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Uptake and Allocation of Newly Absorbed Nitrogen in Young Pear Trees Grafted onto Vigorous Rootstocks (*Pyrus betulifolia*)

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Abstract: Nitrogen is one of the essential elements for fruit tree growth. Therefore, a ^{15}N tracing experiment was conducted to investigate the characteristics of nitrogen uptake and distribution in young pear trees. The experiment included: groups A and B, fertilized with 15 g unlabeled and 5 g labeled N per tree in the spring of 2017; and group C, fertilized with 20 g unlabeled N per tree in the spring of 2017 and 25 g unlabeled and 5 g labeled N per tree in the spring of 2018. Results indicated that new organs grew vigorously before June, and about 50% of the fertilizer nitrogen was concentrated in leaves. From June to November, the growth rate of storage organs was faster than that of new organs, and fertilizer nitrogen was mainly stored in the shoots, trunk, and roots. During the defoliation period, about 84% of the leaf fertilizer nitrogen was mobilized to the shoots, trunk, and roots. There was significant correlation between current fertilizer nitrogen and the dry mass weight of each organ after the completion of the nitrogen cycling of a whole year; the correlation coefficient was 0.98. Fertilizer nitrogen use efficiency increased with the ages of the pear trees. Therefore, nitrogen fertilizer rate should be formulated according to the tree growth characteristics and increased with the ages of the young pear trees; topdressing fertilizer should be applied in summer to ensure trunk and root development.

Keywords: fertilizer nitrogen; senescent leaves; new organs; woody structures; nitrogen use efficiency

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1. Introduction

Nitrogen is the most important mineral element in plant nutrition, including in that of deciduous fruit trees [1–5], and is important for organ differentiation, vegetative growth, and fruit set, affecting the photosynthetic performance of trees and fruit yield [3,6,7]. The nitrogen absorption and allocation characteristics of various organs of deciduous fruit trees are discrepant in different seasons. In early spring, the nitrogen stored in woody organs is remobilized to promote new leaf growth and flowering [8,9] owing to the weak absorption capability of the soil nitrogen resulting from the low soil temperature and the activity of the root systems. The remobilization of nitrogen stops between petal fall and the beginning of the fruit development, then the nitrogen absorbed by the roots increases over time, and the fertilizer nitrogen accounts for approximately 50% of the nitrogen storage in new growth until the middle of June [8–10]. After June, less of the nitrogen uptake in the current year is allocated to the leaves [6,11]. During the leaf senescence stage, the nitrogen in the leaves is transferred to the perennial organs [6,11,12], which support the new growth in the next spring. At the same time, the nitrogen uptake of the roots is stored in the woody structures [13,14].

Previous research has mainly focused on *Pyrus communis* L. grafted onto quince rootstocks (dwarfing rootstocks). The absorption and storage of nutrients in various organs of pear trees are affected by organ growth, and the growth characteristics of different organs are obviously different [8,12,15,16]. The growth characteristics of pear trees grafted onto *Pyrus betulifolia* (vigorous rootstocks) are significantly different from those grafted onto quince rootstocks [17]. Although studies on the absorption and allocation of nitrogen in various organs of pear trees grafted onto vigorous rootstocks have been conducted [3,18–20], most of aforementioned studies mainly focused on the nitrogen distribution in different organs from spring to fruit harvest. However, the nitrogen is mobilized from senescent leaves to the storage organs during the post-harvest period, and the nitrogen uptake of leaves accounts for a high proportion of the total nitrogen absorbed by the whole tree. A study on 6-year-old pear trees concluded that the nitrogen stored in leaves accounts for above 30% of that in the whole tree during the fruit harvest stage [3]; similar results were also reported by Ding et al. [18] and Wu et al. [19]. Therefore, the nitrogen withdrawn from senescent leaves is essential for the nitrogen status of pear trees. Although the nitrogen remobilized from the senescent leaves of mature pear trees was studied by Jiang et al. [15], the vegetative growth of young pear trees is more vigorous compared with that of mature pear trees, while the reproductive growth is opposite. This also leads to the differences in nitrogen absorption and allocation between young and mature pear trees. Moreover, higher nitrogen status is conducive to the vegetative growth of young pear trees [21–23]. Therefore, the investigation of the withdrawal of leaf nitrogen uptake in the current year is of great significance for the application of nitrogen fertilizer, the improvement of nitrogen use efficiency, and promotion of young pear tree growth in the next year.

Therefore, the objective of the present study was to evaluate the absorption and distribution of nitrogen, applied in current year, in various organs of young pear trees grafted onto vigorous rootstocks and the relationship between nitrogen absorption and the growth of young pear trees.

2. Materials and Methods

2.1. Experimental Site and Plant Materials

The experiment was conducted from March 2017 to June 2018 in a pear orchard located in Haidian District (Beijing, China, 39°54′39″ N, 116°24′48″ E). The experimental pear orchard has a continental monsoon climate with a mean annual temperature and annual precipitation sum of about 12.8 °C and 550 mm, respectively. The experimental pear trees (*Pyrus bretschneideri* Rehd 'Huangguan'), grafted onto the rootstock of *P. betulifolia* (vigorous rootstock), were two years old in 2017. The tree spacing was 1 m within rows and 3.5 m between rows (2857 tree·ha^{-1}). The defoliation period lasted from 3 November 2017 to 11 November 2017. The soil in the experimental orchard was loamy with a soil N and organic matter content of 0.5 g·kg^{-1} and 11.6 g·kg^{-1}, respectively, and the soil pH and bulk density were 8.2 and 1.4 g·cm^{-3}, respectively.

2.2. Experimental Design

The experimental trees were divided into groups A, B, and C (Table 1). The trees in groups A and B were fertilized with 20 g N (15 g unlabeled and 5 g labeled, enriched to 10.19 atom% ^{15}N) per tree in 2017. Group C was fertilized with 20 g unlabeled N in 2017 and 30 g N (25 g unlabeled and 5 g labeled) in 2018. The nitrogen fertilizer was dissolved with water and immediately sprayed onto the soil under the tree canopy on 20 March in both experimental years. Then, the fertilized area was immediately covered with soil to prevent ammonia volatilization [8,18]. The experiment had a randomized design and was performed in triplicate; each repetition in every group consisted of two pear trees; six pear trees per group were used to measure the dry mass weight and nitrogen content.

Table 1. The timing and rate of fertilization, the harvest sampling time, and quantity of pear trees in each group.

Group	Fertilization		Harvest Sampling Quantity of Pear Trees		
	2017	2018	2017		2018
			10 June	20 November	10 June
A	15 g/tree○ + 5 g/tree ●	-	6 trees	-	-
B	15 g/tree○ + 5 g/tree ●	-	-	6 trees	-
C	20 g/tree○	25 g/tree○ + 5 g/tree ●	-	-	6 trees

Note: ○ means unlabeled N; ● means labeled N (enriched to 10.19 atom% ^{15}N); - means no fertilization or no trees were harvested.

2.3. Measurements

Trees in groups A, B, and C were removed from the soil on 10 June and 20 November in 2017 and on 10 June in 2018, respectively. Six trees per group were washed, and each whole tree was separated into leaves, shoots (current year), 2- and 3-year-old branches, trunk, roots, and fruits. All organs of every tree were dried to constant weight at 70 °C, and the total dry mass weight of each organ per tree was measured with an electronic scale (YP10002, Shanghai Yueping Scientific Instrument Co., Ltd., Shanghai, China). Some parts of each organ per tree were used to measure the nitrogen content, which was determined with the Kjeldahl azotometer (QSY-II, Beijing Qiangsheng Analysis Instrument Manufacturing Centre, Beijing, China). The %^{15}N enrichment was measured using isotope ratio mass spectrometer (MAT251, Thermo Finnigan, San Francisco, CA, USA).

2.4. Calculations and Data Analysis

The total nitrogen content of each organ per tree was determined according to:

$$\text{N concentration of sample} = \frac{\text{N content of sample(g)}}{\text{dry mass weight of sample(g)}} \tag{1}$$

$$\text{Total N content of each organ (g)} = \text{N concentration of sample} \times \text{total dry mass weight of each organ(g)} \tag{2}$$

where N content of sample means the nitrogen content of the measured parts of each organ, and N concentration of sample means the nitrogen concentration of the measured parts of each organ per tree.

The nitrogen derived from the ^{15}N-fertilizer in each tree structure (%Ndff) represented the contribution percentage of nitrogen uptake from the ^{15}N-fertilizer applied in the current year and was determined according to the formula described by Carranca et al. [24]:

$$\%\text{Ndff} = \frac{\text{at.}\%^{15}\text{ excess of sample}}{\text{at.}\%^{15}\text{ excess of labelled fertilizer}} \times 100 \tag{3}$$

where at.%^{15} excess of sample is equal to atom% ^{15}N enrichment in sample minus the natural atom% ^{15}N enrichment, at.%^{15} excess of sample is equal to atom% ^{15}N enrichment in labeled fertilizer minus the natural atom% ^{15}N enrichment, and natural atom% ^{15}N enrichment is equal to 0.366.

The amount of nitrogen derived from labeled fertilizer applied in current year in different pear tree structures (NDFF) was determined by:

$$\text{NDFF(g)} = \text{total N content in organ (g)} \times \%\text{Ndff} \tag{4}$$

The amount of nitrogen derived from the total fertilizer applied in current year in different pear tree structures (NDFTF) was determined according to Wu et al. [19]:

$$\mathrm{NDFTF(g)} = \mathrm{NDFF(g)} \times \frac{\text{amout of total fertilizer(g)}}{\text{amout of labelled fertilizer(g)}} \tag{5}$$

The nitrogen use efficiency (NUE) was the ratio of NDFTF in the whole pear tree to the total fertilizer nitrogen applied in the current year [19].

2.5. Statistical Analysis

Standard deviation of each parameter was used to assess measuring accuracy and was calculated using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. The Characteristics of Fertilizer Nitrogen Uptake and Distribution in Various Organs of Young Pear Trees

In June 2017, the highest level of newly absorbed fertilizer nitrogen content was observed in the leaves, followed by the trunk, roots, and shoots, and the lowest was detected in the two-year-old branches (Table 2). Especially, about 57% of the fertilizer nitrogen uptake in the current year was taken up by the leaves, which was significantly higher than that taken up by other structures (Figure 1). The leaf nitrogen uptake in the current year decreased by about 84% in November as compared with the June, which was mobilized to the storage structures, including the shoots, roots, and trunk. In November, about 43% of the fertilizer nitrogen uptake from the current year was stored in the trunk, about 22% and 24% of the fertilizer nitrogen was recovered in shoots and roots, respectively, and only about 2% of the fertilizer nitrogen was detected in the two-year-old branches, which was also higher than that in June. In June 2018, about 48% and 22% of the fertilizer nitrogen uptake from the current year was recovered in the leaves and the roots, respectively, and approximately 13%, 9%, and 7% of the fertilizer nitrogen uptake in the current year was observed in the trunk, shoots, and old branches, respectively; only about 2% of the fertilizer nitrogen uptake in the current year was detected in the fruits. The fertilizer nitrogen use efficiency increased with the ages of the young pear trees (Table 3) and was 3.7%, 0.6%, and 7.1% from spring to June 2017, June to November 2017, and spring to June 2018, respectively.

Table 2. The fertilizer nitrogen uptake in the current year distributed in various organs during different stages.

Organs	N Uptake in Current Year (g/Tree)			
	2017			2018
	Spring–June	June–November	Spring–November	Spring–June
Leaves	0.423 ± 0.030	−0.355 ±0.006	0.069 ± 0.006	1.023 ± 0.098
Shoots	0.076 ± 0.009	0.108 ± 0.010	0.184 ± 0.010	0.190 ± 0.011
2-year-old branches	0.016 ± 0.000	0.003 ± 0.001	0.019 ± 0.001	0.117 ± 0.008
3-year-old branches	-	-	-	0.032 ± 0.004
Trunk	0.129 ± 0.010	0.250 ± 0.014	0.379 ± 0.014	0.269 ± 0.019
Root	0.099 ± 0.010	0.106 ± 0.010	0.206 ± 0.009	0.457 ± 0.023
Fruits	-	-	-	0.040 ± 0.004
Total	0.743 ± 0.057	0.112 ± 0.039	0.855 ± 0.039	2.128 ± 0.160

Note: Mean ± standard deviation (n = 6).

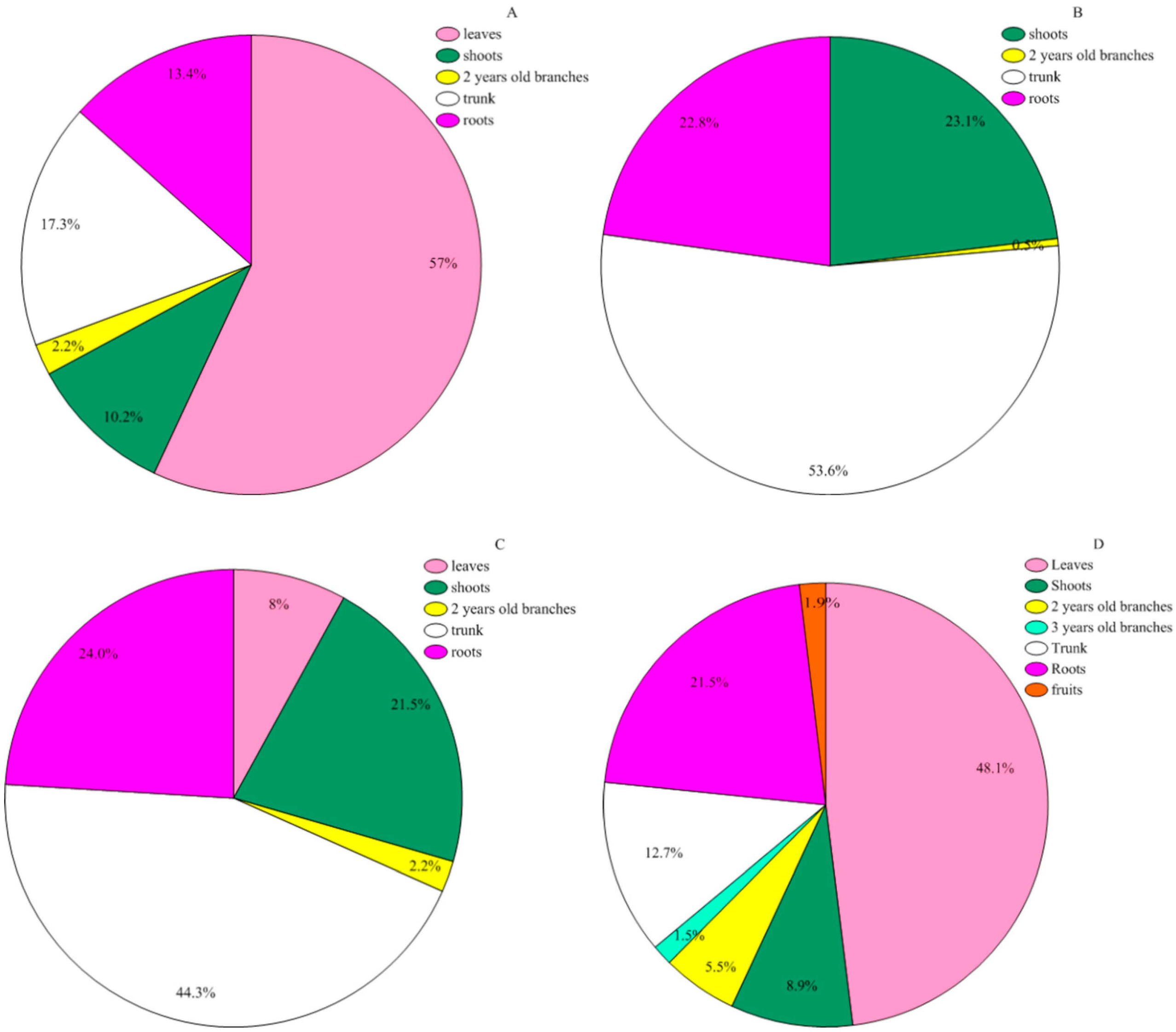

Figure 1. The ratio of fertilizer nitrogen uptake of each organ to the total fertilizer nitrogen uptake of all organs during various stages. (**A**) Spring to June 2017; (**C**) Spring to November 2017; (**D**) Spring to June 2018. (**B**) represents the ratio of fertilizer nitrogen uptake of each organ to the total fertilizer nitrogen uptake of all organs except for leaves from June to November 2017.

Table 3. NUE in each growth stage of young pear trees.

	Stage			
	2017			2018
	Spring–June	**June–November**	**Spring–November**	**Spring–June**
NUE	3.72 ± 0.28%	0.6 ± 0.19%	4.3 ± 0.19%	7.1 ± 0.53%

Note: Mean ± standard deviation (n = 6). NUE means the nitrogen use efficiency.

3.2. The Growth of Various Organs of Young Pear Trees and the Relationship between the Dry Mass Weight and Fertilizer Nitrogen Uptake by Each Organ

The new organs grew rapidly during spring, and the dry mass weight of leaves and shoots accounted for 20% and 8% of the total dry mass weight of the whole tree in June, respectively (Figure 2); the highest dry mass weight was observed in the trunk, which accounted for about 43% and 36% of the whole tree dry mass weight in 2017 and 2018, respectively. The dry mass weight in the perennial branches significantly increased from two to three years after planting, which accounted for about 4% and 14% of the total tree dry mass weight in 2017 and 2018, respectively. In November 2017, except for the leaves, the dry mass weight of each organ increased; in particular, the increase in the dry mass weight of the trunk, roots, and shoots was 287 g, 169 g, and 103 g, respectively, but a subtle difference was observed in the two-year-old branches.

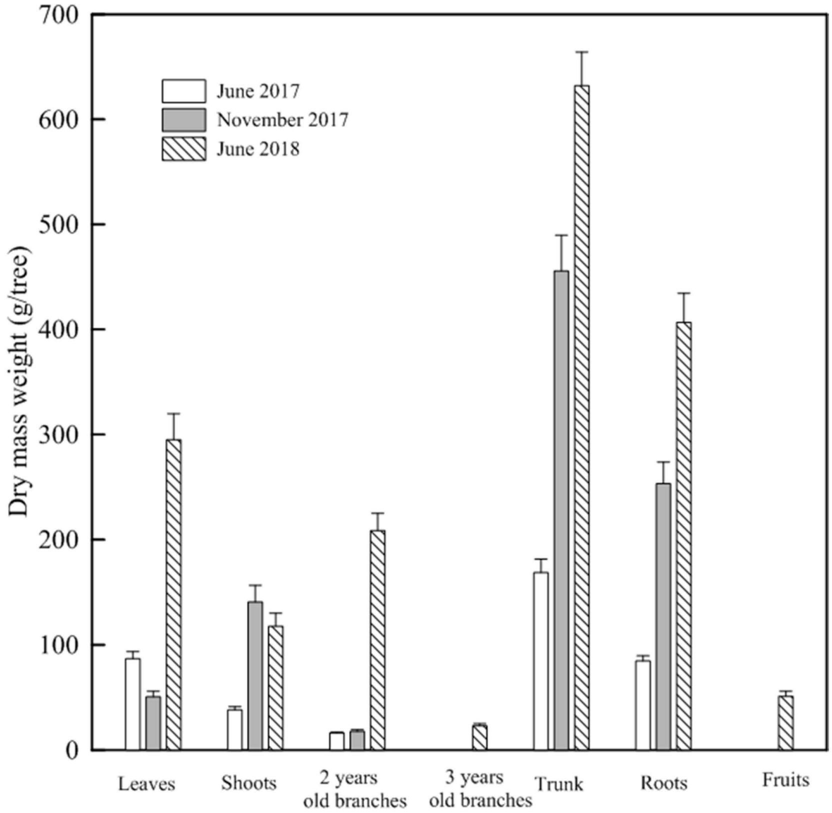

Figure 2. The dry mass weight of each organ during different stages. Vertical bars represent one standard deviation of the mean (n = 6).

In June 2017 and June 2018, there was no significant correlation between the dry mass weight and fertilizer nitrogen uptake of each organ (Table 4). However, a significant correlation between the dry mass weight and fertilizer nitrogen stored in each organ was observed in November 2017, and the fertilizer nitrogen uptake in the current year significantly increased with the dry mass weight.

Table 4. Spearman's correlation coefficient relating fertilizer nitrogen uptake in the current year to the dry mass weight of each organ during different stages.

	Stage		
	2017		**2018**
	Spring–June	**Spring–November**	**Spring–June**
Correlation coefficient	0.305 ^{ns}	0.98 **	0.396 ^{ns}

Note: ** indicates significant at $p < 0.01$, and ^{ns} means not significant.

4. Discussion

4.1. The Characteristics of Fertilizer Nitrogen Uptake and Distribution in Various Organs of Young Pear Trees

The new organs grew vigorously during the spring; thus, above 50% of nitrogen uptake from the fertilizer was absorbed by the leaves and shoots. Although the dry mass weight of the storage structures, including the roots and trunk, was significantly higher than that of the new organs, the fertilizer nitrogen uptake of the roots and trunk was significantly lower than that of the leaves before June, which resulted in a higher nitrogen concentration in the leaves. The leaves mainly grew in spring to generate the photosynthetic products to meet the requirement of the organic matter in the whole tree. Therefore, the nitrogen absorbed by the leaves was not only sustained by the nitrogen stored in the woody structures [9,12,25], but was also taken up from the fertilizer and the soil [9].

In November, the fertilizer nitrogen in the senescent leaves was significantly lower than in June, which resulted from the fact that some of the nitrogen in the leaves was mobilized to the perennial structures of the trees [26–28]. The nitrogen uptake in the current year remained in the senescent leaves and accounted for only 16% of the uptake in June. However, the study of mature pear trees carried out by Jiang et al. [15] reported that the nitrogen remaining in senescent leaves accounts for about 43% of the leaf nitrogen in the fruit harvest stage. The nitrogen storage capability of the woody structure of young pear trees in this study was weaker than that of mature pear trees; thus, more nitrogen was mobilized from the leaves to the perennial structures during the defoliation stage, which was used to meet the nutrient requirements of new growth in the next year. The study of young pear trees carried out by Wu et al. [29] reported that about 38% of leaf nitrogen, detected in June and taken up in previous years, is recovered in senescent leaves after autumn. This meant that, as compared with the leaf nitrogen uptake in previous years, much more leaf nitrogen uptake in current year was mobilized to the perennial organs during the defoliation stage. The study of Jiang et al. [15] also concluded that the nitrogen that reached leaves later in the season was more likely to be recycled than nitrogen that was present in the leaves as a result of accumulation from earlier periods. This may be because the leaf basic structure contains a portion of the nitrogen which cannot be mobilized to other organs. In early spring, before the root systems began to absorb the nutrients, the nitrogen in the leaves was supported by the storage nitrogen uptake in previous years; this portion of nitrogen constituted the basic structure of leaves and cannot be mobilized. Thus, as compared with the nitrogen uptake in the current year, less leaf nitrogen uptake in previous years was mobilized to perennial structures during autumn.

From June to November, the leaves grew slowly; the fertilizer nitrogen applied in current year was mainly taken up and stored in the roots, trunk, and shoots. The nitrogen stored in these organs was used to maintain the self-growth and sustain the new organ growth in the next spring; studies on pear [13], peach [14], sycamore [30], cherry [31–33], apple [34–36], nectarine [37], walnut [38–40], and persimmon [41] trees obtained similar conclusions. However, unlike the shoots, the increase in the fertilizer nitrogen in the two-year-old branches was far lower than that of the roots and the trunk; this may be due to the fact that the perennial branches grew slowly during this period and did not support the new growth in the next spring. This was consistent with the results of Wu et al. [29], who also concluded that less of the remobilized nitrogen recovered in early spring was supported by three-year-old branches, while above 30% of the storage nitrogen remobilized in spring is supplied by two-year-old branches, which were shoots in the previous year. Only about 2% of the fertilizer nitrogen was absorbed by the fruits in June 2018; the vegetative growth of young pear trees was absolutely dominant; the nutrient taken up by the young pear trees was preferentially allocated to the vegetative organs to ensure the growth of the pear tree. Therefore, the nitrogen application in the summer was a necessary condition to promote the growth of the young pear trees and new growth in the next spring.

With the young pear tree ages increased, the root system grew vigorously and distributed deeper and wider; the absorption of the roots was also especially improved. Moreover, the dry mass weight of the whole pear trees and the requirement for the nitrogen increased obviously. Therefore, the fertilizer nitrogen use efficiency significantly increased from 2017 to 2018; moreover, the nitrogen use efficiency from spring to June in 2018 was significantly higher than that from spring to November in 2017. A similar result was obtained by Neto et al. [13], who reported a higher nitrogen use efficiency than that in our study due to split fertilization. Because split fertilization can effectively prevent nitrogen from lodging in the soil and reduce nitrogen losses from volatilization and leaching, it results in an improvement of the nitrogen use efficiency [17,42–45].

4.2. The Relationship between the Nitrogen Uptake from Fertilizer and Dry Mass Weight of Each Organ

The dry mass weight of each organ increased with the young pear trees' ages, which was one of the reasons for the improvement of the nitrogen use efficiency. Before June, the dry mass accumulation was mainly observed in leaves, while the roots and trunk grew preferentially from June to November, which was consistent with the fertilizer nitrogen absorption.

The cycling of nitrogen in a pear tree consists of storage nitrogen remobilization during early spring and leaf nitrogen being withdrawn during autumn [9,12]. Therefore, although the nitrogen was used to meet their own growth of each organ [20], the absorption of nitrogen did not match with the dry mass weight of each organ. In early spring, the nitrogen in the leaves was mobilized from the perennial organs and the soil, which resulted in the relatively high and low nitrogen content in leaves and perennial organs in June, respectively, and the nitrogen concentration in leaves was far higher than that in other organs [19,29]. Therefore, no significant correlation between the fertilizer nitrogen and dry mass weight of each organ was observed in June. In November, the cycling of nitrogen in the whole pear tree over the course of one year was completed, and the nitrogen stored in each organ was a constituent element of this organ. Therefore, significant correlation between the nitrogen uptake from fertilizer and the dry mass weight of each organ was observed.

5. Conclusions

From spring to June, about 50% of nitrogen uptake in the current year was recovered in the leaves, which was far higher than that recovered in other organs. The nitrogen uptake in the current year stored in old branches was significantly lower than that in other structures, and the least nitrogen uptake from the current year was observed in two- and three-year-old branches in June 2017 and 2018, respectively. In autumn, the nitrogen uptake in the current year was mainly concentrated in the perennial organs. As compared with the June, about 84% of the leaf nitrogen uptake in the current year was transferred to the woody structures in the November. The nitrogen in the leaves was mobilized from and to perennial structures in early spring and autumn, respectively. Therefore, a significant correlation between the nitrogen uptake in current year and the dry mass weight of each organ was observed after the completion of the nitrogen cycling of a whole year, but no significant correlation was detected in June. The nitrogen use efficiency gradually increased with the pear trees' ages because of the increase in dry mass weight and the requirement for the nutrition of pear trees and the nitrogen absorption capability of root systems. Therefore, more fertilizer nitrogen should be applied with the increase in young pear trees' ages, and the topdressing should be applied in summer to ensure the trunk and root development of young pear trees.

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