

EFFECTS OF DIFFERENT NITROGEN APPLICATION STRATEGIES ON THE WATER AND NITROGEN USE EFFICIENCY OF GREENHOUSE TOMATOES (*SOLANUM LYCOPERSICUM* L.)

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Abstract. Improvements in nitrogen use efficiency (*NUE*) reduce stress on the environment and improve vegetable production. The effects of the application rate and injection timing of nitrogen (N) on the net photosynthetic rate (P_n), soil enzyme activities, yield (*Y*), water use efficiency (*WUE*), and nitrogen use efficiency (*NUE*) of tomato were studied by conducting an experiment with a split block design in which N was applied at different rates (150 kg·ha⁻¹, N1; 200 kg·ha⁻¹, N2; and 250 kg·ha⁻¹, N3) and timings (first 1/3 of irrigation, S1; middle 1/3 of irrigation, S2; and last 1/3 of irrigation, S3); no N fertilizer was applied in the control. The results showed an appropriate N application rate (i.e., not too high or low) can improve P_n and soil enzyme activity. The *Y* and *WUE* of greenhouse tomato increased non-linearly with the increase in N application rate, but *NUE* first increased and then decreased as the N application rate increased. Compare to S1, S2 and S3 improved the P_n , soil enzyme activities, *Y*, *WUE*, and *NUE* of tomato. These results could be used to promote N conservation and high *Y* of facility agriculture crops.

Keywords: facility agriculture, nitrogen application rate, nitrogen injection timing, soil enzyme activity, yield

Introduction

Facility agriculture is a form of modern agriculture in which engineering technology is leveraged to efficiently produce animals and plants under controllable environmental conditions. Greenhouse technology is commonly used in facility agriculture and plays an important role in meeting the increasing demand for vegetables. For instance, the area of protected vegetable cultivation in China has increased from 10,300 ha in 1982 to 4.05 million ha in 2019, accounting for 30.5% of the total area of vegetable cultivation, and the area of solar greenhouses and large and medium sheds in particular exceeds 2.7 million ha.

Tomato (*Solanum lycopersicum* L.) is one of the main vegetables of facility agriculture and is highly nutritious (Liu et al., 2019; Toor et al., 2006). As of 2019, the cultivated area of tomatoes in the world is 5.03 million ha, accounting for 8.43% of the total area of vegetables cultivation. According to the statistics, the total world production of tomatoes in 2019 was 180.77 million tons (FAOSTAT, 2019). Tomato production requires a large amount of nitrogen (N), especially in the greenhouse production process; however, excessive N input (Rashti et al., 2015; Ju et al., 2006; Min et al., 2012) can increase the risk of N loss (Zhu et al., 2002) and lead to several

environmental problems. One of the main current challenges in vegetable production is finding ways to improve nitrogen use efficiency (*NUE*) and reduce the N application rate without compromising crop yield (*Y*) (Huang et al., 2012; Shi et al., 2020; Barth et al., 2019). Research on greenhouse water and fertilizer technology could make important contributions to the sustainable development of agriculture.

Many studies have examined the effect of N application rate on greenhouse tomatoes. For example, Mahajan et al. (2006) showed that 50% evapotranspiration (*ET*) irrigation rate and 137 kg·ha⁻¹ N application rate could significantly increase tomato *Y* in a greenhouse. Du et al. (2017) showed that the optimal N application rate for maximizing *Y* and water use efficiency (*WUE*) was 250 kg·ha⁻¹, whereas 150 kg N·ha⁻¹ was optimal for maximizing *NUE*. Li et al. (2020) studied the *WUE*, *NUE*, and quality of greenhouse tomatoes under different water and N management strategies and found that the optimal water and N management strategy was 70% *ET* irrigation rate and 150 kg·ha⁻¹ N application rate. These results indicate that the optimal N application rate for greenhouse tomatoes depends on the experimental objectives and environmental factors.

The method of N application can also affect the *NUE* by altering the distribution of N in soil. Khakural et al. (2005) characterized variation in the urea hydrolysis rate at different application depths (0~60 cm) and found that the hydrolysis rate decreased as the N application depth increased. This result indicates that the N supply capacity of different soil layers varies. The uneven distribution of crop roots in soil layers (Outoukarte et al., 2010; Qiu et al., 2017) can also lead to differences in the *NUE* of crops in different soil layers (Matimati et al., 2014). Kaushal et al. (2005) found that applying slow-release N fertilizer at a depth of 20 cm could increase soybean *Y* by increasing the number of pods and grains. As drip irrigation events are usually long (more than 2 h), variation in nutrient injection timing via drip irrigation can affect the N distribution in the soil (Hanson et al., 2006). Thus, a major goal of current research is determining the optimal nutrient injection timing under drip irrigation. Li et al. (2004) conducted laboratory experiments and showed that irrigation in the first quarter, fertilization in the next two quarters, and irrigation in the last quarter of the experimental period were effective for keeping most of the nitrate-nitrogen (NO₃⁻-N) in the root zone of crops. Gärdenäs et al. (2005) simulated the effects of fertilization strategy and soil type on nitrate leaching in four micro-irrigation systems and found that fertilization later in the irrigation process can reduce nitrate leaching. Therefore, the timing of water and fertilizer application can affect the nutrient distribution in the soil and thus the growth and *Y* of crops. However, the effect of the timing of both water and fertilizer application has not been studied in greenhouse tomatoes to date.

Here, the effect of different N application strategies on the N distribution in greenhouse soil and the growth of greenhouse tomatoes was studied through a greenhouse experiment and multi-objective optimization analysis. The results of this study provide new insights that could be used to improve the integration of irrigation and fertilizer technology. These results also have implications for improving N conservation and the *Y* of facility agriculture crops.

Materials and methods

Experimental site

The experiment was conducted from March 23, 2019 to January 30, 2020 in a greenhouse of the Modern Agricultural Science and Technology Exhibition Center of

Xi'an City, Shaanxi Province, China (34°03'N, 108°52'E; 435 m). The region is characterized by a warm temperate semi-humid continental monsoon climate. The annual average temperature is 13.3 °C, the annual average rainfall is 507.7~719.8 mm, the precipitation from August to October accounts for more than 60% of the annual precipitation, the frost-free period is 219~233 days, the maximum annual sunshine time is 2230 h, and the multi-year average wind speed is 2~3. The average bulk density of the 1.0 m soil layer is 1.53 g·cm⁻³, the water holding capacity of the soil is 25.40%, and the depth of the groundwater table is greater than 30 m. The soil is sandy loam. The organic matter, total phosphorus (P), total potassium (K), total N, available N, available P, and available K content in the plough layer before sowing were 10.62 g·kg⁻¹, 7.24 g·kg⁻¹, 3.29 g·kg⁻¹, 0.94 g·kg⁻¹, 68.41 mg·kg⁻¹, 97.74 mg·kg⁻¹, and 74.25 mg·kg⁻¹, respectively.

Field management

The greenhouse (85 m long × 15 m wide) was oriented from north to south. The tomato variety “Jingfan 401” (Jingyan Yinong Seed Sci-tech Co. Ltd., Beijing, China) (Fig. 1) is a hybrid of pink tomato, early maturity, indeterminate growth type, round fruit, single fruit weight 200~260 g, good hardness. The tomato was planted on a ridge with a row spacing of 50 cm and a plant spacing of 40 cm. The length of the ridge was 3.4 m. A 1.0 m deep building waterproof film made of styrene-butadiene-styrene block copolymer was buried in the middle to prevent the horizontal infiltration and movement of soil moisture. Plastic barriers were also placed in the middle to prevent diseases and insect pests associated with the excessive humidity of the irrigation treatments from affecting other plots (Fig. 2). During seeding stage, the vents were opened when the interior temperature was higher than 30 °C. After the middle of April, if the lowest interior temperature stabilized above 15 °C, the vents were opened except on rainy days, until the end of the spring tomatoes experiment. After the middle of October, if the lowest interior temperature was below 10 °C, the warm-air machines were turned on. After management, the maximum daily temperature of spring tomato in the greenhouse generally occurs at 12~14:00 p.m. (the average is 33.6 °C), the lowest temperature generally occurs at 6~7:00 a.m. (the average is 18.4 °C), and the maximum humidity (relative humidity) generally occurs at 6~8:00 a.m. or 19~23:00 p.m. (the average is 84.6%), the lowest humidity generally occurs at 12~14:00 p.m. (the average is 36%). The highest daily temperature of autumn tomato in the greenhouse generally occurs at 12~14:00 p.m. (the average is 24.1 °C). The lowest temperature generally occurs at 7~9:00 a.m. (the average is 13.9 °C). The highest humidity generally occurs at 7~9:00 a.m. or 20~24:00 p.m. (the average is 89.4%), and the lowest humidity generally occurs at 12~14:00 p.m. (the average is 44.1%). Other agronomic practices, such as weeding, pest control, and pollination were same for all treatments, following the local agronomic practices.

The source of the irrigation water was groundwater. To ensure the survival of seedlings on the day of planting, the irrigation amount of first time (I_1 , 32.9 mm for spring and 31.2 mm for autumn) was based on local tomato planting experience. Spring tomatoes were planted on March 27, 2019, and the irrigation treatment began on April 4, 2019 and ended on July 11, 2019. Autumn tomatoes were planted on August 27, 2019, and the irrigation treatment began on September, 4, 2019 and ended on January, 11, 2020. The growth stage of tomato was divided into three stages based on the characteristic features of tomato growth and development: flowering stage (0~30 d after transplanting (W1)), expansion stage (30~90 d after transplanting (W2)), and maturity stage (90 d after transplanting W3).



Figure 1. Field figure of tomato experiment in greenhouse (left) and LCpro-SD portable photosynthesis system (right)

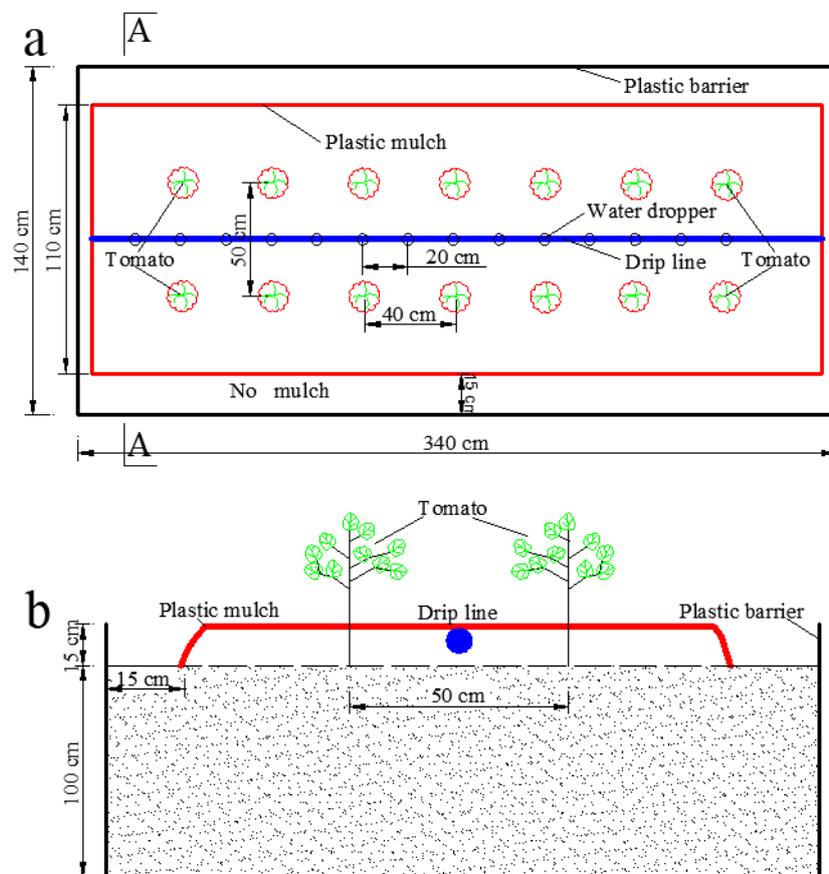


Figure 2. (a) the planform of tomato planting pattern, and drip-line arrangements in a planting plot; (b) section map of "A-A"

The subsequent irrigation amount (I_2) was controlled based on the cumulative evaporation from a 20 cm diameter pan (E_{pan}) (Agbna et al., 2017). The evaporation

amount was measured at 08:00 am every 5 d. Irrigation was carried out after measurements. *Figure 3* shows the I_2 over the experimental period; I_2 was calculated according to *Equation 1* (Liu et al., 2013):

$$I_2 = E_{pan} \times k_{cp} \quad (\text{Eq.1})$$

where E_{pan} is the evaporation between two irrigation events in mm and k_{cp} is the crop-pan coefficient. In this paper, the crop-pan coefficient of k_{cp} was 1.0 (Zhao et al., 2009; Zhang et al., 2020).

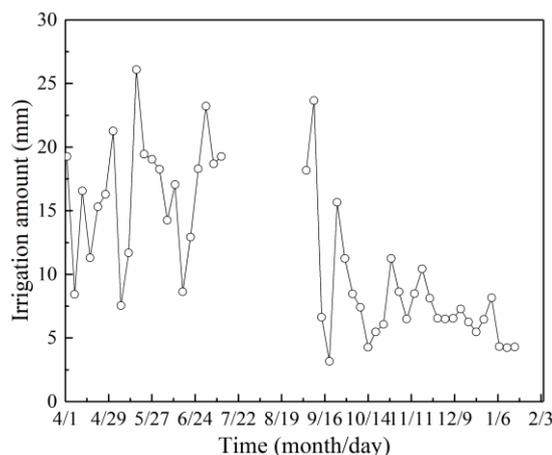


Figure 3. Irrigation records over the experimental period (4/4/2019–7/11/2019 and 9/4/2019–1/11/2020)

Experimental design

Nitrogen application rate (N) and injection timing (S) were the two factors in the experiment; the details of the experimental design are shown in *Table 1*.

Table 1. Test factors and experimental design

Test number	Injection timing of N during irrigation	Nitrogen application rate (kg·ha ⁻¹)
N1S1	First 1/3	150
N1S2	Middle 1/3	150
N1S3	Last 1/3	150
N2S1	First 1/3	200
N2S2	Middle 1/3	200
N2S3	Last 1/3	200
N3S1	First 1/3	250
N3S2	Middle 1/3	250
N3S3	Last 1/3	250
CK	-	0

Similar to the experimental design of Gärdenäs et al. (2005), we evaluated the effect of ten fertigation strategies on *WUE*, *NUE*, and the growth of greenhouse tomatoes. The

experiment was conducted in a split block design consisting of 10 treatments with three replicates per treatment (i.e., 30 plots in total). Organic fertilizer (organic content $\geq 45\%$, NPK $\geq 5\%$, fermentation fertilizer of cattle and sheep excreta), phosphate fertilizer (triple superphosphate, $P_2O_5 \geq 46\%$), and potassium fertilizer (potassium sulfate, $K_2O \geq 51\%$) were applied as the base fertilizer before transplanting tomatoes. The soil in all plots was fertilized with $1500 \text{ kg}\cdot\text{ha}^{-1}$ organic fertilizer, $180 \text{ kg}\cdot\text{ha}^{-1} P_2O_5$, and $120 \text{ kg}\cdot\text{ha}^{-1} K_2O$. N fertilizer (urea) was applied during tomato growth. According to Zhou et al. (2020), tomato plants are sensitive to N 15~60 days after pollination; thus, N application within this window can improve the growth and quality of tomato. The date and amount of N fertilizer application are shown in Figure 4. The three levels of N application included 150 (N1) , 200 (N2) , and $250 \text{ kg}\cdot\text{ha}^{-1} \text{ (N3)}$. The fertilizer was evenly mixed into the water flow with the hydraulic fertilizer applicator. The three injection timings (Fig. 4) were S1, wherein N was injected into drip line during the first 1/3 of the irrigation; S2, wherein N was injected into drip line during the middle 1/3 of the irrigation; and S3, wherein N was injected into drip line during the last 1/3 of the irrigation. No N fertilizer was applied in the control group (CK).

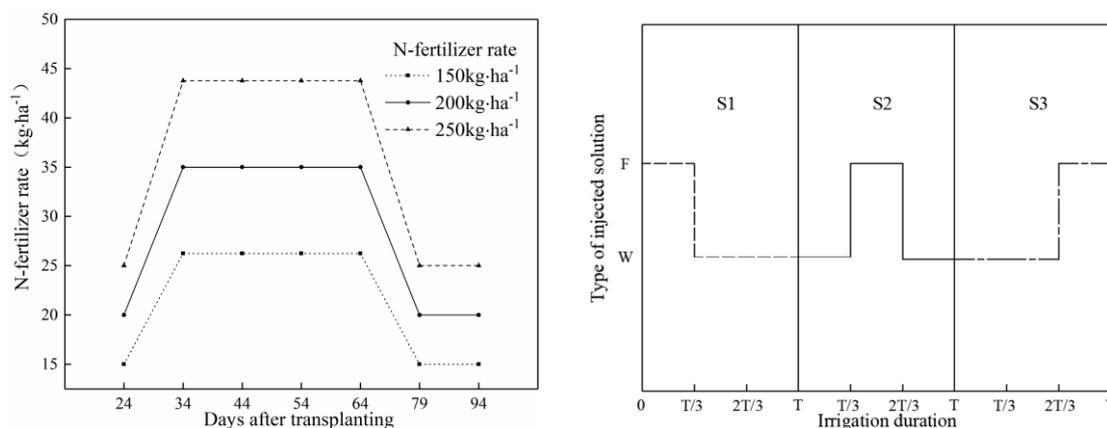


Figure 4. (a) Fertilization records over the experimental period; (b) Three timings of nitrogen injection, T is irrigation duration, F is urea solution, W is water

Sampling and measurements

During the mature period, four tomatoes were randomly selected from each plot, and the quality of mature tomatoes was measured using an electronic scale for obtaining Y per plant. The soil water content θ_0 and θ_1 before and after the growth period were measured by the drying method. The soil was collected at a depth of 100 cm and a soil interval of 20 cm in each plot three times. The crop ET (mm) was calculated using the following water balance equation (Eq. 2; Allen et al., 2011):

$$ET = P + I + U - D - R - \Delta W \quad (\text{Eq.2})$$

where P is the effective precipitation (mm), I is the irrigation amount ($I = I_1 + I_2$, mm), U is the water movement from the deep soil into the root zone (mm), D is the deep percolation (mm), R is the surface runoff (mm), and ΔW is the change in the soil profile water content within the 0~100 cm soil layer (mm), which was calculated according to Equation 3 (Allen et al., 2011):

$$\Delta W = 1000 \times h \times (\theta_0 - \theta_1) \quad (\text{Eq.3})$$

The experiment was conducted in a greenhouse with drip irrigation on flat terrain, and I was small; hence, $P = 0$, $D = 0$, and $R = 0$. The groundwater table was below 5.0 m, and the crop roots were unable to absorb and utilize the groundwater. Thus, the underground water recharge was negligible, $U = 0$.

The WUE was calculated by Equation 4 (Li et al., 2020):

$$WUE = Y / ET \quad (\text{Eq.4})$$

where WUE is the crop WUE ($\text{kg} \cdot \text{m}^{-3}$) and Y is the grain yield of crops ($\text{kg} \cdot \text{plant}^{-1}$).

The NUE was calculated by Equation 5 (Nafi et al., 2019):

$$NUE = (Y - Y_c) / N \quad (\text{Eq.5})$$

where Y_c is the yield of tomato under the CK treatment in $\text{kg} \cdot \text{plant}^{-1}$.

The activity of several soil enzymes was determined from rhizosphere soil collected 70 days after transplantation. Three tomato plants were randomly selected in each plot, the stem of the plant was assumed as the center, and a hole was dug with a straight diameter of about 0.2 m and a depth of about 0.4 m to obtain the root system of the plant. The root was shaken to remove bulk soil, and the rhizosphere soil were collected with a sterile and soft-bristled paintbrush (Ren et al., 2021). Soil urease activity ($\text{mg NH}_4^+\text{-N (g} \cdot 24 \text{ h)}^{-1}$) was estimated using the phenol-sodium hypochlorite colorimetric method; soil sucrase activity ($\mu\text{g glucose (g} \cdot 24 \text{ h)}^{-1}$) was estimated using the dinitrosalicylic acid colorimetric method; and soil phosphatase activity ($\mu\text{g nitrophenol (g} \cdot 24 \text{ h)}^{-1}$) was estimated by the nitrophenylphosphate disodium colorimetric method.

In the experiment, soil was collected 64 d after transplantation (5 d after N application). Soil was collected at the following depths: 0, 10, 20, 30, 40, 50, and 60 cm. Soil was collected from a single location for each plot, which was located 5~10 cm away from the water dropper. The soil $\text{NO}_3^-\text{-N}$ content was determined by a flow analyzer (Bran + Luebbe AutoAnalyzer - III, Germany).

The instantaneous transpiration rate (T_r) and net photosynthetic rate (P_n) of tomato leaves in the greenhouse were measured by an LCpro-SD portable photosynthesis system (ADC, UK) (Fig. 1). Three healthy plants from each plot and three healthy leaves from each plant were selected for measurements. Measurements were taken 30, 64, and 105 d after transplantation. The instantaneous water use efficiency (WUE_L) was calculated by Equation 6 (Zhou et al., 2020):

$$WUE_L = P_n / T_r \quad (\text{Eq.6})$$

where WUE_L is the leaf WUE ($\text{mmol CO}_2 \cdot \text{mol}^{-1} \text{ H}_2\text{O}$); P_n is the leaf net photosynthetic rate ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); and T_r is the leaf instantaneous transpiration rate ($\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

Data analysis

The significant difference of SPSS22.0 (IBM Crop., Armonk, New York, NY, USA) was analyzed by F test, and the significant level was set to $P < 0.05$.

OriginPro2019 (Origin Lab Corporation, Northampton, MA, USA) was used to draw the picture. Except for special annotations, the data are all average \pm standard deviation in the chart.

Results

Distribution of NO_3^- -N

According to Havlin et al. (2006), urea and ammonium-nitrogen (NH_4^+ -N) in the soil are quickly converted into NO_3^- -N. The purpose of this study was to identify the fertilization strategy that results in the most favorable nitrate distribution pattern for tomato growth by analyzing the distribution of NO_3^- -N (depth of 0~60 cm) in the soil of spring and autumn tomatoes 64 d after transplantation (5 d after N fertilizer application).

Figure 5 shows variation in NO_3^- -N with soil depth under different N application strategies. As the soil depth increases, the NO_3^- -N content first increased and then decreased, but the NO_3^- -N content peaked at different depths in the different treatments. At the same timing of N application, the NO_3^- -N content increased as the N application rate increased, but there was no significant difference in the depth corresponding to the peak NO_3^- -N content among treatments. The peak values of NO_3^- -N in S1, S2, and S3 under N1 were 48.46, 53.38, and 46.78 $\text{mg}\cdot\text{kg}^{-1}$, respectively; the peak values of NO_3^- -N in S1, S2, and S3 under N2 were 70.15, 67.22, and 67.56 $\text{mg}\cdot\text{kg}^{-1}$, respectively; and the peak values of NO_3^- -N in S1, S2, and S3 under N3 were 90.28, 89.65, and 84.97 $\text{mg}\cdot\text{kg}^{-1}$, respectively. The depth corresponding to the peak NO_3^- -N content under different N application times was greatest for S1 (40 cm), followed by S3 (30 cm) and S2 (20 cm).

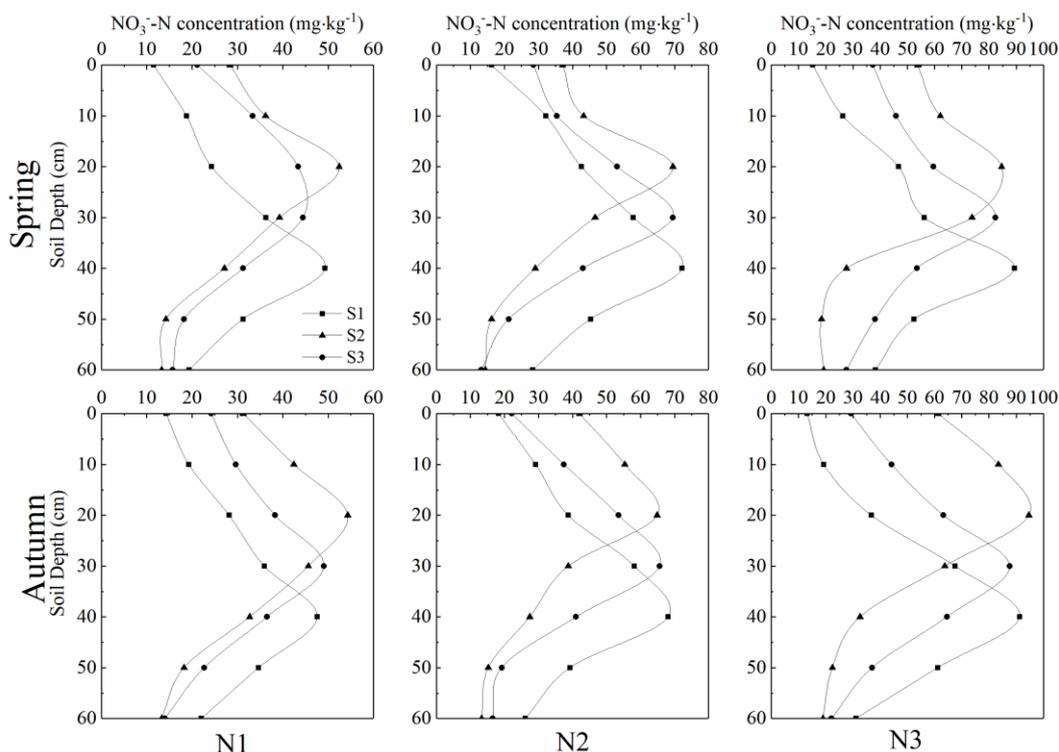


Figure 5. The distribution of NO_3^- -N under different treatments

Photosynthetic parameters

Net photosynthetic rate

The application rate and injection timing of N had significant effects on P_n , but their interaction was not significant. *Table 2* shows the net photosynthetic rate of greenhouse tomatoes at each growth stage under different N application strategies. There were no significant differences in P_n under the different application rate and injection timing of N in the W1 stage. In the W2 stage, the P_n was significantly higher in N1, N2 and N3 than in CK. In spring and autumn tomatoes, the highest P_n was observed in N3 (23.81 and 21.66 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). Compared with CK, the P_n of spring and autumn tomatoes in N3 was increased by 38.51 and 42.22%, respectively. In the W2 stage, the P_n was significantly higher in S1, S2 and S3 than in CK. In spring and autumn tomatoes, the highest P_n were observed in S3 (23.06 and 21.39 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). Compared with CK, the P_n of spring and autumn tomatoes in S3 was increased by 34.15 and 40.45%, respectively. In the W3 stage, the P_n was significantly higher in N1, N2 and N3 than in CK. The P_n of spring and autumn tomatoes were highest for N2 (18.89 and 17.78 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). Compared with CK, the P_n of spring and autumn tomatoes in N2 was increased by 18.21 and 30.54%, respectively. In the W3 stage, the P_n was significantly higher in S1, S2 and S3 than in CK. In spring and autumn tomatoes, the highest P_n was observed in S3 (18.67 and 17.17 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). Compared with CK, the P_n of spring and autumn tomatoes in S3 were increased by 16.83 and 26.06%, respectively. There were no significant differences between S2 and S3 at all stages.

Table 2. Effects of different the application rates (N-R) and injection timings (N-T) of N on the P_n ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) of spring tomatoes and autumn tomatoes

Main effect	Spring			Autumn		
	W1	W2	W3	W1	W2	W3
CK	14.76±1.71aA	17.19±1.61cC	15.98±1.32cC	12.66±0.95aA	15.23±1.52cC	13.62±0.97cC
N-R						
N1	14.38±1.17a	19.64±1.53b	17.08±1.24b	13.17±1.29a	18.20±1.48b	15.23±1.63b
N2	14.86±0.98a	23.16±2.24a	18.89±1.41a	13.16±1.40a	21.71±1.57a	17.78±1.37a
N3	15.02±1.11a	23.81±1.92a	18.47±1.27a	13.16±1.37a	21.66±1.85a	16.94±1.28a
N-T						
S1	14.51±1.20A	20.59±2.13B	17.51±1.32B	13.13±1.30A	19.30±2.35B	15.75±1.65B
S2	14.98±1.10A	22.96±2.75A	18.26±1.36AB	13.22±1.36A	20.88±2.17A	17.04±1.59A
S3	14.77±1.01A	23.06±2.31A	18.67±1.65A	13.35±1.40A	21.39±1.95A	17.17±1.77A
F-value						
N	2.16ns	57.33**	15.71**	0.21ns	54.09**	27.38**
S	1.10ns	22.25**	6.01**	0.19ns	15.85**	9.97**
N*S	0.48ns	1.19ns	0.76ns	0.79ns	0.36ns	0.32ns

The data are all average ± standard deviation in the figure, different letters in the same column meant significant difference at 0.05 level, lowercase letters indicate differences between N application rates, capital letters indicate differences between N injection timings; * indicates significant correlation ($P < 0.05$); ** indicates highly significant correlation ($P < 0.01$), and ns indicates not significant

WUE_L

Table 3 shows the instantaneous water use efficiency (WUE_L , calculated by Eq. 6) of greenhouse tomatoes at each growth stage under different N application strategies. There were no significant differences in WUE_L among the different treatments in the W1 stage. In the W2 stage, the WUE_L first increased and then decreased as the N application rate increased. The WUE_L of spring and autumn tomatoes were highest for N2 (2.25 and 2.43 mmol CO₂·mol⁻¹ H₂O, respectively). Compared with CK, the WUE_L was increased by 25.70% and 48.17% in N2 for spring and autumn tomatoes, respectively. In the W2 stage, the WUE_L was significantly higher in S1, S2 and S3 than in CK. In spring and autumn tomatoes, the highest WUE_L were observed in S3 (2.21 and 2.36 mmol CO₂·mol⁻¹ H₂O, respectively). Compared with CK, the WUE_L of spring and autumn tomatoes in S3 was increased by 23.46 and 43.90%, respectively. In the W3 stage, the WUE_L first increased and then decreased as the N application rate increased. The WUE_L of spring and autumn tomatoes were highest for N2 (2.33 and 2.49 mmol CO₂·mol⁻¹ H₂O, respectively). Compared with CK, the WUE_L was increased by 24.60% and 31.75% in N2 for spring and autumn tomatoes, respectively. In the W3 stage, the WUE_L was significantly higher in S1, S2 and S3 than in CK. In the W3 stage, the WUE_L for spring tomatoes was highest in S3 (2.30 mmol CO₂·mol⁻¹ H₂O), and that for autumn tomatoes was highest in S2 (2.43 mmol CO₂·mol⁻¹ H₂O). Compared with CK, the WUE_L was increased by 24.60% and 31.75% in N2 for spring and autumn tomatoes, respectively. In the W3 stage, the WUE_L was significantly higher in S1, S2 and S3 than in CK. In the W3 stage, the WUE_L for spring tomatoes was highest in S3 (2.30 mmol CO₂·mol⁻¹ H₂O), and that for autumn tomatoes was highest in S2 (2.43 mmol CO₂·mol⁻¹ H₂O). Compared with CK, the WUE_L was increased by 22.99% in S3 and 28.57% in S2 for spring and autumn tomatoes, respectively.

Table 3. Effects of different the application rates (N-R) and injection timings (N-T) on the WUE_L (mmol CO₂·mol⁻¹ H₂O) of spring tomatoes and autumn tomatoes

Main effect	Spring			Autumn		
	W1	W2	W3	W1	W2	W3
CK	2.12±0.24aA	1.79±0.27cB	1.87±0.17cB	1.87±0.17aA	1.64±0.16cC	1.89±0.09cC
N-R						
N1	2.01±0.19a	1.97±0.22b	2.11±0.23b	2.13±0.21a	2.04±0.18b	2.18±0.18b
N2	2.10±0.17a	2.25±0.34a	2.33±0.21a	2.36±0.19a	2.43±0.21a	2.49±0.22a
N3	2.15±0.24a	2.23±0.21a	2.23±0.29ab	2.26±0.30a	2.35±0.22a	2.40±0.17a
N-T						
S1	2.04±0.22A	2.05±0.25A	2.12±0.23A	2.20±0.25A	2.16±0.25B	2.24±0.22B
S2	2.14±0.19A	2.19±0.31A	2.25±0.27A	2.25±0.27A	2.30±0.25A	2.43±0.22A
S3	2.08±0.21A	2.21±0.29A	2.30±0.25A	2.30±0.25A	2.36±0.25A	2.41±0.20A
F-value						
N	3.09ns	10.26**	6.24**	1.51ns	33.93**	24.05**
S	1.63ns	2.94*	4.15*	1.87ns	8.50**	10.67**
N*S	0.43ns	1.48ns	1.00ns	1.18ns	1.03ns	0.51ns

The data are all average ± standard deviation in the figure, different letters in the same column meant significant difference at 0.05 level, lowercase letters indicate differences between N application rates, capital letters indicate differences between N injection timings; * indicates significant correlation (P < 0.05); ** indicates highly significant correlation (P < 0.01), and ns indicates not significant

Rhizosphere soil enzyme activity

The application rate and injection timing of N had significant effects on soil urease activity, but their interaction was not significant (*Table 4*). Therefore, these two factors were analyzed separately. Soil urease activity increased as the N application rate increased, but there was no significant difference between N2 and N3. The soil urease activity of spring and autumn tomatoes was the highest in N3, which was 0.74 and 0.67 mg·(g·24 h)⁻¹, respectively. Compared with CK, the soil urease activity was increased by 29.82 and 31.37% in spring and autumn tomatoes under N3, respectively. Soil urease activity was higher under S2 and S3 than under S1, but there was no significant difference between S2 and S3. The soil urease activity of spring and autumn tomatoes was the highest in S3, which was 0.73 and 0.65 mg·(g·24 h)⁻¹, respectively. Compared with CK, the soil urease activity was increased by 28.07 and 27.45% in spring and autumn tomatoes under S3, respectively.

Table 4. Effects of different application rates (N-R) and injection timings (N-T) on rhizosphere soil urease activity

Main effect	Soil urease mg·(g·24 h) ⁻¹	
	Spring	Autumn
CK	0.57 ± 0.04cC	0.51 ± 0.06cC
N-R		
N1	0.65 ± 0.06b	0.59 ± 0.07b
N2	0.71 ± 0.06a	0.64 ± 0.06a
N3	0.74 ± 0.08a	0.67 ± 0.07a
N-T		
S1	0.66 ± 0.07B	0.60 ± 0.07B
S2	0.71 ± 0.07A	0.64 ± 0.07AB
S3	0.73 ± 0.08A	0.65 ± 0.06A
F-value		
N	14.16**	9.59**
S	7.75**	4.85*
N*S	0.34ns	0.15ns

The data are all average ± standard deviation in the figure, different letters in the same column meant significant difference at 0.05 level, lowercase letters indicate differences between N application rates, capital letters indicate differences between N injection timings; * indicates significant correlation (P < 0.05); ** indicates highly significant correlation (P < 0.01), and ns indicates not significant

The application rate and injection timing of N had significant interaction effects on soil sucrase activity (*Table 5*). Under S1, soil sucrase activity increased as the N application rate increased. Under S2 and S3, soil sucrase activity first increased and then decreased as the N application rate increased. At the same N application rate, soil sucrase activity was significantly higher under S2 and S3 than under S1, but there was no significant difference between S2 and S3. The soil sucrase activity of spring and autumn tomatoes was the highest under N2S2 (4.42 and 3.74 μg·(g·24 h)⁻¹, respectively). Compared with CK, the soil sucrase activity was increased by 75.4 and 79.81% in spring and autumn tomatoes under N2S2, respectively.

The application rate and injection timing of N had significant interaction effects on soil phosphatase activity (Table 5). Under S1, soil phosphatase activity increased as the N application rate increased, but there was no significant difference between N2S1 and N3S1. Under S2 and S3, soil acid phosphatase activity first increased and then decreased as the N application rate increased. At the same N application rate, soil phosphatase activity was significantly higher under S2 and S3 than under S1, but there was no significant difference between S2 and S3. Soil phosphatase activity was highest in spring tomatoes under N2S3 and in autumn tomatoes under N2S2 (33.25 and 25.57 $\mu\text{g}\cdot(\text{g}\cdot 24\text{ h})^{-1}$, respectively). Compared with CK, the soil phosphatase activity was increased by 64.44 and 97.45% in spring tomato under N2S3 and autumn tomato under N2S2, respectively.

Table 5. Effects of different treatments on rhizosphere soil sucrose and phosphatase activity

Treatment	Spring		Autumn	
	Soil sucrose $\mu\text{g}\cdot(\text{g}\cdot 24\text{ h})^{-1}$	Soil phosphatase $\mu\text{g}\cdot(\text{g}\cdot 24\text{ h})^{-1}$	Soil sucrose $\mu\text{g}\cdot(\text{g}\cdot 24\text{ h})^{-1}$	Soil phosphatase $\mu\text{g}\cdot(\text{g}\cdot 24\text{ h})^{-1}$
N1S1	3.02 ± 0.51c	22.67 ± 2.38e	2.53 ± 0.19d	14.3 ± 1.39f
N1S2	3.31 ± 0.19c	24.74 ± 2.02e	2.93 ± 0.22c	16.84 ± 1.43de
N1S3	3.31 ± 0.13c	24.82 ± 1.59d	2.94 ± 0.25c	16.73 ± 1.91e
N2S1	3.73 ± 0.28b	27.2 ± 1.14c	3.26 ± 0.21b	18.22 ± 1.62cd
N2S2	4.42 ± 0.24a	33.17 ± 2.04a	3.74 ± 0.24a	25.57 ± 1.51a
N2S3	4.34 ± 0.19a	33.25 ± 1.92a	3.61 ± 0.33a	24.95 ± 1.51a
N3S1	3.9 ± 0.36b	28.99 ± 1.76b	3.28 ± 0.23b	18.82 ± 1.44bc
N3S2	3.96 ± 0.5b	29.97 ± 1.57b	3.31 ± 0.2b	19.93 ± 1.63b
N3S3	3.94 ± 0.22b	29.88 ± 2.62b	3.3 ± 0.18b	19.89 ± 1.42b
CK	2.52 ± 0.31d	20.22 ± 1.28f	2.08 ± 0.21e	12.95 ± 0.73f
F-value				
N	65.46**	106.60**	73.19**	149.35**
S	9.75**	23.14**	13.53**	71.18**
N*S	2.72*	5.99**	3.07*	10.66**

The data are all average ± standard deviation in the figure, different letters in the same column meant significant difference at 0.05 level, lowercase letters indicate differences between N application rates, capital letters indicate differences between N injection timings; * indicates significant correlation ($P < 0.05$); ** indicates highly significant correlation ($P < 0.01$), and ns indicates not significant

Y, WUE, and NUE

The application rate and injection timing of N had significant effects on spring and autumn tomato Y, WUE (calculated by Eq. 4), and NUE (calculated by Eq. 5; Table 6). As no interaction effects were detected, the two factors were analyzed separately. The Y of spring and autumn tomatoes increased as the N application rate increased. The Y was significantly higher under N1, N2 and N3 than under CK, but there was no significant difference between N2 and N3. The Y of spring and autumn tomatoes was the highest in N3, which was 2.75 and 2.39 $\text{kg}\cdot\text{plant}^{-1}$, respectively. Compared with CK, the Y was increased by 55.37 and 52.23% in spring and autumn tomatoes under N3, respectively. The Y was higher in S1, S2 and S3 than under S1, but there was no significant difference between S2 and S3. The Y for spring tomatoes was highest in S3

(2.62 kg·plant⁻¹), and that for autumn tomatoes was highest in S2 (2.29 kg·plant⁻¹). Compared with CK, the *Y* was increased by 48.02 and 45.86% in spring and autumn tomatoes under S3 and S2, respectively. The variation in *WUE* was similar to that observed for *Y*. The *Y* of spring and autumn tomatoes increased as the N application rate increased. The *WUE* of spring and autumn tomatoes was highest under N3, which was 21.18 and 26.64 kg·m⁻³, respectively. Compared with CK, the *WUE* was increased by 49.58 and 47.18% in spring and autumn tomatoes under N3, respectively. The *WUE* was higher in S1, S2 and S3 than under S1, but there was no significant difference between S2 and S3. The *WUE* for spring and autumn tomatoes was highest in S3 (20.24 and 25.61 kg·m⁻³, respectively). Compared with CK, the *WUE* was increased by 42.94 and 41.49% in spring and autumn tomatoes under S3, respectively. *NUE* first increased and then decreased as the N application rate increased. *NUE* was highest under N2. Compared with N1, the *NUE* was increased by 52.19 and 31.43% in spring and autumn tomatoes under N2, respectively. The *NUE* for spring tomatoes was highest in S3 (122.22 kg·kg⁻¹), and that for autumn tomatoes was highest in S2 (105.59 kg·kg⁻¹). Compared to S1, the *NUE* was increased by 53.70 and 31.87% in spring and autumn tomatoes under S3 and S2, respectively.

Table 6. Effects of different the application rates (N-R) and injection timings (N-T) on the *Y*, *WUE*, and *NUE* of spring tomatoes and autumn tomatoes

Main effect	Spring			Autumn		
	<i>WUE</i> (kg·m ⁻³)	<i>NUE</i> (kg·kg ⁻¹)	<i>Y</i> (kg·plant ⁻¹)	<i>WUE</i> (kg·m ⁻³)	<i>NUE</i> (kg·kg ⁻¹)	<i>Y</i> (kg·plant ⁻¹)
CK	14.16±1.26dC	-	1.77±0.16dC	18.10±1.54cC	-	1.57±0.13cC
N-R						
150	17.17±1.20c	81.48±31.96b	2.19±0.16c	22.75±1.98b	83.33±34.50b	1.99±0.18b
200	20.30±1.71b	123.96±33.70a	2.61±0.23b	25.45±1.95a	109.52±25.81a	2.32±0.18a
250	21.18±1.56a	115.67±26.26a	2.75±0.21a	26.64±2.16a	95.85±22.67ab	2.39±0.19a
N-T						
S1	18.32±2.01B	79.52±29.04B	2.34±0.27B	23.72±1.98B	80.07±22.36B	2.12±0.20B
S2	20.09±2.12A	119.37±29.37A	2.60±0.29A	25.49±2.32A	105.59±26.75A	2.29±0.22A
S3	20.24±2.26A	122.22±30.96A	2.62±0.31A	25.61±3.00A	103.04±33.19A	2.29±0.28A
F-value						
N	73.62**	20.80**	89.92**	32.11**	6.75**	43.72**
S	17.59**	24.04**	25.98**	9.06**	7.77**	9.37**
N*S	0.975ns	0.78 ns	1.21ns	0.91ns	0.56ns	0.77ns

The data are all average ± standard deviation in the figure, different letters in the same column meant significant difference at 0.05 level, lowercase letters indicate differences between N application rates, capital letters indicate differences between N injection timings; * indicates significant correlation (P < 0.05); ** indicates highly significant correlation (P < 0.01), and ns indicates not significant

Discussion

Effects of the rate and timing of N application on photosynthetic characteristics

In this study, the *P_n* of greenhouse tomatoes under different treatments differed in different growth stages. In the W1 stage, the application rate and injection timing of N had little effect on the *P_n* of greenhouse tomato. This may stem from the fact that

tomato plants were small and have a low demand for N, and the N level in all treatments was sufficient for meeting the growth demands of the tomato plants. In the W2 stage, the P_n of greenhouse tomatoes increased as the N application rate increased; this finding is consistent with those of Ruiz et al. (2008). There was no significant difference in the P_n between N3 and N2. The P_n was higher under S2 and S3 than under S1, which stems from the fact that the distribution of N under S2 and S3 was more conducive to the absorption and utilization of N by tomato plants. In the W3 stage, the P_n of greenhouse tomatoes first increased and then decreased as the N application rate increased. Previous studies (Wang et al., 2018; Huang et al., 2003) have shown that greater applications of fertilizer in the early and middle stages of tomato growth may lead to the overgrowth of tomato plants, resulting in shortages of water and fertilizer in the late growth stage. Variation in the P_n among different N application timings in the W1 stage was similar to that in the W2 stage. In the W3 stage, variation in the P_n under different N application timings was similar to that in the W2 stage.

WUE_L is an important parameter used to describe WUE at the leaf scale (Centritto et al., 2000). The variation in WUE_L of greenhouse tomatoes under different treatments in this study differed among growth stages. In the W1 stage, there was no significant difference in the WUE_L of greenhouse tomatoes under different treatments. In the W2 and W3 stages, the WUE_L first increased and then decreased as the N application rate increased. This indicates that an appropriate N application rate (i.e., not too high or low) can improve tomato WUE_L , a finding that is consistent with those of Zhou et al. (2020).

Effects of the rate and timing of N application on soil enzyme activities

Soil enzymes are derived from the secretions of animals, plants, and microbial cells and their decomposed residues in soil, and microbial cells are the main source of soil enzymes. There is a close correlation between soil enzyme activities and soil physicochemical properties (Acosta et al., 1999). In general, high enzyme activity is considered an important indicator of soil fertility (Hussain et al., 2017). There were significant differences in soil enzyme activities between all treatments (Tables 4 and 5). The soil enzyme activity was higher in spring tomatoes than in autumn tomatoes. This mainly stems from the fact that the soil temperature was higher for spring tomatoes than for autumn tomatoes. Lower temperature reduces cell activity, which is not conducive to the respiration of microorganisms and crops (Luo et al., 2020), and thus affects the activity of soil enzymes.

Soil urease catalyzes the hydrolysis of urea to NH_3 (Ibrahim et al., 2020). As the N application rate increased, the soil urease activity increased, and the soil urease activity was significantly higher under N2 and N3 than under N1. Previous studies have shown that the response of soil urease activity to increases in N varies in different ecosystems. For example, some studies have shown that soil urease activity significantly increases when the N fertilizer applied increases (Dalal et al., 1975; Wang et al., 2008). By contrast, other studies have shown that increases in N application can inhibit soil urease activity (Wang et al., 2014; Khakural et al., 1995). This is because (1) soil microbial activity is not exclusively dependent on the total soil N content but is also restricted by the soil organic carbon content and (2) soil types, vegetation types, and nutrient availability vary in different ecosystems. In this paper, the application of organic fertilizer before planting tomato increased the soil organic carbon content; consequently, the increased application of N fertilizer increased soil urease activity, which was consistent with the results of Marcote et al. (2001) and Liu et al. (2010).

There was no significant difference in urease activity between N2 and N3. The excessive accumulation of $\text{NH}_4^+\text{-N}$ in the soil as the N application rate increased is thought to inhibit soil urease activity. Given that an N application rate of $250 \text{ kg}\cdot\text{ha}^{-1}$ was not sufficient for inhibiting soil urease activity, additional work is needed to determine whether there is eventually an N application rate beyond which soil urease activity is reduced. Compared with S1, the urease activity under S2 and S3 was significantly increased, which mainly stemmed from the difference in the timing of N application and N distribution in the soil (*Fig. 5*).

Soil sucrase is directly involved in the metabolism of organic matter and the release of low molecular weight sugars in soil, which is an important index for characterizing the rate of soil carbon cycling. There were significant differences in soil sucrase activity under different N application rates (*Table 5*). Compared with the control group, the application of N fertilizer increased the activity of sucrase, and as the N application rate increased, the soil sucrase activity first increased and then decreased. This may stem from the fact that as the N application rate increases, soil organic carbon content gradually becomes the main factor restricting soil microbial activity. According to Kim et al. (2011), when the background value of soil N content is high, the increase in N application tends to inhibit enzyme activity; in all other cases, soil N content improves enzyme activity, and the results of this paper confirm this observation. Soil sucrase activity under S2 and S3 was significantly different from that under S1, which may stem from the fact that the different distributions of N among treatments affected the intensity of the activity of microorganisms near plant roots and soil sucrase activity.

The secretion of soil phosphatase is an important adaptive response of plants to low P environments (Taylor et al., 1993; Duff et al., 1994; Tran et al., 2010a). Previous studies (e.g., Teixeira et al., 2021) have shown that soil organic matter, total N, and total P have significant effects on the activity of acid phosphatase. Significant differences were observed in all experimental treatments in this study. This may stem from the fact that the growth of plant roots varies under different soil N supply capacities. Large roots have a higher demand for P, which promotes the secretion of acid phosphatase by plant roots.

Effects of the rate and timing of N application on Yield, WUE, and NUE

Fertilizer plays an important role in crop growth and development. Tomato absorbs moisture and nutrients from the soil through its roots, carries out photosynthesis, and synthesizes carbohydrates. N can regulate crop physiological processes and support crop growth (Bartkowiak et al., 2015) and is one of the most important nutrient elements for crop growth. In this study, the tomato *Y* under irrigation was significantly higher under S2 and S3 than under S1. This mainly stems from the fact that under S1, urea is more prone to leaching downward, which concentrates the $\text{NO}_3^-\text{-N}$ in the soil layer below 40 cm. Under S2 and S3, $\text{NO}_3^-\text{-N}$ was mostly concentrated in the 20~30 cm soil layer, and the taproot zone during tomato growth mainly occurs in the 20~30 cm soil layer, which facilitates the absorption and utilization of N. As the N application rate increased, the tomato *Y* gradually increased. There was no significant difference in tomato *Y* between N3 and N2. As the N application rate increased, the effect of N on tomato *Y* gradually decreased. Du et al. (2017) found that the *Y* and *WUE* of greenhouse tomatoes were at their highest when the N application rate was $250 \text{ kg}\cdot\text{ha}^{-1}$ in northwestern China; this is consistent with our results.

In this study, the *WUE* of greenhouse tomatoes gradually increased as the N application rate increased, but there was no significant difference between N2 and N3. In other words, further increases in the N application rate slow the increasing trend in *WUE*. The *NUE* first increased and then decreased as the N application rate increased. The reason is that increases in the N application rate can promote the growth of crop roots (Wang et al., 2011), keep the stomata open (Bucci et al., 2006), and enhance photosynthesis (Ruiz et al., 2008), thus improving the *WUE* and *NUE* of crops.

However, as the N application rate increases, the main factors limiting crop growth change, and the dry matter formation efficiency of crops reduces, which slows the increase in *WUE* and decreases *NUE* (Wang et al., 2018; Huang et al., 2003). Compared to S1, the distribution of N under S2 and S3 could improve *WUE* and *NUE*. The reason is that N under S2 and S3 was concentrated in the 20~30 cm layer, which is the layer in which N is most accessible to crops. In the W2 stage, maintaining the NO_3^- -N content between 70 and 80 $\text{mg}\cdot\text{kg}^{-1}$ in the 20~30 cm soil layer helped maintain higher *WUE* and *NUE* and achieve higher tomato *Y*.

Conclusions

There are three main conclusions of this study. First, the effects of N application strategies on photosynthesis vary among tomato growth stages. Excessive N application may decrease the P_n and WUE_L of tomato at later growth stages. In the W2 and W3 stages, the P_n and WUE_L of tomato were higher under S2 and S3 than under S1. Second, soil urease activity increased non-linearly as the N application rate increased; the soil sucrase activity first increased and then decreased as the N application rate increased; soil phosphatase activity increased significantly as the N application rate increased. The soil enzyme activities were significantly higher under S2 and S3 than under S1. Third, the *Y* and *WUE* of greenhouse tomatoes increased non-linearly as the N application rate increased, and there were no significant differences between N3 and N2. Compared to S1, S2 and S3 improved the *Y* and *WUE* of greenhouse tomato. Considering our results comprehensively, the combination of N injection timing of S2 or S3 (middle or last 1/3 of irrigation) under N application rate of N2 (200 $\text{kg}\cdot\text{ha}^{-1}$) is recommended as technical parameters for greenhouse tomato cultivation in arid and semi-arid sandy loam soils. However, the nitrogen application strategies need to be further studied in different regions, tomato genotypes, and soil textures.

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