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## Fate of nitrogen-15 in the subsequent growing season of greenhouse tomato plants (*Lycopersicon esculentum* Mill) as influenced by alternate partial root-zone irrigation

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Alternate partial root-zone irrigation (APRI) has profound impacts on the distribution of nitrogen fertilizer applied in-season. However, the fate of previous residual nitrogen in the subsequent crop growing season under APRI has seldom been studied. Our objective in this study was to investigate the effects of APRI on the reutilization, redistribution and loss of residual nitrogen in the subsequent season. To achieve this objective, in the previous season, greenhouse tomatoes (*Lycopersicon esculentum* Mill) were chosen as a plant material, and were treated with two irrigation patterns (APRI and conventional irrigation (CI)), two <sup>15</sup>N labeling depths (K<sup>15</sup>NO<sub>3</sub> with a <sup>15</sup>N abundance of 10.57%, labeled in the 0–20 cm and 40–60 cm soil layers, respectively) and two transplant times (early and late summer). In the subsequent season, we adopted the same irrigation patterns, but with no <sup>15</sup>N labeled in the soil. Our results showed that 81.3–90.7% of the residual <sup>15</sup>N from the previous season still remained in the 0–100 cm soil layer, 4.1–7.3% was absorbed by the subsequent-season tomatoes, and 2.9–14.6% was lost. The <sup>15</sup>N reutilization rates (defined as the ratio of <sup>15</sup>N uptake by the subsequent tomatoes to the total applied <sup>15</sup>N) were 2.20–4.73% under the different treatments (the <sup>15</sup>N utilization rates of the in-season tomatoes were 18.8–27.9%). Compared to CI, APRI significantly ( $p < 0.05$ ) increased the plant <sup>15</sup>N uptake and <sup>15</sup>N reutilization rate, and APRI also contributed to a greater mineral and organic <sup>15</sup>N amount in shallower soil layers. Overall, the tomato <sup>15</sup>N reutilization rate was found to be significantly ( $p < 0.05$ ) higher when <sup>15</sup>N labeling was performed in the 0–20 cm soil layer compared to that in the 40–60 cm layer. Moreover, the <sup>15</sup>N reutilization rate had a significant positive relationship with the root dry weight ( $R = 0.74^*$ ), root length density ( $R = 0.72^*$ ), soil mineral <sup>15</sup>N ( $R = 0.91^{**}$ ) and total residual <sup>15</sup>N amount ( $R = 0.88^{**}$ ).

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## Introduction

Chinese farmers have been using inorganic fertilizers extensively since the 1980s due to increasing labor costs and the relatively lower efficiency of organic fertilizers.<sup>1</sup> Inorganic fertilizer application is one of the important factors of the so-called “Miracle in China”, using 7% of the arable land to feed 22% of the population of the world. The high residues of inorganic fertilizers, particularly nitrogen fertilizers, have induced a series of ecological and environmental problems, such as soil acidification,<sup>2</sup> salinization<sup>3,4</sup> and crop nitrate/nitrite and ammonia poisoning.<sup>5,6</sup> These problems are of great concern,

not only in China, but also in many other countries.<sup>7–9</sup> For greenhouse-covered arable fields, the situation of nitrogen residues is worsening. China can apply 569–2000 kg ha<sup>−1</sup> of pure nitrogen during one season of production of greenhouse crops, which is several times or even dozens of times over that applied to ordinary field crops, leading to a large quantity of nitrogen residues<sup>10</sup> and losses.<sup>11</sup> Among different forms of residual nitrogen, nitrate-nitrogen (NO<sub>3</sub><sup>−</sup>-N) is characterized as easily leached;<sup>12</sup> NO<sub>3</sub><sup>−</sup>-N is hard to convert to other forms in deeper soil layers, and therefore it not only pollutes surface water through runoffs, but also poses a serious threat to underground water environments.<sup>13</sup>

Greenhouse agriculture in northern China suffers from high NO<sub>3</sub><sup>−</sup>-N residues, as well as severe water shortages. The distribution of water resources in China is geographically uneven, with 81% of the total water resources being intensively distributed in the Yangtze River basin and southern regions. Specifically, there is ten times more fresh water per capita in the

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south than the north.<sup>14</sup> Thus, for agricultural production in northern China, it is of great importance to utilize water resources efficiently and control the outputs of agricultural contaminants.

Efforts have been made to employ innovative irrigation methods to promote the growth of crop roots, and to recover the residual nitrogen fertilizer in the soil.<sup>15,16</sup> A study using <sup>15</sup>N labeling has revealed that water-saving irrigation is conducive to winter wheat recovering nitrogen fertilizer in the deeper soil layer at 100–150 cm.<sup>17</sup> Alternate partial root-zone irrigation (APRI) is one component of partial root-zone irrigation (PRI). As a relatively new water-saving irrigation technique, APRI has now been applied in the production of soybean, peppers, apples, potatoes, tomatoes, cotton, grapes, *etc.*<sup>18</sup> In APRI, half of the root-zone is irrigated while the other half is allowed to dry, and then the previously well-watered side of the root system is allowed to dry while the previously dried side is irrigated when the next irrigation occurs.<sup>15</sup> Earlier results have demonstrated that APRI can significantly save irrigation water without significantly decreasing the yield.<sup>19,20</sup> APRI has also been proved to promote dry matter accumulation in the roots and increase the root length density.<sup>21</sup> Although many studies have investigated the impact of APRI on crop yield and water use, few studies have focused on how APRI influences the crop NO<sub>3</sub><sup>−</sup>-N uptake, and no research has studied the fate and balance of applied nitrogen fertilizers in the subsequent growing season of the crop under the influence of APRI.

In our previous study in 2014, we labeled K<sup>15</sup>NO<sub>3</sub> (abundance of 10.57%) in different soil layers at 0–20 and 40–60 cm, and studied the impact of alternate partial root-zone irrigation on the <sup>15</sup>N uptake of greenhouse tomatoes. We found that APRI had a profound impact on the distribution of in-season applied fertilizer nitrogen.<sup>22</sup> The objective of this study (conducted in 2015) was to investigate the fate and balance of previous residual <sup>15</sup>N in the subsequent growing season of greenhouse tomatoes, as influenced by APRI. Details included: (1) the reutilization of <sup>15</sup>N by the subsequent tomatoes; (2) the distribution of <sup>15</sup>N in different soil layers; (3) the balance of <sup>15</sup>N in the subsequent growing season. The study conclusions are expected to provide useful information for those in areas suffering from an agricultural water shortage and excessive nitrogen residues.

## Materials and methods

### Experimental site description and the previous experiment

The experiments were conducted in 2015 at the Production Base of Greenhouse Vegetables (longitude 126°22'E, latitude 46°12'N) of Lanxi county, Suihua city, Heilongjiang province. Suihua belongs to the northern hemisphere temperate zone; it has four clear seasons, with snow cover in winter, while being warm and humid in summer. The annual average temperature from 2000 to 2013 in Suihua ranged from 1.3 °C to 4.0 °C. There is an annual duration of 120–140 days in the frost-free season, and a sunshine duration of 2600–2900 h. The annual average precipitation in Suihua is 483 mm. Precipitation occurs intensively in July and August. The experiment was carried out in a solar greenhouse. The span of the greenhouse is 10 m, the

length is 8 m and the height of the back wall is 3 m. For ventilation and cooling in summer, several vents were installed in the back wall with 1 m height above the ground; for details, see Fig. 1. Crop seedlings were transplanted separately in early and late summer. The day/night average temperature was 24.7/20.2 °C in early summer, and 21.3/18.5 °C in late summer, during the whole growth stage of the crop.

Since this experiment constitutes a second part of our previous work, here we briefly introduce the previous experiment:<sup>22</sup>

The previous experiment was carried out in 2014 in the above-mentioned greenhouse. The physical and chemical attributes of the original soil were determined as shown in Table 1. The tomato (*Lycopersicon esculentum* Mill) cultivar “Red Ruby” was used as the plant material. The experiment included two irrigation patterns (APRI and conventional irrigation (CI)), two <sup>15</sup>N labeling locations in the soil layers (0–20 and 20–40 cm), and two transplant times (early and late summer). The transplant dates in 2014 for early and late summer were June 18 and August 22, respectively. For details of the experimental design, see Table 2. As was recorded, the total irrigation amount of CI and APRI was 498 and 324 mm, respectively, at the transplant time of early summer, and 476 and 310 mm at that of late summer.

Several soil columns that were pre-buried in the soil were used for the experiment. The soil columns were prepared using a PVC cylindrical mold. The height of the PVC mold was 1 m and the diameter was 40 cm, with the bottom unsealed. Plastic film was employed and kept close to the inner side of the mold. The soils were dug out by 20 cm in each layer and then filled back into the mold as the field's original soil layers. The backfilled soils inside and outside of the mold were kept at the same height during the filling process in order to avoid the deflection of the mold. To provide the nutrients that are needed by the tomato plants, the 0–20 cm layer of the soil was mixed with 100 mg kg<sup>−1</sup> N, 150 mg kg<sup>−1</sup> P<sub>2</sub>O<sub>5</sub> and 150 mg kg<sup>−1</sup> K<sub>2</sub>O. These pure nutrients came from NH<sub>4</sub>NO<sub>3</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and K<sub>2</sub>SO<sub>4</sub>, respectively. The <sup>15</sup>N used for labeling was K<sup>15</sup>NO<sub>3</sub> (abundance of 10.57%), and the <sup>15</sup>N was labeled to a thickness of 10 cm, as is displayed in Fig. 2. The dosage of <sup>15</sup>N was 466 mg for each soil column. After finishing the soil backfilling and <sup>15</sup>N labeling, the molds were taken out from the field, only leaving the plastic film to separate the soils inside and outside the columns. At 55 and 76 DAT, dissolved urea was applied two times as additional fertilizer, and each time the application amount was 60 mg kg<sup>−1</sup> N. For each APRI treatment, a film separator (20 cm height) was

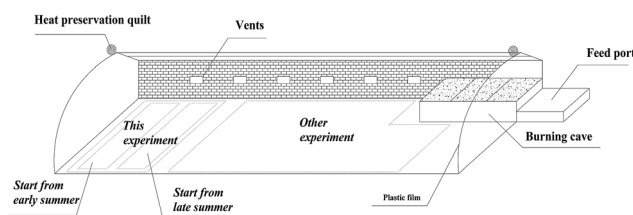


Fig. 1 The solar greenhouse used in this experiment (the day/night average temperature was 24.7/20.2 °C in early summer, and 21.3/18.5 °C in late summer, during the whole growth stage of the crop).<sup>22</sup>



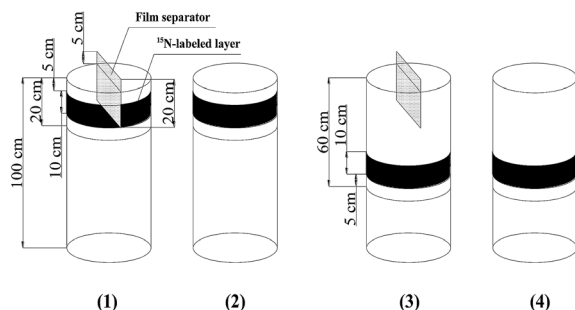
**Table 1** The physical and chemical attributes of the soil in the greenhouse

Soil depth (cm)	pH	Bulk density (g cm <sup>-3</sup> )	Organic matter (g kg <sup>-1</sup> )	Available N (mg kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )
0–10	7.37	1.39	14.71	122.4	18.81	121.3	1.40
10–20	7.44	1.42	10.93	105.7	14.92	106.2	1.25
20–60	7.65	1.55	8.62	91.6	5.33	63.4	0.78
60–100	7.91	1.51	5.36	61.3	3.21	35.5	0.39

**Table 2** Experimental design of <sup>15</sup>N labeling, irrigation method and transplanting time in the 2014 season<sup>a</sup>

Transplant time	Treatment	Transplant date	Irrigation pattern	Depth of <sup>15</sup> N labeling (cm)
Early summer	APRI10	June 18	APRI	0–20
	CI10	June 18	CI	0–20
	APRI50	June 18	APRI	40–60
	CI50	June 18	CI	40–60
Late summer	APRI10	August 22	APRI	0–20
	CI10	August 22	CI	0–20
	APRI50	August 22	APRI	40–60
	CI50	August 22	CI	40–60

<sup>a</sup> Note: APRI represents alternate partial root-zone irrigation, and CI represents conventional irrigation.

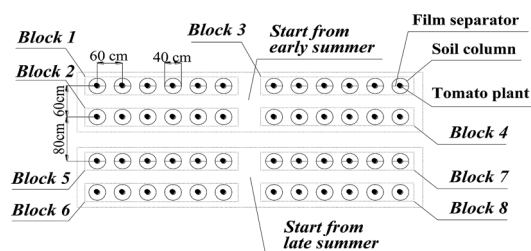
**Fig. 2** Diagrammatic sketch of <sup>15</sup>N labeling in the soil columns in 2014 (soil columns (1) and (3) are for the plants with alternate partial root-zone irrigation, and columns (2) and (4) are for the plants with conventional irrigation).<sup>22</sup>

buried in the middle of each soil column, leaving 5 cm height out of the soil surface (Fig. 2). The film separator had a gap in its center for transplanting the tomato seedlings. Fig. 3 displays the arrangement of the soil columns. The distance between two adjacent columns was 20 cm, and the distance between two plots for different transplant times was 40 cm. Fig. 1–3 can also be seen in our previous study.<sup>22</sup>

After the experiment, the total amount of <sup>15</sup>N remaining in the soil at 0–100 cm was 251.4–309.6 mg per column; for details, see ref. 22.

## Experimental design

This study (2015) was conducted to investigate the fate of residual <sup>15</sup>N from the previous experiment (2014), therefore this

**Fig. 3** The arrangement of soil columns (in blocks 1 and 5, the plants are treated with alternate partial root-zone irrigation, and <sup>15</sup>N is labeled at 10 cm depth in the soil; in blocks 3 and 7, the plants are treated with alternate partial root-zone irrigation, and <sup>15</sup>N is labeled at 50 cm depth in the soil; in blocks 2 and 6, the plants are treated with conventional irrigation, and <sup>15</sup>N is labeled at 10 cm depth in the soil; in blocks 4 and 8, the plants are treated with conventional irrigation, and <sup>15</sup>N is labeled at 50 cm depth in the soil).<sup>22</sup>

experiment was carried out *in situ*. After the experiment in 2014, no crops were planted in the soil columns until starting this experiment. The soils in the 0–20 cm layer for each soil column were ploughed before the tomato planting of this season.

This experiment adopted the same irrigation patterns as our previous study in 2014, namely APRI and CI. An earlier study conducted in a solar greenhouse in northern China proved that the tomato water use efficiency, yield and quality could reach an optimal compromise when controlling the lower limit of soil moisture at 70%  $\theta_f$  (field capacity) and the upper limit at 90%  $\theta_f$ .<sup>23</sup> Thus, during the whole growth stage of the tomato plants, the soil moisture of CI in this study was controlled at a lower limit of 70%  $\theta_f$ , and an upper limit of 90%  $\theta_f$ . Moreover, earlier results demonstrated that APRI saved 40% irrigation water, while not significantly reducing the crop yield.<sup>21,24</sup> According to this information, the total irrigation amount of APRI in our study was designed as 60% of the amount of CI. An irrigation amount of 62 mm was applied for the survival of the seedlings during 0–28 DAT (days after transplant), for the transplant times of both early and late summer. After that, the tomatoes were irrigated with different patterns of APRI and CI.

Moreover, the fertilization and transplant times in this experiment were also the same as those in 2014, while in this study no <sup>15</sup>N was labeled in the soil. The transplant date was June 15 for the transplant time of early summer, and August 17 for that of late summer. The total irrigation amount of CI and APRI was recorded as 501 and 325 mm for the tomatoes transplanted in early summer, and 486 and 316 mm for those transplanted in late summer.



## Sampling and measurement

For both transplant times in 2015, plant samples were collected separately as root, stem, fruit and leaf (including ordinary leaves and fallen leaves) samples after the last harvest; meanwhile, soil samples were collected using a diminutive soil auger at 10 cm per layer. Ten samples were collected in the 0–100 cm soil layer for each soil column.

(1)  $^{15}\text{N}$  atom percentage excess: air-dried soil samples were ground and passed through a 0.15 mm sieve for measuring the  $^{15}\text{N}$  atom percentage excess. Mineral nitrogen in fresh soil samples was extracted using 2 M KCl and distilled using micro Kjeldahl apparatus, in the presence of MgO and Devarda alloy.<sup>25</sup> The  $^{15}\text{N}$  atom percentage excess in the sample was determined using a mass spectrometer (Finnigan-Mat-251, Mass-Spectrometers, Finnigan, Germany) at Nanjing Institute of Soil Science, CAS.

(2) Root length density: root samples from the tomato plants were cleaned and scanned using an EPSON EXPRESSION 1680 scanner, and then analyzed using WinRHIZO software to obtain the data on root length density.

(3) Root dry matter: fresh root samples from tomato plants were placed in an oven, and dried firstly at 105 °C for 30 min, and then at 70 °C until a constant weight was achieved.

## Calculations and statistical analysis

(1) Reutilization rate of  $^{15}\text{N}$  ( $^{15}\text{NUE}$ , %).  $^{15}\text{NUE}$  was calculated as:<sup>1</sup>

$$N_{\text{diff}} = C_s \times \frac{E_s}{E_f} \quad (1)$$

$$^{15}\text{NUE} = \left( \frac{N_{\text{diff}}}{M_f} \right) \times 100\% \quad (2)$$

where  $N_{\text{diff}}$  is the  $^{15}\text{N}$  amount in the tomato plants (mg),  $C_s$  is the total N amount in the tomato plants (mg),  $E_s$  is the  $^{15}\text{N}$  atom percentage excess in the tomato plants,  $E_f$  is the  $^{15}\text{N}$  atom percentage excess in the  $^{15}\text{N}$  labeled fertilizer, and  $M_f$  is the amount of  $^{15}\text{N}$  fertilizer applied in 2014 (mg).

(2) Organic  $^{15}\text{N}$ . The organic  $^{15}\text{N}$  content in each soil layer was calculated as the difference between the total  $^{15}\text{N}$  minus the mineral  $^{15}\text{N}$  in the respective soil layer.<sup>25</sup>

(3)  $^{15}\text{N}$  recovery amount (mg per soil column). The amount of  $^{15}\text{N}$  recovery was the sum of the residual  $^{15}\text{N}$  in the 0–100 cm soil layer and the  $^{15}\text{N}$  uptake by the tomato plants.<sup>26</sup>

(4)  $^{15}\text{N}$  loss (mg per soil column). The amount of  $^{15}\text{N}$  loss is calculated using the applied  $^{15}\text{N}$  amount minus the  $^{15}\text{N}$  recovery amount.<sup>11</sup>

The data were compared statistically using SPSS software Version 17.0.<sup>27</sup>

## Results

### $^{15}\text{N}$ uptakes in different plant parts

APRI significantly ( $p < 0.05$ ) increased the  $^{15}\text{N}$  accumulation in all plant parts except for the stem in the plants transplanted in early summer (Table 3). The  $^{15}\text{N}$  amount under APRI was increased by 37.9–53.4%, 10.1–38.3%, 36.6–73.1% and 47.1–61.9%, respectively in the leaves, stems, roots and fruits of the tomato plants, when compared to those under CI.

For each plant part, the difference in  $^{15}\text{N}$  amount at the different labeling depths but under the same irrigation pattern was significant ( $p < 0.05$ ), except for some unrepresentative cases. In treatments with  $^{15}\text{N}$  labeled at the soil depth of 10 cm, the  $^{15}\text{N}$  amount in the leaves, stems, roots and fruits of the tomato plants was 15.0–42.3%, 7.7–56.2%, 20.5–59.2% and 13.2–44.8% higher than that labeled at a depth of 50 cm.

Although a delay in the transplant time slightly decreased the  $^{15}\text{N}$  accumulative amount in the respective plant parts, the decrease was not significant ( $p > 0.05$ ) overall. A noticeable decrease in  $^{15}\text{N}$  caused by transplant time was found in the stem under APRI50 and CI50, and in the roots under APRI50.

### Total $^{15}\text{N}$ , mineral $^{15}\text{N}$ and organic $^{15}\text{N}$ in the soil layers

Fig. 4 displays the profiles of total  $^{15}\text{N}$ , mineral  $^{15}\text{N}$  and organic  $^{15}\text{N}$  in the 0–100 cm soil layer under different treatments. From the distribution of total  $^{15}\text{N}$ , it was found that the peak value under APRI10, CI10, APRI50 and CI50 appeared at a soil depth of 30, 40, 50 and 60 cm, respectively. Similar trends were obtained with the transplant time of both early and late summer. Compared to CI, APRI reserved 48.6–176.3% more  $^{15}\text{N}$  in the respective labeled layer. Under the same treatment (irrigation and labeling), the distribution of total  $^{15}\text{N}$  was similar between

**Table 3** The amount of  $^{15}\text{N}$  absorbed by different plant parts in the 2015 season<sup>a</sup>

Transplant time	Treatment	Leaf (mg per plant)	Stem (mg per plant)	Root (mg per plant)	Fruit (mg per plant)
Early summer	APRI10	7.68 ± 0.34a	2.14 ± 0.10a	1.23 ± 0.09a	10.99 ± 1.40a
	CI10	5.00 ± 0.45b	1.94 ± 0.07ab	0.82 ± 0.07bc	6.89 ± 1.28c
	APRI50	5.40 ± 0.35b	1.99 ± 0.11ab	0.93 ± 0.03b	8.95 ± 0.36b
	CI50	3.75 ± 0.28c	1.54 ± 0.20d	0.68 ± 0.03cd	6.08 ± 0.74cd
Late summer	APRI10	6.99 ± 0.41a	2.05 ± 0.13a	1.26 ± 0.04a	10.78 ± 0.73a
	CI10	4.59 ± 0.26bc	1.80 ± 0.04bc	0.73 ± 0.06c	6.78 ± 0.70c
	APRI50	5.51 ± 0.90b	1.59 ± 0.04cd	0.79 ± 0.04c	7.37 ± 0.37bc
	CI50	3.99 ± 0.15c	1.15 ± 0.06e	0.58 ± 0.09d	4.55 ± 0.59d

<sup>a</sup> Note: APRI10 and APRI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths, respectively, under alternate partial root-zone irrigation, and CI10 and CI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths, respectively, under conventional irrigation in the 2014 season. In the same column, means followed by the same letter (a, b, c, d, or e) do not differ significantly at the 5% level, according to Duncan's multiple range test. Each value is the mean ± SD.



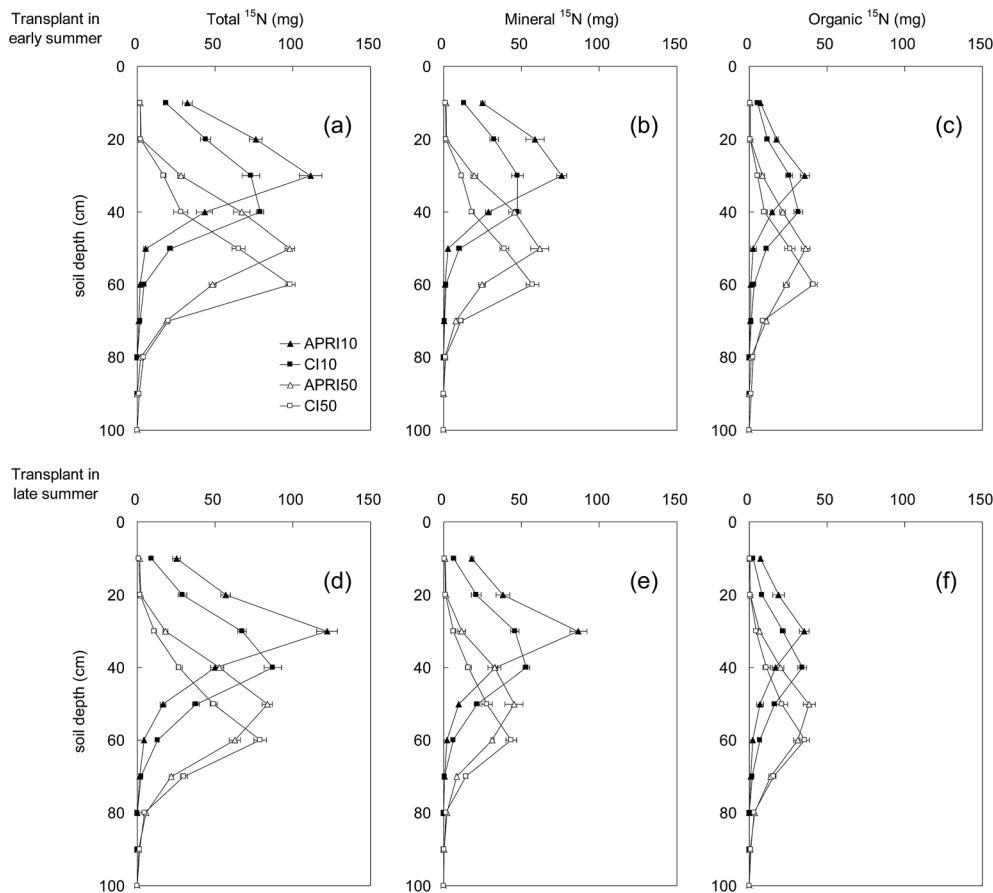


Fig. 4 Profiles of total  $^{15}\text{N}$ , mineral  $^{15}\text{N}$  and organic  $^{15}\text{N}$  in the 0–100 cm soil layer at the transplant time of early summer (a–c) and late summer (d–f) (APRI10 and APRI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths, respectively, under alternate partial root-zone irrigation, and CI10 and CI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths, respectively, under conventional irrigation in the 2014 season; error bars are standard errors of the mean).

the different transplant times, while the amount of total  $^{15}\text{N}$  was found to be lower with the transplant time of late summer.

Mineral  $^{15}\text{N}$  and organic  $^{15}\text{N}$  were distributed analogously in the soil layers to the total  $^{15}\text{N}$ . As is shown in Fig. 4(b–e), the  $^{15}\text{N}$  in the soil was mainly present in the mineral form. When the  $^{15}\text{N}$  was labeled at 10 cm depth, the mineral  $^{15}\text{N}$  in the 0–30 cm layer under APRI was significantly ( $p < 0.05$ ) higher than that under CI. And when the  $^{15}\text{N}$  was labeled at 50 cm depth, the mineral  $^{15}\text{N}$  in the 0–50 cm layer under APRI was significantly ( $p < 0.05$ ) higher than that under CI.

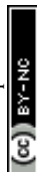
Overall, the transplant time in our study did not significantly change the distribution of total  $^{15}\text{N}$ , mineral  $^{15}\text{N}$  or organic  $^{15}\text{N}$  in the 0–100 cm soil layer. However, the irrigation pattern had clear effects on the  $^{15}\text{N}$  accumulation and distribution in the soil layers. APRI contributed greatly to the preservation of  $^{15}\text{N}$  in a shallower soil layer relative to CI, for the  $^{15}\text{N}$  labeling depths of both 10 and 50 cm.

#### Distribution and balance of $^{15}\text{N}$

The distribution and balance of  $^{15}\text{N}$  is shown in Table 4. After the experiment in 2015, the accumulation amount of  $^{15}\text{N}$  in the 0–100 cm soil layer was in the range of 204.5–276.4 mg per soil

column, accounting for 81.3–90.7% of the original  $^{15}\text{N}$  amount. The greatest  $^{15}\text{N}$  accumulation amount in the soil was achieved by APRI10, and the difference between the two transplant times was not significant ( $p > 0.05$ ). The irrigation pattern had an obvious influence on the accumulation amount of  $^{15}\text{N}$  in the soil, as APRI increased it significantly ( $p < 0.05$ ) by 10.8–18.0%; the greatest increment was found between APRI50 and CI50 when the tomato plants were transplanted in late summer. Otherwise, a deeper  $^{15}\text{N}$  labeling depth resulted in a lower accumulation amount of  $^{15}\text{N}$  in the soil; this was particularly obvious for the transplant time of late summer. Overall, the plant  $^{15}\text{N}$  uptake was significantly ( $p < 0.05$ ) increased by APRI in comparison to CI, and was also significantly ( $p < 0.05$ ) increased by a 10 cm labeling depth in comparison to 50 cm. Although a slight increase in  $^{15}\text{N}$  uptake was also detected in the tomatoes transplanted in early summer compared to those transplanted in late summer, the increase was not significant ( $p > 0.05$ ).

After the experiment in the 2015 season, the amount of  $^{15}\text{N}$  recovery under the different treatments was found to range from 214.7 to 297.5 mg, and the amount of  $^{15}\text{N}$  loss ranged from 8.7 to 36.7 mg. The recovery and loss amount of  $^{15}\text{N}$  in the 2015

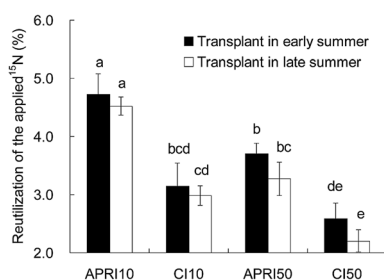




**Table 4** The balance of  $^{15}\text{N}$  under different treatments in the 2015 season<sup>a</sup>

Transplant time	Treatment	Residual $^{15}\text{N}$ from the 2014 season (mg)	Recovery (mg)		
			Soil residual (mg)	Plant uptake (mg)	Loss (mg)
Early summer	APRI10	302.3	271.5 $\pm$ 6.8a	22.0 $\pm$ 1.6a	8.7
	CI10	267.0	242.2 $\pm$ 5.6b	14.7 $\pm$ 1.9bcd	10.2
	APRI50	304.0	267.5 $\pm$ 9.9a	17.3 $\pm$ 0.8b	19.2
	CI50	278.4	238.1 $\pm$ 11.3b	12.1 $\pm$ 1.2de	28.2
Late summer	APRI10	309.6	276.4 $\pm$ 9.0a	21.1 $\pm$ 0.7a	12.1
	CI10	271.4	246.2 $\pm$ 8.9b	13.9 $\pm$ 0.8cd	11.3
	APRI50	286.3	249.2 $\pm$ 5.8b	15.3 $\pm$ 1.3bc	21.8
	CI50	251.4	204.5 $\pm$ 7.2c	10.3 $\pm$ 0.9e	36.7

<sup>a</sup> Note: APRI10 and APRI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths, respectively, under alternate partial root-zone irrigation, and CI10 and CI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths, respectively, under conventional irrigation in the 2014 season. In the same column, means followed by the same letter (a, b, c, d, or e) do not differ significantly at the 5% level, according to Duncan's multiple range test. Each value is the mean  $\pm$  SD. The amount of  $^{15}\text{N}$  refers to the amount in each soil column. The amount of soil residual  $^{15}\text{N}$  includes that in the 0–100 cm soil layer.



**Fig. 5** Reutilization of the applied  $^{15}\text{N}$  by the tomato plants (APRI10 and APRI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths, respectively, under alternate partial root-zone irrigation, and CI10 and CI50 represent that  $^{15}\text{N}$  was labeled at 10 and 50 cm soil depths respectively under conventional irrigation in the 2014 season; means followed by the same letter (a, b, c, d, or e) do not differ significantly at the 5% level according to Duncan's multiple range test, when the eight means were compared together, and each value is the mean  $\pm$  SD).

season accounted for 85.4–97.1% and 2.9–14.6%, respectively, of the total residual  $^{15}\text{N}$  from 2014. Among the different treatments, APRI10 gave the greatest  $^{15}\text{N}$  recovery and the lowest  $^{15}\text{N}$  loss. With the same irrigation pattern and labeling depth, the  $^{15}\text{N}$  loss was lower when the plants were transplanted in early summer, relative to late summer.

### $^{15}\text{N}$ reutilization rate and its influencing factors

The  $^{15}\text{NUE}$  ranged from 2.20 to 4.73% under the different treatments (Fig. 5). The highest  $^{15}\text{NUE}$  of 4.73% was obtained for the tomato plants transplanted in early summer under the APRI10 treatment. Compared to CI, APRI significantly ( $p < 0.05$ ) increased the  $^{15}\text{NUE}$  by 43.2–51.7%. However, the transplant time had no significant ( $p > 0.05$ ) effect on  $^{15}\text{NUE}$ , although a slight decrease in  $^{15}\text{NUE}$  was observed for the transplant time of late summer. In addition, it should be noticed that the  $^{15}\text{NUE}$  was higher when the  $^{15}\text{N}$  was labeled in a shallower soil layer. Compared to APRI10, a significant ( $p < 0.05$ ) reduction in  $^{15}\text{NUE}$  was found for APRI50, for both transplant times. A similar trend was also detected between CI10 and CI50.

The relationship between  $^{15}\text{NUE}$  and its influencing factors is shown in Table 5.  $^{15}\text{NUE}$  had a significant positive relationship with the root dry matter amount ( $R = 0.74^*$ ), root length density ( $R = 0.72^*$ ), total  $^{15}\text{N}$  ( $R = 0.90^{**}$ ), mineral  $^{15}\text{N}$  ( $R = 0.91^{**}$ ) and total residual  $^{15}\text{N}$  amount from the 2014 season ( $R = 0.88^{**}$ ). This indicated that the  $^{15}\text{NUE}$  was closely related to the plant roots, as well as the  $^{15}\text{N}$  amount and its availability.

## Discussion

Much research has been done on the reuse of applied nitrogen by succeeding crops. Liang<sup>1</sup> observed a reutilization rate of

**Table 5** Factors influencing the reutilization rate of  $^{15}\text{N}$  in the soil–plant system

	$^{15}\text{N}$ reutilization rate	Root dry matter	Root length density	Total $^{15}\text{N}$	Mineral $^{15}\text{N}$	$^{15}\text{N}$ residual by 2014
$^{15}\text{N}$ reutilization rate	1.00	0.74 <sup>b</sup>	0.72 <sup>b</sup>	0.90 <sup>a</sup>	0.91 <sup>a</sup>	0.88 <sup>a</sup>
Root dry matter		1.00	0.68	0.66	0.65	0.54
Root length density			1.00	0.48	0.45	0.60
Total $^{15}\text{N}$				1.00	0.98 <sup>a</sup>	0.81 <sup>b</sup>
Mineral $^{15}\text{N}$					1.00	0.77 <sup>b</sup>
$^{15}\text{N}$ residual by 2014						1.00

<sup>a</sup> Represents significant correlation at the 0.01 level. <sup>b</sup> Represents significant correlation at the 0.05 level. Total  $^{15}\text{N}$ , mineral  $^{15}\text{N}$  and  $^{15}\text{N}$  residual from 2014 are that of the 0–60 cm soil layer.



fertilizer nitrogen of 2–9%. Macdonald<sup>28</sup> reported that approximately 6% of residual nitrogen was taken up by the succeeding crop. Bhogal<sup>29</sup> found that equivalent to 8–20% of the fertilizer nitrogen applied in the previous season was absorbed by the crops in the succeeding season. A four-year case study in eastern China also demonstrated that the total reutilization of fertilizer nitrogen applied in the first season was 11–15% during the later three seasons, almost reaching half of that in the first season.<sup>30</sup> Presently, many earlier studies have evaluated the reuse and the redistribution of previous applied nitrogen in the subsequent season. The main difference between our study and those earlier works is that we investigated the fate of previous residual nitrogen under the influence of APRI, a promising irrigation pattern for areas suffering from a water shortage.

We observed a significantly higher <sup>15</sup>N amount in the tomato leaves under APRI when compared to CI; this might be explained as follows: (1) APRI enhanced the availability of soil <sup>15</sup>N,<sup>31</sup> and (2) before this experiment, the soil <sup>15</sup>N amounts in the original soil under different treatments were different, and more <sup>15</sup>N remained in the shallow soil layer under APRI in the previous season (2014). In the previous season, the soil total <sup>15</sup>N amount under APRI treatment was 34.3% higher compared to that under CI treatment.<sup>22</sup> The higher <sup>15</sup>N amount in other plant parts, including the stem, roots and fruits, also proved the advantages of APRI in promoting crop nitrogen reuptake. The high plant nitrogen uptake under APRI might also be explained by the higher microbial biomass and nitrogen immobilization in the soil.<sup>32</sup> An earlier study indicated that the plant nitrogen uptake decreased as the temperature decreased,<sup>33</sup> possibly due to a reduction in soil mineral nitrogen under lower temperatures.<sup>34</sup> We found a slight <sup>15</sup>N decrease in the plants transplanted in late summer compared to those transplanted in early summer, though the decrease was not significant ( $p > 0.05$ ).

Besides the <sup>15</sup>N residual effects from the previous season, the redistribution of total <sup>15</sup>N in the 0–100 cm soil layer may have been affected by the irrigation pattern and <sup>15</sup>N labeling location. Under APRI, the position of the <sup>15</sup>N peak in the soil profile was approximately 10 cm shallower than that under CI, indicating that nitrogen leaching was weakened by APRI. This confirmed the result of Wang's<sup>21</sup> study. Besides, Wang also reported that 61.3% of the <sup>15</sup>N labeled at a 45 cm depth was moved upwards under APRI. However, in our study, the rate was 26.0–36.8%, possibly due to the fact that we labeled the <sup>15</sup>N in a deeper soil layer, and that our experiment was carried out in the subsequent season when some of the <sup>15</sup>N had been lost in the previous season.

The form of residual nitrogen in the soil greatly affects its bioavailability.<sup>35,36</sup> Bhogal<sup>29</sup> pointed out that large amounts of residual fertilizer nitrogen were in the mineral form, while the study by Macdonald<sup>37</sup> found that major amounts of residual nitrogen were in the organic form, and only small amounts were in the mineral form. These differences primarily related to the amount and type of the applied nitrogen, as well as the soil attributes.<sup>38,39</sup> After the experiment in the 2015 season, more than 50% of the residual <sup>15</sup>N in our study was in the mineral form, remaining available in the soil for utilization by the succeeding crop. The higher mineral <sup>15</sup>N under APRI could possibly

be explained by the fact that the dry and wet cycles stimulate the mineralization of soil nitrogen.<sup>40</sup> It was also observed in our study that the mineral <sup>15</sup>N at the transplant time of late summer was lower than that of early summer, which might be attributed to a relatively lower temperature in late summer. Early findings by Tian<sup>41</sup> indicated that soil temperature had the greatest contribution to the mineralization of total nitrogen compared to other environmental factors, presenting a positive relationship with the amount of mineral nitrogen.

In our study, APRI significantly ( $p < 0.05$ ) increased <sup>15</sup>N recovery. Namely, APRI contributed to a higher recovery of residual nitrogen, which remained in the soil from the previous season. The reason might be that the relatively lower irrigation amount under APRI limited the amount of <sup>15</sup>N that could leach into the deeper soil layer when compared to CI, thus reducing the risk of <sup>15</sup>N loss. Previous studies by Vázquez<sup>42</sup> and Sims<sup>43</sup> reported that nitrogen losses from the soil occurred primarily when excessive irrigation occurred, leading to variations in the residual nitrogen in different soil layers. Our result was similar to that obtained by Wang;<sup>21</sup> the main difference was that his experiment was on nitrogen recovery by the in-season crops under APRI.

Nitrogen use efficiency (NUE) is one of the key indicators for evaluating irrigation regimes, together with the water use efficiency, crop yield, quality, *etc.*<sup>44,45</sup> This study observed the nitrogen reuse efficiency as influenced by different irrigation patterns and transplant times, as well as different residual nitrogen amounts. In our study, the transplant time appeared to have little effect on the <sup>15</sup>NUE; however, the <sup>15</sup>NUE was significantly ( $p < 0.05$ ) increased by APRI in comparison to CI, for both <sup>15</sup>N labeling depths. It may be that the dry and wet alternate conditions caused by APRI promote pre-stored carbon remobilization, and in most situations, increases in carbon remobilization from vegetative tissues are closely associated with a higher NUE.<sup>46,47</sup> Except for the mineral <sup>15</sup>N mentioned earlier, it was found that <sup>15</sup>NUE correlated significantly with the root dry matter ( $R = 0.74^*$ ), as well as the root length density ( $R = 0.72^*$ ). The higher <sup>15</sup>NUE under APRI might also be explained by the fact that APRI could cause alternate water stress in the root-zone and promote compensatory root growth, thereby regulating the functioning of the crop root system.<sup>15</sup> Significantly higher root dry weights under APRI or dry-wet cycling have been reported by many previous studies.<sup>45,48,49</sup> It cannot be ignored that the <sup>15</sup>NUE was also closely related to the total <sup>15</sup>N residual amount from the previous season ( $R = 0.88^{**}$ ), which is in line with the findings in tobacco.<sup>30</sup>

Our study revealed the impact of APRI on the fate of <sup>15</sup>N in the subsequent growing season. However, caution should be taken as this experiment was conducted under greenhouse conditions, and the environments might be different under field conditions; thus more research under various growth conditions needs to be carried out in the future.

## Conclusion

After the experiment in the 2015 season, it was found that 81.3–90.7% of the residual <sup>15</sup>N from the 2014 season remained in the



0–100 cm soil layer, 4.1–7.3% was absorbed by the 2015-season tomato plants, and 2.9–14.6% was lost. The  $^{15}\text{N}$  reutilization rates (defined as the ratio of plant  $^{15}\text{N}$  uptake to total  $^{15}\text{N}$  applied in 2014) were 2.20–4.73% under the different treatments. Compared to CI, APRI significantly ( $p < 0.05$ ) increased the accumulation amount of  $^{15}\text{N}$  in the 0–100 cm soil layer, as well as the plant  $^{15}\text{N}$  uptake and reutilization rate, and APRI also contributed to a greater  $^{15}\text{N}$  distribution in the shallower soil layers. Overall, the tomato  $^{15}\text{N}$  reutilization rate was found to be significantly ( $p < 0.05$ ) higher with  $^{15}\text{N}$  labeled in the 0–20 cm soil layer in comparison to the 40–60 cm layer, and insignificantly higher when transplanting in early summer compared to late summer. Furthermore, the  $^{15}\text{N}$  reutilization rate had a significant positive relationship with the root dry matter ( $R = 0.74^*$ ), root length density ( $R = 0.72^*$ ), mineral  $^{15}\text{N}$  ( $R = 0.91^{**}$ ) and total residual  $^{15}\text{N}$  from 2014 ( $R = 0.88^{**}$ ). It was concluded from our study that an enlarged root system and a high nitrogen availability under APRI might have contributed to the higher  $^{15}\text{N}$  reutilization rate.

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