

# N Utilization, Residual and Loss Characteristics of Spring-Topdressing ( $^{15}\text{N}$ -urea) Pear Orchards in the Old Course of the Yellow River Area

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**Abstract:** In the old course of the Yellow River area, most orchards are over-applied with nitrogen (N) fertilizers. To improve N management in this area, a  $^{15}\text{N}$  tracing experiment was conducted to investigate the absorption, distribution and loss of spring-topdressing urea in pear orchards from March to August 2019. The 7-year-old Sucui 1 pear was used as the test material, and 277.5 g of ordinary urea and 15 g of  $^{15}\text{N}$ -urea N were evenly applied to each plant. The N absorption, distribution and utilization efficiency of different organs from the flowering stage to the post-harvest stage were analyzed, and the residual and loss of N in the soil were also discussed. The N fertilizer utilization rate increased with the advancement of the phenological period. The N fertilizer utilization rate in the full bloom period is 10.39%, which is the fastest growing period, and reached a maximum of 23.62% in the post-harvest stage. In the young fruit stage, the amount of N derived from labeled fertilizer (%Ndff) of the fruit was only 1.02%, and most of the new vegetative organs were above 1%. Residual amount in the 20–40 cm soil layer was significantly higher than that in other soil layers. Direction of N fertilizer is N fertilizer loss>soil residue>tree absorption. N loss in the fruit expansion stage and the harvest stage is higher, which are 3.76 g and 3.74 g, respectively. N utilization rate in this area is low throughout the year. There is nutrient competition between reproductive growth and vegetative growth, which can be effectively alleviated by spring top-dressing. The N loss during fruit expansion and harvesting is serious. Attention should be paid to split fertilizer application and the timely supplementation of an appropriate amount of N fertilizer to improve N use efficiency.

**Keywords:** pears;  $^{15}\text{N}$ -urea; nitrogen absorption; N residual; nitrogen loss



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## 1. Introduction

China is the main producer of pears in the world. In China, the cultivation area and yield of pears are the first in the world, accounting for 69.1% and 68.4% of the world's total cultivation area and total output, respectively [1]. In China, fruit growers have unilaterally pursued high yield and large fruit, resulting in an amount of nitrogen (N) fertilizer application far exceeds the needs of pear trees. At present, the amount of N fertilizer used in pear orchards was as high as 540 to 967 kg·ha<sup>-1</sup> [2]. However, the N fertilizer utilization rate in Chinese pear orchards was only 1/3 to 1/2 of that in foreign countries [3]. Excessive application of N fertilizers will not only reduce fertilizer use efficiency (FUE) and fruit quality [4–6], but also cause problems such as soil quality degradation and environmental pollution [7,8].

Topdressing in pear orchards is usually performed in two stages: before flowering stage and the fruit expansion stage. Jordan et al. [9] showed that N application in spring is crucial for the growth of flower buds and fruits, and can effectively alleviate nutrient competition. According to the survey, the proportion of pear orchards that applied N fertilizer before germination was as high as 54.3% [10].

N utilization efficiency is affected by many factors. After N fertilizer is applied to the soil, part of it is absorbed by fruit trees; the other part remains in the form of inorganic N or organically combined in the soil; another portion of N is lost to the environment through ammonia volatilization, nitrification, denitrification, and runoff [11]. Studies have shown that soil organic matter content and pH can affect ammonia volatilization and nitrification and denitrification in soil, which in turn affects soil N retention and loss [11–14]. In addition, Ren et al. found that different climate types have an impact on the N utilization efficiency of chemical fertilizers and organic fertilizers, which is manifested as temperate continental climate zone > temperate monsoon climate zone > subtropical monsoon climate zone [15]. Under the same soil fertility, Liu et al. found that the yield and N utilization efficiency of wheat in Fujian were significantly lower than those in Sichuan. This is probably because the rainfall in the wheat season in Fujian (815 mm) was much higher than that in Sichuan (180 mm). The loss of nitrate N was intensified under the driving of high rainfall. So the nitrate N available for wheat in Fujian was insufficient [16]. Therefore, under different soil and climate conditions, the ways and intensities of N transformation and loss are different, and the N utilization efficiency will also be greatly different [17].

The old course of the Yellow River area (including Henan Province, Anhui Province and Jiangsu Province) is one of the four major pear production areas in China. It belongs to the dominant production area of white pear and sand pear. According to statistics, the area of pear cultivation in the old course area of the Yellow River reached  $1.43 \times 10^5$  ha, and the annual output  $3.16 \times 10^6$  t [18]. The soil in this area originates from the river alluvium formed by the flooding and diversion of the Yellow River many times. The farmland type is mainly sandy soil, which is characterized by heavy sandiness, low organic matter content and poor physical structure [19]. The old course of the Yellow River area is located in the transition zone from warm temperate zone to subtropical zone. Most farmers in this area usually apply nitrogen fertilizer once in spring [20]. The average annual urea input of pear orchard was 2.10 kg per plant, and the average input of three-nutrient compound fertilizer (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O = 15:15:15) is 7.87 kg per plant. The input N through organic fertilizer only accounts for 18.18% of the total input N [10]. Under the high intensity of N fertilizer input, soil available N content in most pear orchards was still at a low level, with an average of  $49.49 \text{ mg} \cdot \text{kg}^{-1}$ , which was lower than the lower limit of the suitable range ( $60\text{--}130 \text{ mg} \cdot \text{kg}^{-1}$ ) [21]. Due to the high and concentrated precipitation in summer, the phenomenon of N loss and low N use efficiency in pear orchards is common [22]. It is of great significance to elucidate the nitrogen absorption, utilization and loss in orchard production system for the better development of the pear industry in this region.

Nitrate N was the main form of nitrogen absorbed and utilized by pear roots [23]. Part of the absorbed nitrate N was reduced and utilized in roots, and the remaining nitrate N was transported in the xylem to shoots for utilization and storage. The ability of absorbing and accumulating N in different organs of fruit trees shifted with the change of growth center [24]. After N fertilizer was applied to apple trees in spring, the distribution rate of N in leaves and annual branches of the trees increased rapidly during the shoot growth period. The N were mainly transported to the fruit at the fruit expansion and fruit maturity stage. After fruit harvesting, N was mainly transported to the trunk, root and other organs of trees. The N competition ability of different growth centers was significantly affected by climate, fertilization and tree growth potential. The distribution of <sup>15</sup>N reflects the nutrient competition of organs in different periods of the tree. The fertilization plan formulated according to the requirements of trees at different periods can effectively reduce the application of N fertilizer and improve utilization rate.

At present, the research on N nutrition and N fertilizer residue and loss rules of pear trees is mostly concentrated in Xinjiang and the Bohai Bay area of China, but the relevant research in the old course Yellow River area is still blank. Climate and soil factors can affect the duration and effect of N fertilizer. We hypothesized that N absorption and distribution of pear trees in different phenological periods and N loss of fertilizer are different in different areas. In this study, we used <sup>15</sup>N fertilizer to label early-maturing sand pears. The

results of experiment do not concern the utilization of nitrogen from fertilizers in the form of urea but N originated from the small doses of nitrogen in the form of labeled urea. The method of application N-fertilizer make possible to analyze the fate of nitrogen from the spring application of urea. The objective of our research is: (i) the N fertilizer absorption and utilization by trees after spring topdressing and the duration of N fertilizer effect in the old course of the Yellow River area; (ii) the residue and loss status of N fertilizer in soil. We expect that this study will help improve N management in pear orchards in this region.

## 2. Materials and Methods

### 2.1. Site Description

The experiment was carried out in the experimental demonstration base (117°11' E, 34°15' N) of Xuzhou Agricultural Science Research Institute in Xuhuai District, Jiangsu Province. The average annual precipitation is 841 mm, the rainfall is mainly concentrated from July to October. The annual average temperature is 14.5 °C. The total precipitation from March to August 2019 was 641.2 mm. The daily average rainfall and daily average temperature during the test period are shown in Figure 1. The orchard soil is fluvo-aquic soil. The basic physical and chemical properties of soil were: organic matter 12.00 g·kg<sup>-1</sup>, pH 7.1, nitrate N 24.12 mg·kg<sup>-1</sup>, ammonia N 55.28 mg·kg<sup>-1</sup>, available phosphorus 65.48 g·kg<sup>-1</sup>, and available potassium 148.41 mg·kg<sup>-1</sup>. Soil bulk densities for 0–20, 20–40, 40–60, 60–80, 80–100 and 100–120 cm soil layers are 1.34, 1.53, 1.42, 1.43, 1.44, 1.42 kg·m<sup>-3</sup>, respectively.

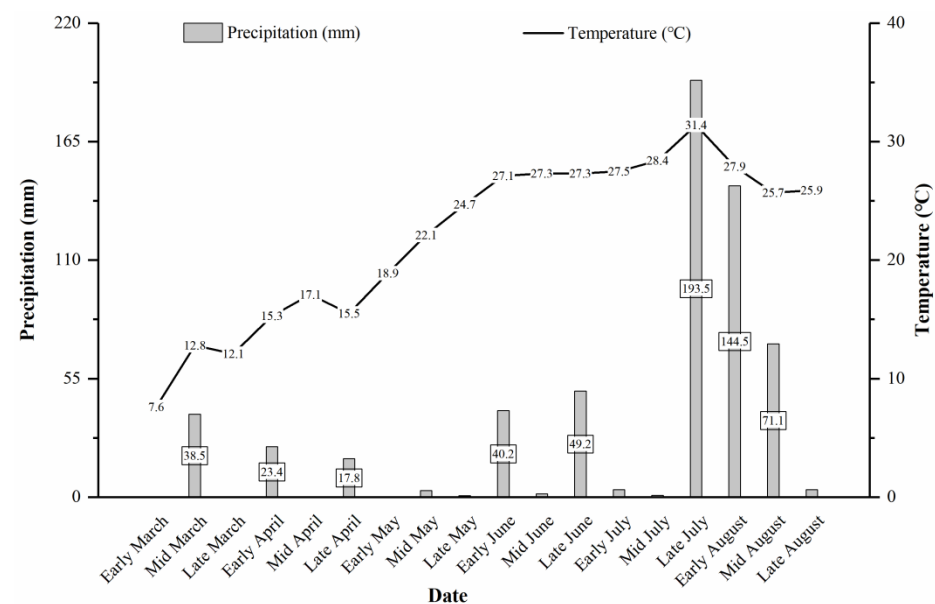


**Figure 1.** The daily mean air temperature (line) and precipitation (histogram) during the experimental period.

### 2.2. Orchard Management

The orchard was built in 2007. The orchard was planted with Sucui 1 pear (*Pyrus pyrifolia* (Burm.f.) Nakai. cv. Sucui 1), grafted on *Pyrus betulifolia* Bunge rootstock. Row spacing was 5 m and plant spacing within rows was 3 m. Selected 15 Sucui 1 pears with basically the same growth potential and no pests and diseases. In the autumn of 2018, 20 kg of fully decomposed cow dung and 1 kg of compound fertilizer (N:P:K = 15:15:15) were applied to each plant. The ditch was opened on one side for fertilization. The distance between the ditch and the tree trunk was 0.7 m. The length, width and depth of the fertilization ditch were 0.6 m, 0.4 m and 0.5 m, respectively.

### 2.3. Experimental Materials and Experimental Design

Fertilized on 7th March 2019. The method of fertilization involved digging a circular trench with a radius of 30 cm and a width and depth of 20 cm around each pear tree.

Applied 277.5 g ordinary urea and 15 g  $^{15}\text{N}$ -urea ( $\text{CO}(^{15}\text{NH}_2)_2$ , 10.16% abundance, Shanghai Research Institute of Chemical Industry) to each tree at the same time. Evenly mix ordinary urea and  $^{15}\text{N}$ -urea before application. Then applied  $\text{K}_2\text{SO}_4$  266 g and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  345 g to each tree. The fertilized area was covered immediately with soil to prevent ammonia volatilization. Water 10 L immediately after fertilization. No other fertilizers were applied during the whole experiment. Other management measures of orchard were administered in the usual manner.

#### 2.4. Plant and Soil Sampling

The whole plant was sampled at full flowering stage (4 April), young fruit (11 May), fruit expansion (12 June), fruit maturity (6 July) and post-harvest (4 August), and 3 plants were analyzed each time. The collection of pear trees: first fruit or flowers, then shoot leaves were collected. One-year-old shoots, perennial branches, and the trunk of each tree were collected using shears and saws. Lastly, roots were collected by excavating the whole tree; the root sampling area was a rectangle (5 m (length)  $\times$  3 m (breadth)) centered on the trunk; the depth was 1.2 m. The whole plant was divided into: fine root ( $d \leq 0.2$  cm), thick root ( $d > 0.2$  cm), the trunk, perennial branches, biennial branches, annual branches, new shoots, leaves, flowers (fruits), among which the trunk, perennial branches, biennial branches and annual branches are subdivided into xylem and bark.

Soil collection was performed as follows: 3 evenly distributed sampling sites were selected in the canopy projection area. In the vertical direction of each sampling site, soil samples at depths of 0–20, 20–40, 40–60, 60–80, 80–100 and 100–120 cm were obtained, and 3 soil samples in each layer were evenly mixed into one replicate.

After each sample was washed in the order of clean water  $\rightarrow$  detergent  $\rightarrow$  clean water  $\rightarrow$  1% hydrochloric acid  $\rightarrow$  deionized water three times. All samples were oven-dried at 105 °C for 30 min, then dried at 80 °C to constant weight, ground by an electric mill, passed through a 60 mesh sieve, mixed and bagged for standby. The soil samples were dried in the shade, crushed by an electric mill, screened through a 60 mesh sieve, and bagged for standby.

#### 2.5. Laboratory Analysis

Soil and plant total N were determined by micro-Kjeldahl method [25]. The liquid that remained after determining total N was concentrated by heating in a water bath with a few drops of dilute sulfuric acid for  $^{15}\text{N}$  determination.  $^{15}\text{N}$  abundance was determined by isoprime100 stable isotope ratio mass spectrometer, and soil bulk density was determined by the ring knife method.

#### 2.6. Computations

Sources of total N absorbed by the plant include fertilizers (normal urea and  $^{15}\text{N}$ -urea) and soil reserves. The labeled urea has the same chemical formula and chemical properties as the common urea. After mixing and watering, ordinary urea and  $^{15}\text{N}$ -urea were absorbed by the tree with the same probability. Therefore, the absorption and utilization of  $^{15}\text{N}$  urea can reflect the absorption and utilization of ordinary urea. The  $^{15}\text{N}$  formula is calculated according to [26]:

$$\text{Total N in each organ (g)} = \frac{\text{each organ N concentration (\%)}}{100 \times \text{organ dry weight (g)}}$$

$$\%N_{dff} = \frac{\text{abundance of } ^{15}\text{N} \text{ in plant} - \text{natural abundance of } ^{15}\text{N}}{\text{abundance of } ^{15}\text{N} \text{ in fertilizer} - \text{natural abundance of } ^{15}\text{N}} \times 100\%$$

$$^{15}\text{N distribution rate (\%)} = \frac{^{15}\text{N absorbed by each organ from fertilizer (mg)}}{\text{total } ^{15}\text{N absorbed by plant from fertilizer (mg)}} \times 100\%$$

$$^{15}\text{N distribution rate}(\%) = \frac{^{15}\text{N absorbed by each organ from fertilizer (mg)}}{\text{total } ^{15}\text{N absorbed by plant from fertilizer (mg)}} \times 100\%$$

$$^{15}\text{N absorbed by each organ from fertilizer (mg)} = \text{total N in each organ (g)} \times \% \text{Ndff}$$

$$^{15}\text{N utilization rate}(\%) = \frac{\text{Ndff}(\%) \times \text{total N of organs (g)}}{^{15}\text{N fertilizer (g)}} \times 100\%$$

$$^{15}\text{N residual rate}(\%) = \frac{^{15}\text{N residue in soil (g)}}{^{15}\text{N fertilizer (g)}} \times 100\%$$

$$^{15}\text{N loss rate}(100\%) = 100\% - ^{15}\text{N utilization rate}(\%) - ^{15}\text{N residual rate}(\%)$$

where natural abundance of  $^{15}\text{N}$  was equal to 0.366%.

### 2.7. Statistical Analysis

The data are presented as means ( $\pm$ SE). Statistical analyses of the data were performed using the SAS. Data were analyzed using one-way factorial analysis of variance (MEAN). Differences were considered significant at a probability level of  $p < 0.05$ . Use Origin 2017b to draw the charts in this article

## 3. Results

### 3.1. N Uptake and Utilization of Pear Trees in Different Phenological Periods

Total N of tree increased gradually with the advance of phenological period (Figure 2A). It reached 77.8, 109.6, 169.2, 207.5 and 219.9 g in the five stages respectively. The total N of the tree increased the most in the fruit expansion stage, 59.6 g more than that in the previous stage. The trend of  $^{15}\text{N}$  uptake was similar to that of total N (Figure 2B), and the total uptake of  $^{15}\text{N}$  was 3.5 g during the whole year. The utilization rate of N fertilizer by the tree gradually increased during the whole fruit growth and development period (Figure 2C). From 7th March to the full flowering stage (4th April), the tree absorbed relatively fast, and the fertilizer utilization rate was 10.39%. In the following four stages, the cumulative N utilization rate of the tree was 16.55%, 19.88%, 22.13% and 23.62%. At the young fruit stage, the fruit expansion stage, the fruit maturity stage and the post-harvest stage, the N utilization rate in each period was 6.16%, 3.33%, 2.25% and 1.49%. The N utilization efficiency was the highest at full flowering stage, followed by the young fruit stage and post-harvest stage.

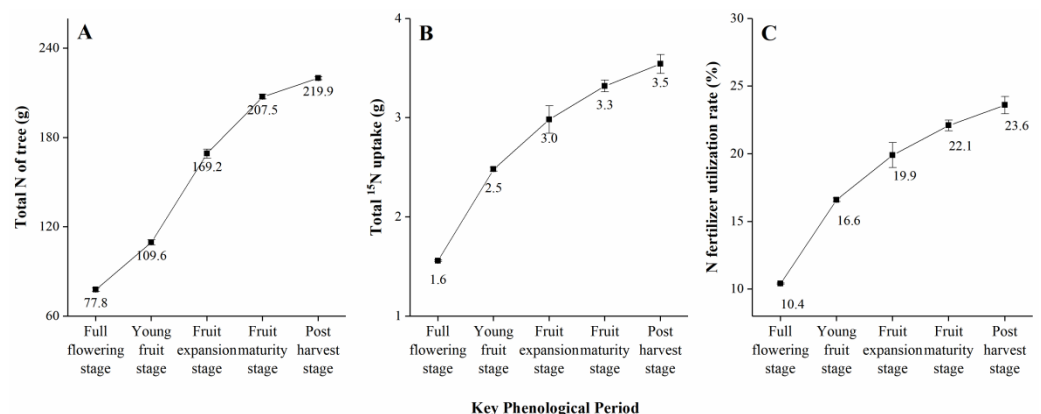


**Figure 2.** Total N of tree (A), total  $^{15}\text{N}$  uptake of tree (B), N utilization rate (C) in different phenological period.

### 3.2. N Utilization and Allocation by Trees in Different Phenological Stages

#### 3.2.1. %Ndff of $^{15}\text{N}$ in Different Organs in Different Periods

%Ndff reflects the ability of plant organs to absorb  $^{15}\text{N}$  from fertilizer.  $^{15}\text{N}$  was determined in the first sample collected (Table 1), indicating that it can be absorbed by plants 3–4 weeks after the application of N fertilizer in spring. In full flowering stage, the %Ndff of fine roots was the highest, reaching 23.78%. Followed by thick root, trunk xylem, respectively, 4.02%, 3.66%. During this stage, the roots began to absorb  $^{15}\text{N}$  applied in spring and transferred part of  $^{15}\text{N}$  to the new aboveground organs. Leaf %Ndff value was the lowest, only 0.17%. Only 1.09% spent. Flowers are only 1.09%. It shows that the N used was mainly storage N in the full flowering stage. After entering the young fruit stage, the %Ndff of the fruit was only 1.02%, and most of the new vegetative organs were above 1%. There was nutritional competition in this period; the %Ndff of thick roots decreased from 4.02% to 3.19%, and the %Ndff of fine roots decreased from 23.78% to 4.62%. In this period, roots were mainly used as nutrient output organs. During fruit maturity stage, fruit %Ndff reached the maximum (4.00%), the  $^{15}\text{N}$  distribution of vegetative organs decreased. The reproductive growth center had strong competitiveness for N.

**Table 1.** The %Ndff of different organs during key phonological phase.

| Tree Organs        |            | Full Flowering Stage | Young Fruit Stage    | Fruit Expansion Stage | Fruit Maturity Stage | Post-Harvest Stage |
|--------------------|------------|----------------------|----------------------|-----------------------|----------------------|--------------------|
| Leaves             |            | $0.17 \pm 0.01^e$    | $2.48 \pm 0.03^b$    | $1.98 \pm 0.02^c$     | $1.43 \pm 0.03^d$    | $4.1 \pm 0.08^a$   |
| Flowers (Fruit)    |            | $1.09 \pm 0.00^c$    | $1.02 \pm 0.02^c$    | $3.74 \pm 0.00^b$     | $4.00 \pm 0.02^a$    |                    |
| New shoots         |            | $1.07 \pm 0.01^d$    | $1.10 \pm 0.00^d$    | $2.00 \pm 0.05^c$     | $3.11 \pm 0.01^b$    | $5.68 \pm 0.06^a$  |
| Annual branches    | xylem      | $2.63 \pm 0.00^b$    | $2.20 \pm 0.00^c$    | $2.21 \pm 0.01^c$     | $1.82 \pm 0.02^d$    | $3.56 \pm 0.07^a$  |
|                    | bark       | $1.24 \pm 0.02^d$    | $2.11 \pm 0.00^b$    | $2.16 \pm 0.03^b$     | $2.03 \pm 0.02^c$    | $2.95 \pm 0.04^a$  |
| Biennial branches  | xylem      | $2.40 \pm 0.03^c$    | $1.93 \pm 0.02^d$    | $0.96 \pm 0.01^e$     | $3.12 \pm 0.01^b$    | $4.83 \pm 0.06^a$  |
|                    | bark       | $1.74 \pm 0.03^d$    | $1.91 \pm 0.00^c$    | $0.78 \pm 0.01^e$     | $2.37 \pm 0.04^b$    | $4.02 \pm 0.06^a$  |
| Perennial branches | xylem      | $2.19 \pm 0.01^b$    | $1.27 \pm 0.00^d$    | $0.90 \pm 0.01^e$     | $2.00 \pm 0.00^c$    | $2.59 \pm 0.06^a$  |
|                    | bark       | $1.11 \pm 0.02^b$    | $1.07 \pm 0.03^{bc}$ | $0.31 \pm 0.04^d$     | $1.04 \pm 0.03^c$    | $2.35 \pm 0.04^a$  |
| The trunk          | xylem      | $3.66 \pm 0.00^a$    | $1.93 \pm 0.02^c$    | $0.62 \pm 0.07^e$     | $1.42 \pm 0.01^d$    | $3.18 \pm 0.03^b$  |
|                    | bark       | $0.44 \pm 0.01$      | $0.94 \pm 0.00$      | $0.31 \pm 0.08$       | $0.39 \pm 0.01$      | $2.44 \pm 0.90$    |
| Roots              | Thick root | $4.02 \pm 0.04^a$    | $3.19 \pm 0.00^a$    | $2.12 \pm 0.02^b$     | $0.97 \pm 0.00^c$    | $0.29 \pm 0.02^d$  |
|                    | Fine root  | $23.78 \pm 0.33^a$   | $4.62 \pm 0.04^c$    | $4.44 \pm 0.04^c$     | $6.19 \pm 0.01^b$    | $3.73 \pm 0.17^d$  |

Note: Each value is the mean of three replication  $\pm$  SE ( $n = 15$ ); different small letters on the same row indicate significant differences between the same organs at different sampling periods ( $p < 0.05$ ).

#### 3.2.2. Distribution Rate of $^{15}\text{N}$ in Different Organs in Different Periods

The percentage of  $^{15}\text{N}$  in each organ to the total amount of  $^{15}\text{N}$  in the whole plant reflects the distribution of fertilizer in the tree and the law of migration in each organ. After the application of  $^{15}\text{N}$  to pear trees, in full flowering stage, the distribution of  $^{15}\text{N}$  was mainly in the roots, among which fine roots were most distributed (Table 2). The roots accounted for 56.59% of the total distribution rate, while the above-ground parts accounted for 43.41% of the total distribution rate. With the advance of phenology stage, the distribution rate of  $^{15}\text{N}$  in all organs above ground (flowers and fruits, new shoots and leaves and non-new organs) showed an upward trend. At the same time, the distribution rate of  $^{15}\text{N}$  in the root decreased gradually. The distribution rate of fine roots decreased significantly, by 37.14%, 37.12%, 36.72%, and 40.18% respectively. On the contrary, the distribution rate of leaves  $^{15}\text{N}$  rises faster. Compared with the full flowering stage, the distribution of leaves  $^{15}\text{N}$  increased by 38.95%, 33.26%, 19.12%, 47.01% in the following four stages. From the fruit expansion stage, the distribution of N in leaves began to decrease, while the distribution proportion of fruit began to increase. During the fruit maturity stage, the N distribution of leaves was at a low level, and the distribution of  $^{15}\text{N}$  to other organs

also gradually decreased. The distribution rate of fruits  $^{15}\text{N}$  rapidly increased to 40.08%, an increase of 188.40%. After the fruit was harvested, the leaves became the N distribution reservoir again. The partitioning rate of  $^{15}\text{N}$  was 47.88%.

**Table 2.** Proportion of  $^{15}\text{N}$  in different tissue to total  $^{15}\text{N}$  uptake in different key phenological periods (%).

| Tree Organs        |            | Full Flowering Stage | Young Fruit Stage  | Fruit Expansion Stage | Fruit Maturity Stage | Post-Harvest Stage |
|--------------------|------------|----------------------|--------------------|-----------------------|----------------------|--------------------|
| Leaves             |            | $0.87 \pm 0.02^e$    | $39.82 \pm 0.41^b$ | $34.13 \pm 0.65^c$    | $19.99 \pm 0.81^d$   | $47.88 \pm 1.00^a$ |
| Flowers (Fruit)    |            | $4.63 \pm 0.15^c$    | $1.54 \pm 0.08^d$  | $16.65 \pm 0.44^b$    | $39.78 \pm 0.54^a$   |                    |
| New shoots         |            | $3.44 \pm 0.26^b$    | $0.92 \pm 0.02^e$  | $2.73 \pm 0.2^d$      | $4.88 \pm 0.03^c$    | $10.02 \pm 0.73^a$ |
| Annual branches    | xylem      | $2.33 \pm 0.05^a$    | $1.24 \pm 0.08^b$  | $0.96 \pm 0.05^c$     | $1.11 \pm 0.04^{bc}$ | $2.34 \pm 0.18^a$  |
|                    | bark       | $1.6 \pm 0.01$       | $0.91 \pm 0.06$    | $0.55 \pm 0.02$       | $0.77 \pm 0.04$      | $0.45 \pm 0.03$    |
| Biennial branches  | xylem      | $3.74 \pm 0.11^a$    | $1.09 \pm 0.06^c$  | $0.85 \pm 0.04^d$     | $1.36 \pm 0.02^b$    | $3.61 \pm 0.07^a$  |
|                    | bark       | $1.47 \pm 0.00^a$    | $0.68 \pm 0.03^c$  | $0.36 \pm 0.01^e$     | $0.55 \pm 0.01^d$    | $1.04 \pm 0.03^b$  |
| Perennial branches | xylem      | $11.47 \pm 0.27^a$   | $3.98 \pm 0.07^c$  | $3.23 \pm 0.09^d$     | $2.36 \pm 0.1^e$     | $4.73 \pm 0.29^b$  |
|                    | bark       | $4.64 \pm 0.09^a$    | $1.79 \pm 0.2^b$   | $0.23 \pm 0.03^e$     | $0.59 \pm 0.04^d$    | $0.94 \pm 0.03^c$  |
| The trunk          | xylem      | $7.39 \pm 0.25^c$    | $11.93 \pm 0.05^b$ | $6.42 \pm 0.66^c$     | $4.59 \pm 0.16^d$    | $18.89 \pm 1.12^a$ |
|                    | bark       | $1.84 \pm 0.04^c$    | $2.72 \pm 0.13^b$  | $0.84 \pm 0.17^d$     | $0.52 \pm 0.04^e$    | $4.53 \pm 0.28^a$  |
| Roots              | Thick root | $14.1 \pm 0.55^c$    | $28.02 \pm 0.55^a$ | $27.68 \pm 0.82^a$    | $17.73 \pm 0.58^b$   | $3.26 \pm 0.14^d$  |
|                    | Fine root  | $42.49 \pm 0.57^a$   | $5.35 \pm 0.12^c$  | $5.37 \pm 0.33^c$     | $5.77 \pm 0.18^b$    | $2.31 \pm 0.19^d$  |

Note: Each value is the mean of three replication  $\pm$  SE; different small letters on the same row indicate significant differences between the same organs at different sampling periods ( $p < 0.05$ ).

### 3.3. $^{15}\text{N}$ -Urea Uptake, Residues and Losses in Different Phenological Stages

The N uptake showed an increasing trend with the passage of the phenological period. The utilization rate of N fertilizer reached the highest after harvest, which was 3.54 g (Figure 3), while the uptake of N fertilizer was only 1.56 g in the full flowering stage. The soil residual rate of  $^{15}\text{N}$ -urea showed a downward trend. The residual amount reached 12.43 g in the full flowering stage, and the residual rate was as high as 82.87%. After the fruit was harvested, the residuals were the lowest at 1.22 g. N loss and soil residue showed an opposite trend, and the N loss was as high as 10.24 g after fruit harvest.

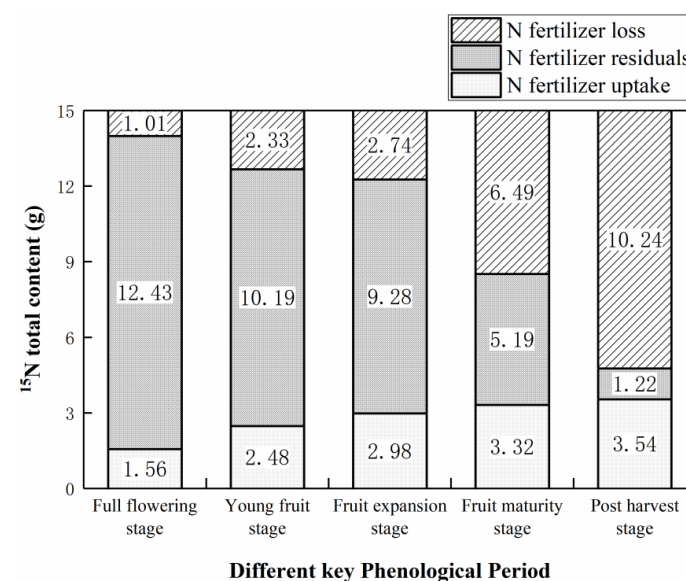


**Figure 3.**  $^{15}\text{N}$ -urea uptake, remains and loss of different key phenological phase.

### 3.4. Residue of $^{15}\text{N}$ -Urea in Soil

It can be seen from Figure 4 that the 20–40 cm soil layer has the highest residual amount, and the residual amounts in each phenological period were 8.49, 5.25, 5.16, 2.52, and 0.61 g, respectively. The second was the 0–20 cm soil layer, 2.78, 2.33, 1.49, 1.34 and 0.13 g respectively. The N residue in the 40–60 cm soil layer first increased and then decreased. The residual amount of the 40–60 cm soil layer was slightly higher than that in the 0–20 cm soil layer during the fruit expansion stage and post-harvest stage. The  $^{15}\text{N}$  content in each soil layer below 60 cm showed a downward trend. The annual average residual amount of  $^{15}\text{N}$  in 80–100, 100–120 cm soil layers is only 0.09 and 0.02 g, indicating that N infiltration is slow.

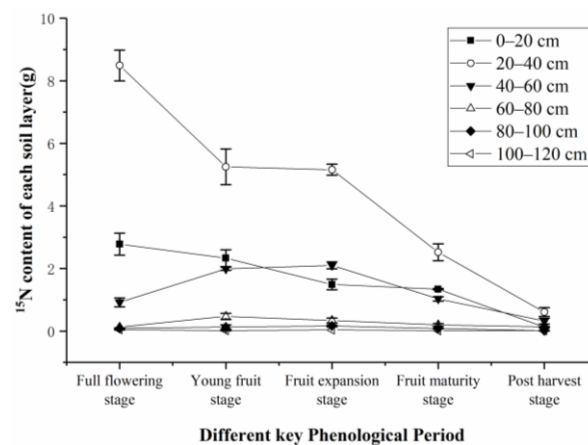


Figure 4. Profiles of  $^{15}\text{N}$  content at different soil depths.

## 4. Discussion

At the young fruit stage, the %Ndff,  $^{15}\text{N}$  distribution rate of above-ground organs (including flowers, fruits, new shoots and leaves, and non-nascent organs) showed an upward trend (Tables 1 and 2). The %Ndff of thick roots decreased from 4.02% to 3.19%, and that of fine roots decreased from 23.78% to 4.62% (Table 1). At this stage, roots were mainly used as nutrient export organs, indicating that after the full flowering stage, the N required for the growth of shoot tissues was gradually changed from the stored N to the N absorbed by roots in the current year. The %Ndff of most nascent vegetative organs was more than 1%, and the Neff% of fruits (only 1.02%) was lower than that of nascent organs, which was consistent with the results of Wu et al. [26] and Zhang et al. [27] (Table 1). During this period, the ability of nascent vegetative organs to recruit N was much greater than that of fruits, and vegetative morphogenesis and reproductive morphogenesis were easy to form nutrient competition. The results of Curette et al. [28] showed that spraying N on blooming leaves could increase the size of pear fruits and the number of cells per fruit, indicating that spring topdressing was conducive to reducing nutrient competition, which was very important for improving pear yield and quality.

There was significant difference in  $^{15}\text{N}$  distribution rate of tree organs in different periods. During the whole growth period, the  $^{15}\text{N}$  content in perennial organs decreased first and then increased, and the  $^{15}\text{N}$  content in new organs increased continuously. This is mainly due to the change of  $^{15}\text{N}$  absorption ability of each organ and nitrogen displacement distribution between individual organs. In this study, the  $^{15}\text{N}$  distribution rate and %Ndff of fine roots reached their maximum during the full blooming period (Table 1), when the roots began to absorb and transport the spring-applied  $^{15}\text{N}$  to the shoots. This result is consistent with the findings of Jiang et al. [29] Therefore, the N used in the full flowering stage was not based on the N absorbed in the year as the main N source. During this stage, the tree mainly used the N stored in the tree in the autumn of the previous year. Studies showed that most of the N in the new organs in spring came from the tree itself, which was the remobilization of the tree's own nitrogen. This part of N was stored in the body of the

tree last autumn. That means more than 2/3 of the nitrogen in the new organs in spring comes from the fertilizer last autumn [29,30]. However, our experimental results showed that the organ with the highest  $^{15}\text{N}$  distribution rate was the leaf at the post-harvest stage (Table 2). Tartachnyk and Blanke [31] reported on the apple tree that the N concentration in leaves showed a decreasing trend from fruit maturity to defoliation. 57% of  $^{15}\text{N}$  was mobilized from leaves and recirculated to perennial organs and roots  $^{15}\text{N}$  [29]. Therefore, this may be due to the earlier sampling period, when N has not been fully transferred to perennial organs, resulting in higher  $^{15}\text{N}$  in leaves.

Wu et al. [32] found that the  $^{15}\text{N}$ -urea utilization rate at the fruit maturity stage was 26.23%, the residual rate was 27.97%, and the loss rate was 45.80% after top dressing in spring in Beijing. Wang et al. [33] studied the fate of N in pear orchards in Xinjiang, and found that the utilization rate of urea in the fruit maturity stage was only 18.5%, the residual rate was 22.31%, and the loss rate was as high as 59.0% (Because the test only measured the residual amount of inorganic N in the soil, and some of the residual urea may be converted into organic N and other forms, so the N loss rate was too high). The results of our study showed that the fertilizer utilization rate of urea in the old Yellow River area reached 22.13% at the fruits maturity stage, the residual rate was 34.57%, and the loss rate was 43.27% (Figure 3). The  $^{15}\text{N}$ -urea utilization rate of trees in the old Yellow River area was lower than that in Beijing area, but higher than that in Xinjiang area; the urea loss rate was roughly the same as that in Beijing area. The large difference in utilization rate was mainly caused by the frequency of fertilization and climate. In our experiment, urea was all applied to the soil in spring, while Wu et al. [26] divided urea into spring top dressing (60%) and fruit expansion top dressing (40%). Xinjiang was arid and less rainy, which was not conducive to the movement and absorption of N.

The main ways of nitrogen fertilizer loss in orchards were ammonia volatilization, nitrification, denitrification, leaching and surface runoff. The rate of nitrogen loss was only 18.3% from full flowering stage to fruit expansion stage. It is worth noting that urea is rapidly lost during fruit expansion stage and harvest stage. The loss rates in these two periods were more than 20% respectively (Figure 3). Wang et al. [34] found that the urea loss reached 24.42% in the expansion stage of apple fruit in Shandong orchards, which was similar to the results of this experiment. Ammonia volatilization and nitrate leaching were considered as the main ways of nitrogen loss in orchards. High temperature will aggravate ammonia volatilization and precipitation will accelerate the leaching of nitrate nitrogen [35,36]. This region had low temperatures and little rain before June, and the temperature and precipitation have increased significantly since June (Figure 1). This can explain that the urea loss is very small from the flowering stage to the fruit expansion stage, and the reason for the sharp increase of nitrogen loss after the middle of June. Therefore, in the later stage of fruit expansion, effective measures should be taken to improve the N loss caused by high temperature and rain, and to improve the N fertilizer utilization rate.

To sum up, our suggestions for fertilization in this area are as follows: pay attention to divided fertilization, do not take the spring one-time fertilization measures. Split fertilization can significantly increase N efficiency and reduce N loss [37]. Reducing fertilizer and increasing the proportion of organic fertilizer can reduce N loss [38,39]. A large number of studies has shown that slow release or controlled release fertilizers, coated fertilizers and fertilizer synergists can significantly improve FUE. [40–44]. The methods of increasing the depth of fertilization have also been proved to be effective [45,46].

## 5. Conclusions

The utilization rate of urea reached the maximum at the post-harvest stage, which was 23.62%. The growth center of the tree was constantly shifting from the root system to leaves and fruits, then to fruits, and finally to leaves. There was N nutrient competition at the young fruit stage between leaf and fruit. The fate of N fertilizer from full flowering stage to fruit harvest stage showed loss > soil residue > tree uptake. N loss was more than 25% during fruit maturity stage and post-harvest stage.

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