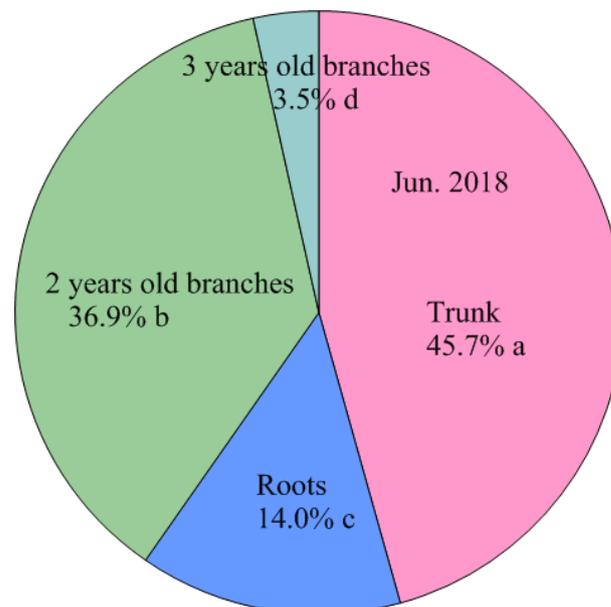


Figure 5. The ratio of $N_{2016\&2017}$ supplied by each perennial structure of pear trees to total remobilized $N_{2016\&2017}$ in spring of 2018. $N_{2016\&2017}$ means amount of fertilizer-N derived in 2016 and 2017. Different small letters indicate significant differences ($p < 0.05$) among organs.

From June to November 2017, the increase in N_{2016} in the trunk was 26.5 mg (Figure 2), which accounted for 56.7% of the N_{2016} withdrawn from leaves (Figure 6). The N_{2016} withdrawn from the senescent leaves to the shoots and roots was 11.9 and 8.0 mg, respectively, which were significantly lower than that to the trunk. No significant differences were observed in N_{2016} of the 2 year old branches between June and November in 2017.

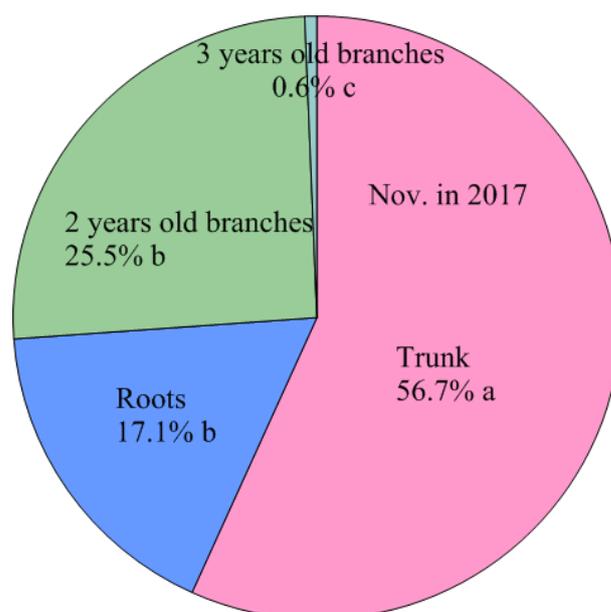


Figure 6. The distribution of N_{2016} , withdrawn from senescent leaves, among various perennial structures of pear trees in autumn 2017. N_{2016} means amount of fertilizer-N derived in 2016. Different small letters indicate significant differences ($p < 0.05$) among organs.

4. Discussion

4.1. N Absorbed from Soil and Fertilizer

The total N uptake from native soil and fertilizer increased with the age of pear trees, which is consistent with the results of Neto et al. [10]. From 2016 to 2018, the N requirement increased with dry mass weight of pear trees (Table 3), and the N absorption capacity of root systems improved, which resulted in more N being taken up by pear trees. In 2017, the increment in the total N of the whole trees from June to November was significantly higher than that from spring to June, because the capacity of N uptake from the soil was low due to the low soil temperature and activity of root systems in early spring [10,42,43]. Especially, the higher temperature and precipitation during the summer was helpful to the mineralization of soil organic matters [44–46], resulting in higher inorganic N content in soil, which can be taken up by pear trees. However, the opposite phenomenon was observed in the N derived from fertilizer, which resulted from the fertilizer being supplied in early spring and the fertilizer N concentration in the soil being relatively low after June.

4.2. Remobilization of the Stored N during Spring

N taken up in the former years was the main N source of leaves during the early spring. In June 2017, the N_{2016} in the leaves approximately accounted for 28.1% of the total N_{2016} in a whole tree, the corresponding value was 26.0% in June 2018 (Figures 2 and 3). Due to low soil temperature in early spring, the activity of root systems was low and N absorption capacity was weak, thus the growth of new organs was supported by the N stored in perennial tissues in pear trees. Similar results were determined by Neto et al. [10] for young pear trees. However, approximately 45% of storage N was partitioned to new growth of 5 years-old pear trees [30]. The reason for this discrepancy may be the weak capability of N storage of young pear trees, due to low biomass of perennial organs in our study. The storage N remobilized in the shoots and fruits was far lower than that in the leaves, and similar results were obtained by Kim et al. [19] and Bravo et al. [29]. The dry mass weight and N concentration in leaves were far higher than that in shoots and fruits, which resulted in the higher total N and stored N remobilized in leaves.

4.3. Storage N Derived from Perennial Structures in Spring

The storage N in the trunk was important to sustain the new growth in the next spring, approximately providing 32.7% of N_{2016} and 30.5% of $N_{2016\&2017}$ stored in the trunk to new organs in early spring 2017 and 2018 (Figures 2 and 3), respectively. Approximately 81.8% of N_{2016} and 58.4% of $N_{2016\&2017}$ stored in the 2 year old branches were provided for new growth in spring of 2017 and 2018, respectively. These indicated that the trunk and 2 year old branches of young pear trees play an essential role in the process of storage N remobilization. Similar conclusions were reported by Sanchez et al. [30] and Neto et al. [10] for pear trees, Millard and Neilsen [28] for apple trees and Bravo et al. [29] for walnut trees. A significantly lower N concentration in the trunk and 2 year old branches were observed in June than that in last November (Table 3). From the November to next June, the decline in the N concentration and increase in dry mass of the trunk and 2 year old branches indicated that mass stored N in these tissues was remobilized by new organs during the spring.

Approximately 16.7% of the N_{2016} stored in the roots was consumed by new organs in the spring of 2017, but only 14.2% of the $N_{2016\&2017}$ stored in the roots was recovered in new organs in the spring of 2018. The root system grew faster in 2018 than that in 2017, which was proven by the higher ratio of root dry weight to an entire tree in 2018. These resulted in more N being stored in roots. The N concentration was constant (1.2–1.3%) from November to next June (Table 3), the dry mass weight and N content in roots increased, in particular the significant differences from November of 2017 to June of 2018 were observed. These indicated that although the N stored in roots was also the source of new growth in spring, the N taken up by roots from the soil was higher than that supplied by the roots. The N restored in root systems during the autumn mainly met the requirement of root growth in young pear trees grafted on the vigorous rootstocks during the next spring.

In the spring of 2017 and 2018, the stored N remobilized in new organs were supplied by the trunk, 2 year old branches and roots in our study (Figures 4 and 5). However, the studies on the pear trees (*Pyrus communis* L.) grafted on the quince rootstocks show that almost all stored N recovered in new growth is supplied by the trunk and old shoots [10]. In our study, the trunk dry mass weight increased by 52% and 59% during the first and second year after planting, respectively, while the corresponding values are 39% and 19% in the studies on the dwarfing rootstocks [10], and similar trends were observed in the shoots. The aboveground of young pear trees grafted on the vigorous rootstocks grew more vigorously than that on the dwarfing rootstocks. However, slower growth rate of the root systems in young trees grafted on the vigorous rootstocks was observed by Zhao et al. [35], as compared with that on the dwarfing rootstocks. Therefore, more nutrients were used to meet the requirement of the vigorous structures growth, and less N remobilized in new organs was derived from the trunk and old shoots of the trees grafted on the vigorous rootstocks, as compared with the that of the dwarfing rootstocks. The studies on the seven year old pear trees grafted on quince rootstocks indicated that the reserve N stored in the roots is utilized for new growth, the aboveground parts grow vigorously, but biomass of the root system keeps constant [30,31]. These results also showed that when the root system stops growing and the nutrient requirement of their own growth decreases, the roots provide N for supporting the new growth of mature pear trees grafted on the dwarfing rootstocks. The study on the mature pear trees grafted onto vigorous rootstocks indicated that the remobilized N in spring were mainly supported by the root [36], maybe because the mass weight of each structure in mature pear trees was relatively stable, therefore the N taken up in previous years was mainly stored in root systems.

4.4. Redistribution of the Stored N Withdrawn from Senescent Leaves

Approximately 46.7 mg N_{2016} in the leaves was mobilized to the perennial tissues during autumn 2017, which accounted for approximately 61.5% of the N_{2016} in the leaves in the June. The similar results were reported by Sanchez and Righetti [8] for pear trees and Niederholzer et al. [9] for peach trees. The N content in leaves was significantly higher

than that in other tissues in June, which approximately accounted for 40% of total N in a whole tree, thus the N withdrawn from leaves effectively avoided the N losses during deciduous periods and increased the N use efficiency.

Until November, approximately 56.7% of the N_{2016} withdrawn from the senescent leaves were recovered in the trunk, which were far higher than that recovered in the branches and roots (Figure 4). A significant increase in total N content was also detected in the trunk, which resulted in the improvement in N concentration during this period (Table 3). These results proved that the trunk absorbed N from the soil and senescent leaves during the summer and autumn, which sustained new growth in the next spring.

From June to November 2017, approximately 25.5% of the N_{2016} withdrawn from the senescent leaves was transferred to the shoots (Figure 4), which led to a significant increase in N_{2016} in the shoots. An increase in the total N in the shoots was also observed, which was helpful in supporting the new growth during the next spring. However, there were no significant increases in N_{2016} in the 2 year old branches. The total N increase in this tissue only accounted for 1% of that in a whole tree, indicating that the 2 year old branches of young pear trees were not the main N storage tissues for supporting new growth in the next spring. N uptake of 2 year old branches was only used to meet the requirements of growth during this period. These results were confirmed by the fact that the $N_{2016\&2017}$ derived from the 3 year old branches only accounted for 3.5% of $N_{2016\&2017}$ recovered in new tissues in the spring of 2018 (Figure 6).

In our study, the total N in the roots significantly increased from June to November 2017, indicating that the root system was one of the main N storage structures during the winter. Studies on apple [47], sycamore [22], pear [31] and nectarine tree [6] have reported similar conclusions. About 17.1% of NFF withdrawn from the senescent leaves was recovered in roots (Figure 4), which were also significantly lower than that in trunk and shoots. These also proved that the trunk and 2 year old branches played a more important role than the roots in the cycling process of storage N, the N stored in root systems was mainly used to supply the root growth in young pear trees grafted on vigorous rootstocks during the next spring.

5. Conclusions

During the early spring, more than 25% of the N taken up in former years within a whole young pear tree was remobilized in the leaves, which was far higher than that in the shoots and fruits. Most of the storage N remobilized in new growth was supplied by the trunk and shoots (n-1), which were significantly higher than that by the root systems. From June to November, approximately 82.2% of the storage N withdrawn from senescent leaves was recovered in trunk and shoots, and only about 17.1% of storage N withdrawn from senescent leaves was recovered in roots. The trunk and 2 year old branches played a more important role than the roots in the cycling process of storage N in young pear trees. However, as compared with that on the dwarfing rootstocks, more storage N remobilized in new growth was supplied by the root system of the young pear trees grafted on the vigorous rootstocks. This may be because more N was needed to meet the requirement of the vigorous aboveground structures' growth, and less N remobilized in new organs was derived from the aboveground structures of young pear trees grafted on the vigorous rootstocks.

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