



**ARTICLE**

## Adjusting Nitrogen Application in Accordance with Soil Water Availability Enhances Yield and Water Use by Regulating Physiological Traits of Maize under Drip Fertigation

Mingda Yang<sup>1</sup>, Shouchen Ma<sup>2</sup>, Fujian Mei<sup>1</sup>, Li Wei<sup>1</sup>, Tongchao Wang<sup>1,\*</sup> and Xiaokang Guan<sup>1,\*</sup>

<sup>1</sup>Collaborative Innovation Center of Henan Grain Crops, Agronomy College of Henan Agricultural University, Zhengzhou, 450002, China

<sup>2</sup>Field Scientific Observation and Research Base of Land Use, Ministry of Land and Resources, Henan Polytechnic University, Jiaozuo, 454000, China

\*Corresponding Authors: Tongchao Wang. Email: wtcwrn@126.com; Xiaokang Guan. Email: guanxk06@126.com

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### ABSTRACT

Knowledge of the interactive effects of water and nitrogen (N) on physio-chemical traits of maize (*Zea mays* L.) helps to optimize water and N management and improve productivity. A split-plot experiment was conducted with three soil water conditions (severe drought, moderate drought, and fully water supply referring to 45%–55%, 65%–75%, and 85%–95% field capacity, respectively) and four N application rates ( $N_0$ ,  $N_{150}$ ,  $N_{240}$ , and  $N_{330}$  referring to 0, 150, 240, 330 kg N ha<sup>-1</sup> respectively) under drip fertigation in 2014 and 2015 in the Huang-Huai-Hai Plain of China. The results indicated that drought stress inhibited physiological activity of plants (leaf relative water content, root bleeding sap, and net photosynthetic rate), resulting in low dry matter accumulation after silking, yield, and N uptake, whereas increased WUE and NUE. N application rates over than 150 kg ha<sup>-1</sup> aggravated the inhibition of physiological activity under severe drought condition, while it was offset under moderate drought condition. High N application rates ( $N_{330}$ ) still revealed negative effects under moderate drought condition, as it did not consistently enhance plant physiological activity and significantly reduced N uptake as compared to the  $N_{240}$  treatment. With fully water supply, increasing N application rates synergistically enhanced physiological activity, promoted dry matter accumulation after silking, and increased yield, WUE, and N uptake. Although the  $N_{240}$  treatment reduced yield by 5.4% in average, it saved 27.3% N under full water supply condition as compared with  $N_{330}$  treatment. The results indicated that N regulated growth of maize in aspects of physiological traits, dry matter accumulation, and yield as well as water and N use was depended on soil water status. The appropriate N application rates for maize production was 150 kg ha<sup>-1</sup> under moderate drought or 240 kg ha<sup>-1</sup> under fully water supply under drip fertigation, and high N supply (>150 kg ha<sup>-1</sup>) should be avoided under severe drought condition.

### KEYWORDS

Interaction of water and nitrogen; net photosynthetic rate; root bleeding sap; nitrogen uptake; water use efficiency



## 1 Introduction

The Huang-Huai-Hai Plain (3HP) of China is one of the most important cereal producing regions in China. Maize production from this area accounts for 35.5% of total national cereal production [1]. To support relatively high yield in this region, large amounts of irrigation water and chemical fertilizers (especially nitrogen, N) have been applied for several decades. However, due to the increased of water demand from industry and domestic purposes, agricultural irrigation water availability has been shrunk in the 3HP [2]. Besides, over fertilization of N and extensive irrigation method in agriculture, such as flood irrigation and broadcasting of fertilizer, reduce the efficiency of water and N use in agriculture, which making the effective irrigation methods and corresponding with water and N management become the most urgent perspective in the 3HP of China [3–5]. Drip irrigation has been approved as an efficient irrigation technology in crop production system and is widely used in the 3HP of China [4–6]. The advantages of drip irrigation technology, including high water use efficiency (WUE), reducing ineffective losses and environmental risks (e.g., surface soil evaporation and deep percolation) has been substantially approved in the 3HP of China and worldwide [4–7].

Water and nutrients are the most important factors in crop growth, development, and yield formation [8]. Sufficient water supply induces higher net photosynthetic rate ( $P_n$ ) of plant leaves and maintains photosystem II activity [9], which results in a higher dry matter accumulation and grain yield [10]. N as the essential nutrient, it involves in various physiological and biochemical processes of plants [11], and finally determines crops yield [12]. N plays vital role in the development of crop root system, and the feedback effects have been showed in water and N absorption of crops [13–15]. However, huge amount of N application with inappropriate ways (approaches, e.g., manual broadcasting of granules or is applied in bulk quantities at one time) resulted into minimized plant N uptake and maximized waste of fertilizer through leaching and volatilization pathways [16,17], thus causing potential environmental risk and farming costs [18]. Therefore, precise water and N management is urgently needed in intensive cultivated region such as 3HP of China. The functioning of N fertilizer in crop growth and development is regulated by soil water status. Frederick et al. [19] revealed that fully yield potential would be obtained with N application under well-watered conditions, while the severity of water stress would be aggravated under limited irrigation conditions with N application. Moreover, increasing N application rate is not an appropriate way to compensate the biomass reduction from water stress in maize [20]. Judicious management of water and N can enhance their synergistic interaction, which promotes growth and enhances the photosynthesis, thereby increasing the yield and the efficiency of water and N [21,22].

Drip fertigation has been incorporated with water and N management in together by precisely supplied water and N as per plant demand, which has been proved as an efficient way in water and N use efficiency improvement. Tian et al. [5] noted that maize yield had been increased for 3.8% with 30.4% N and 40% irrigation water saving under drip fertigation when compared with flood irrigation and urea broadcasted. Wang et al. [23] also found that application rate of 190–240 kg N ha<sup>-1</sup> obtained the highest yield and agronomic N efficiency under mulched drip fertigation, which was almost the same yield produced compared with conventional irrigation and fertilization methods of 340 kg N ha<sup>-1</sup> in Xinjiang province of China. The reason for yield increase and water, N use efficiency promotion was mainly due to the interaction of water and N [8,20,24].

Photosynthetic assimilation as the most important physiological process of plant would be responded to water and N interaction [21,25], which would potentially influence the dry matter accumulation and remobilization of maize. The physiological traits, such as  $P_n$  and leaf relative water content are good indicators in plants resistance to dehydration of stress [26]. Under water stress, maintaining high leaf relative water content is beneficial for regulating plant physiological and biochemical metabolism of plant by stomatal closure, decrease transpiration and maintenance of PSII activity [27]. Bleeding sap is a manifestation of the root pressure, which reveals the root function of its absorption activities [28]. With

these indicators, plant physiological response of water and N interactions can be revealed in different water and N conditions. Most researches on the physiological traits of field maize focus on the effects of single factor such as irrigation [9,29] or fertilization [30,31]. There was little information about the interactive effects of water and N in terms of fertigation on physiological traits of maize, especially under the condition of drip fertigation.

We hypothesized that proper water and N management would promote maize yield and water productivity by improving physiological traits of maize. The objectives of this study were: (1) To clarify the interactive effects of water and N on the physiological process of maize; (2) To reveal the potential yield, water, and N use efficiency increase of summer maize under drip fertigation.

## 2 Materials and Methods

### 2.1 Descriptions of Experimental Site

A field experiment was conducted in summer maize growing seasons from June to October of 2014 and 2015 at the Experimental Station of Henan Agricultural University, Henan Province, China (34°47'51"N, 113°38'3"E). Based on 30 years of meteorological data, the annual average minimum and maximum air temperature were 10.0°C and 20.4°C, respectively, and annual mean precipitation was 640 mm. The soil type was sandy loam in the experiment site. The main physical characteristics and content of sand, silt, and clay in the 0–160 cm soil layers are listed in Tab. 1. Before sowing in 2014, the main properties of the 0–30 cm soil layer were as follow: organic matter content 11.6 g kg<sup>-1</sup>, alkaline-hydrolysable nitrogen 128.7 mg kg<sup>-1</sup>, available phosphorus 27.5 mg kg<sup>-1</sup>, and available potassium 235.8 mg kg<sup>-1</sup>. Furthermore, the basic soil properties are presented in Tab. 1 and the meteorological data in the summer maize growing seasons is shown in Fig. 1.

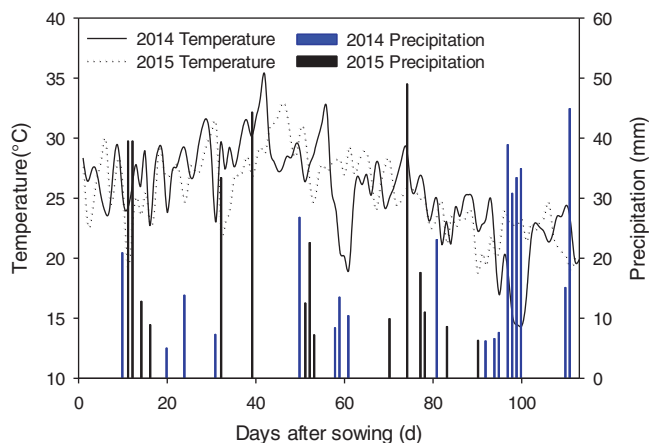
**Table 1:** Main physical characteristics of soil in the experimental plots

Soil layer (cm)	Field capacity (%)	Bulk density (g cm <sup>-3</sup> )	Physical properties		
			Sand (%)	Silt (%)	Clay (%)
0–20	23.6	1.31	55.9	29.0	15.1
20–40	21.8	1.46	53.6	29.4	17.0
40–60	22.0	1.31	50.7	30.9	18.4
60–80	23.4	1.36	59.0	29.9	11.1
80–100	23.8	1.31	56.4	29.0	14.6
100–160	23.0	1.35	55.1	29.6	15.3

Summer maize was grown under a movable transparent rain-shelter structure, and it was closed when there was rain. Each plot was isolated by a 14 cm width concrete wall to prevent water seepage, and the bottom of the plot was a waterproof layer. The depth of each plot was 2.0 m, and total measured area of each plot was 6.6 m<sup>2</sup> (2.2 m × 3 m).

### 2.2 Experimental Design and Field Management

A split plot design was adopted with different water treatments, including soil water content within 45%–55% of field capacity (FC) (severe drought, SD), 65%–75% FC (moderate drought, MD), and 85%–95% FC (fully water supply, FW) after third leaf stage of maize. In addition, there were different N rates in the sub-plots as 0 kg ha<sup>-1</sup> (N<sub>0</sub>), 150 kg ha<sup>-1</sup> (N<sub>150</sub>), 240 kg ha<sup>-1</sup> (N<sub>240</sub>), and 330 kg ha<sup>-1</sup> (N<sub>330</sub>). A total of 12 treatments were set up, and each treatment was replicated three times.



**Figure 1:** Daily average temperature and precipitation at the experimental site in maize growing seasons from June to October of 2014 and 2015

The irrigation system consisted of a pump, filter, flow meter, control valves, and pressure gauges, and the system was operated at 0.1 MPa within irrigation operation period. The drip irrigation laterals with inside diameter of 15.9 mm and pressure-compensating emitters were spaced at 60 cm (placed at every row of maize planted) on the soil surface. Irrigation water was supplied with 1.1 L h<sup>-1</sup> inline drippers located at the laterals with interval of 30 cm apart. A water meter (nominal diameter: 15 mm) was used to control the applied water for each irrigation level. The irrigation amount was calculated according to the following equation based on the pre-irrigation soil volumetric water content (SWC) (cm<sup>3</sup> cm<sup>-3</sup>) in each measured soil depth:

$$I = 0.1 \times (I_{ul} \times \gamma_{bd} - 1000\theta_b) \times D_h \quad (1)$$

where  $I$  (mm) is the irrigation amount,  $I_{ul}$  (%) is the irrigation upper limit,  $\theta_b$  (cm<sup>3</sup> cm<sup>-3</sup>) is the SWC before irrigation,  $\gamma_{bd}$  (g cm<sup>-3</sup>) is the soil bulk density, and  $D_h$  (cm) is the wetness depth of soil profile. Based on maize root system distribution in different growth stages, the irrigation amount has been calculated within 0–40 cm, 0–60 cm, and 0–80 cm before the jointing stage, from the jointing to silking stage, and after silking stage respectively. The initial irrigations were delivered in equal quantities for all plots at one day after sowing to ensure seed emergence and seedling establishment. The drip irrigation of MD and FW were performed 11 times in 2014 and 10 times in 2015 respectively with the same time, and the drip irrigation of SD were performed 9 times in 2014 and 8 times in 2015, respectively. Irrigation amount at different growth periods for different treatments are showed in [Tab. 2](#).

Calcium superphosphate for phosphorus (16% P<sub>2</sub>O<sub>5</sub>) and potassium oxide for potassium (60% K<sub>2</sub>O) were applied before soil preparation (60 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were applied in each summer maize season) as basal fertilizer application. Soluble urea (N content of 46%) was used in the experiment with the Venturi injector by drip fertigation. 10%, 30%, 40%, and 20% of soluble urea was applied at third leaf stage, jointing, tassel, and filling stage according to each N application rates, i.e., 0, 150, 240, and 330 kg ha<sup>-1</sup>.

### 2.3 Crop Management

The summer maize variety, *cvs Zhengdan 958* was sown on 10 June, 2014, and 14 June 2015. The seeding density was 75,000 plants ha<sup>-1</sup> with a row spacing of 60 cm and plant spacing of 22.2 cm (each plot had four rows, and each row had 13 plants). Summer maize was harvested on 30 September 2014, and 3 October 2015. Weeds, pests, and diseases were well controlled.

**Table 2:** Drip irrigation amount (mm) of summer maize at different growth periods under drip fertigation during 2014 and 2015

Treatments	2014				2015				
	From sowing to jointing stage	From jointing to silking stage	From silking to maturity	Total	From sowing to jointing stage	From jointing to silking stage	From silking to maturity	Total	
SD	N <sub>0</sub>	63.4	69.0	62.9	195.2	64.9	63.5	68.9	197.2
	N <sub>150</sub>	64.5	75.9	55.8	196.1	64.3	67.4	73.8	205.5
	N <sub>240</sub>	62.7	73.4	61.6	197.7	66.2	67.5	68.7	202.4
	N <sub>330</sub>	64.8	67.7	60.4	192.9	68.3	60.1	72.0	200.4
MD	N <sub>0</sub>	97.0	141.8	81.9	320.7	91.7	103.8	120.2	315.9
	N <sub>150</sub>	98.4	149.6	79.6	327.6	93.7	102.9	126.6	323.3
	N <sub>240</sub>	104.8	149.3	82.8	337.0	96.8	110.5	129.0	336.3
	N <sub>330</sub>	100.1	148.0	68.7	316.80	93.0	110.0	123.4	326.4
FW	N <sub>0</sub>	130.5	205.1	103.1	438.6	135.6	168.9	163.4	467.9
	N <sub>150</sub>	130.9	210.0	113.0	453.9	124.4	172.5	162.0	458.9
	N <sub>240</sub>	129.7	209.0	116.8	453.9	124.2	179.1	173.2	476.5
	N <sub>330</sub>	133.0	215.9	108.1	457.0	124.2	180.8	175.2	480.2

Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC. N<sub>0</sub>: N application rate 0 kg ha<sup>-1</sup>, N<sub>150</sub>: N application rate 150 kg ha<sup>-1</sup>, N<sub>240</sub>: N application rate 240 kg ha<sup>-1</sup>, N<sub>330</sub>: N application rate 330 kg ha<sup>-1</sup>.

## 2.4 Parameter Measurements

SWC was measured using time-domain reflectometry device (TRIME-PICO IPH, Germany) from the soil surface to 160-cm depth with 20-cm intervals. The measurements were made every 7 days from sowing to the jointing stage and every 5 days after the jointing stage.

Leaf relative water content was measured at the jointing (the first fully expanded leaf from top to bottom) and silking stages (ear leaf). Briefly, three leaves were cut from each plot of three individual plants. The initial fresh weight was weighed immediately, and then the leaf was transferred into clear water and saturated for 5 h. The leaf samples were removed from the water, and the saturated fresh weight was weighed. The leaf samples were placed in an oven and dried at 105°C for 30 min and then at 70°C until they reached a constant weight, which was recorded. Leaf relative water content was calculated as follow: Leaf relative water content (%) = (initial fresh weight–dry weight)/(saturated fresh weight–dry weight) × 100.

Root bleeding sap collection was carried out by a modified method according to Guan et al. [28] at the jointing, silking, and filling stages (25 days after silking) of maize. Briefly, three plants of each treatment were cut at the first internode (about 12 cm above the soil surface) at 18:00. The absorbent cotton was pressed on the incision to absorb the bleeding sap for 12 h. Each incision with absorbent was covered with a polyethylene sheet to keep out dust and insects. The absorbent cotton was measured at 06:00 the following morning. Root bleeding sap was recorded as the difference in the weight of the cotton bag before and after absorption. The delivery rate was expressed as concentration per time unit per root (g h<sup>-1</sup> root<sup>-1</sup>) according to Wang et al. [32].

$P_n$  of ear leaf was measured from 09:00 to 11:00 using an LI-6400 portable open-flow gas exchange system (Li-COR Inc., NE, USA) under controlled light intensity (1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in silking and filling stages of maize.

The above-ground biomass of summer maize was cut from the soil surface and separated into four components (stem + sheath, leaf, cob, and bract) in the silking stage and five components (stem + sheath,

leaf, cob, bract, and grain) at physiologically maturity. The plant samples were oven dried at 105°C for 30 min and at 70°C until they reached a constant weight. Dry matter weight in the silking stage and at physiologically maturity, and grain weight were recorded. Dry matter accumulation after silking, dry matter remobilization into grain, contribution of remobilization to grain, and contribution of dry matter accumulation after silking to grain were calculated as follow:

Dry matter accumulation after silking ( $\text{kg ha}^{-1}$ ) = dry matter weight at maturity – dry matter weight in the silking stage

Dry matter remobilization into grain ( $\text{kg ha}^{-1}$ ) = Dry matter weight in the silking stage – (dry matter weight at maturity-grain yield)

Contribution of remobilization to grain (%) = Dry matter remobilization into grain/grain yield  $\times$  100

Contribution of dry matter accumulation after silking to grain (%) = Dry matter accumulation after silking/grain yield  $\times$  100

Grain yield was measured at physiologically maturity by harvesting two rows in the middle of the plot and grain yield was expressed as dry weigh with 15.5% moisture content.

Evapotranspiration (ET) was calculated using the soil water balance equation:

$$ET = I + P + F - R - D + \Delta W \quad (2)$$

where  $ET$  (mm) is evapotranspiration,  $I$  (mm) is drip irrigation amount,  $P$  (mm) is the amount of precipitation,  $R$  (mm) is the surface runoff,  $D$  is drainage (mm), which was calculated as  $D = (\theta_j - FC)$ , where  $\theta_j$  are the SWC of the root zone (120–160 cm) in stages  $j$ , and  $D$  is set to zero if  $\theta_j < FC$ ;  $F$  (mm) is the capillary rise to the root zone, and  $\Delta W$  (mm) is the soil water depletion, calculated as the total soil water storage in the profile (0–160 cm) at sowing minus that at maturity. Since our experiment was conducted under a movable transparent rain-shelter and bottoms of all plots were sealed, the  $R$  and  $F$  were neglected here. Data analysis showed that  $D$  is zero for all treatments. So, the equation can be simplified as:

$$ET = I + \Delta W \quad (3)$$

WUE was defined as follows:

$$WUE = \text{Grain yield} / ET \quad (4)$$

Plant samples of different organs at physiologically maturity were analyzed for N concentrations by Kjeldahl method. N uptake was defined by multiplying N concentrations of plant by dry matter accumulation at physiologically maturity. Nitrogen use efficiency (NUE) was defined as follows [33]:

$$NUE = \text{Grain yield} / N \text{ uptake} \quad (5)$$

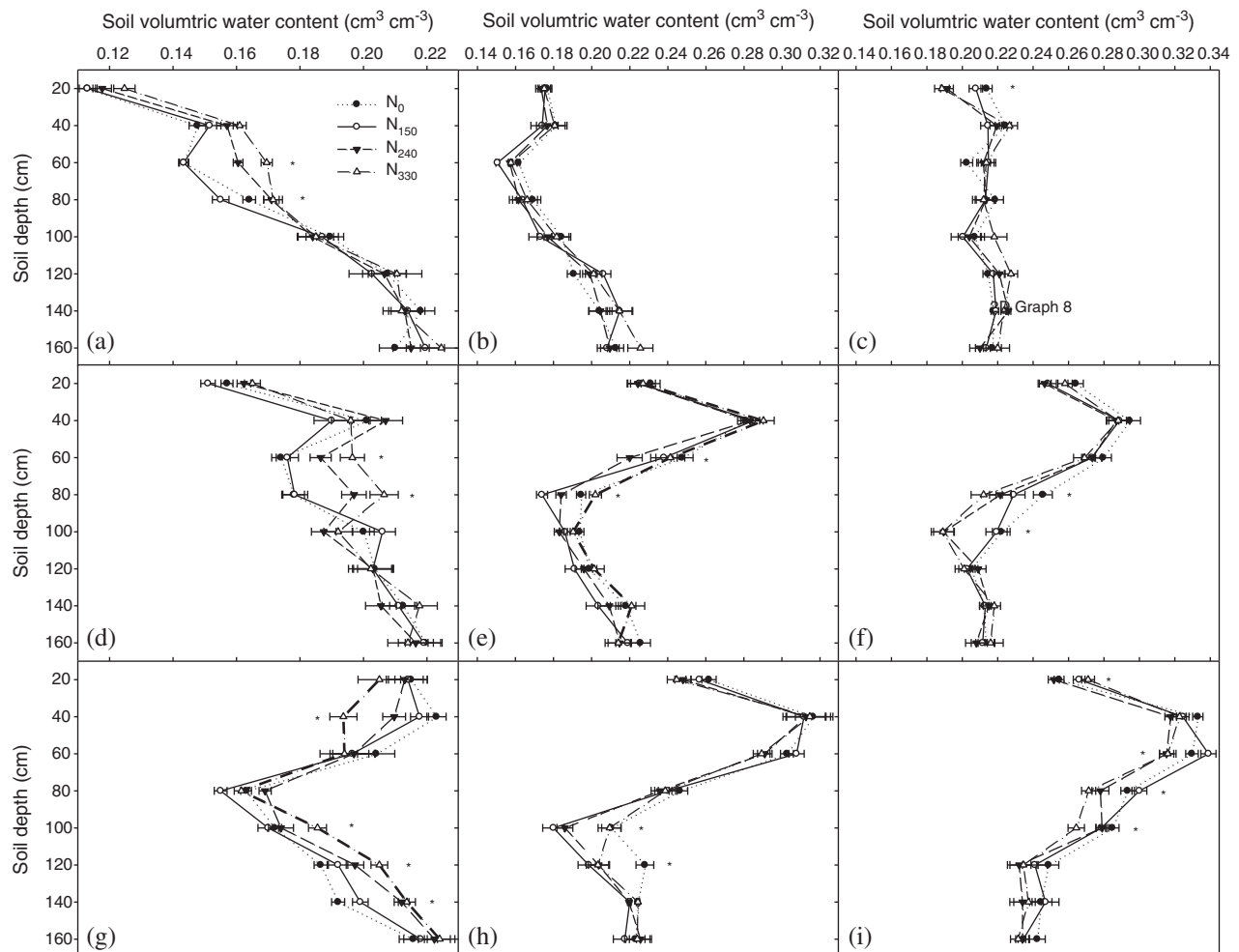
## 2.5 Statistical Analysis

ANOVA was performed to determine the effects of irrigation, application N rate, and their interaction effects on leaf relative water content, root bleeding sap,  $P_n$ , dry matter remobilization into grain and its contribution to grain, dry matter accumulation after silking and its contribution to grain, grain yield, WUE, N uptake, and NUE with the significance difference (LSD) at 5% probability level. PROC GLM (General Linear Model) was conducted to assess the relationship between N application rates and grain yield, and N application rates and N uptake by SAS 8.0 (SAS Institute, Cary, NC, Version 8.02).

### 3 Results

#### 3.1 Soil Volumetric Water Content

SWC of different N treatments at the jointing, silking and maturity under SD condition are shown in Figs. 2a, 2d and 2g. At the jointing stage (Fig. 2a), SWC of the  $N_{330}$  treatment at 40 cm and 60 cm soil layers were significantly higher than those of the  $N_0$  and  $N_{150}$  treatments. SWC in the deep soil layers (>80–cm) fluctuated little. In the silking stage (Fig. 2d), the  $N_{330}$  treatment had higher SWC at 60 cm and 80 cm soil layers than other treatments. Compared with the  $N_0$  treatment, SWC of the  $N_{330}$  treatment at 20 cm and 40 cm soil layers decreased significantly, while SWC of the  $N_{330}$  treatment at 100 cm, 120 cm and 140 cm soil layers increased at maturity (Fig. 2g). Under MD condition, SWC of the  $N_{240}$  treatment at 60 cm and 80 cm soil layers was lower than those of the  $N_0$  treatment in silking stage (Fig. 2e). At maturity (Fig. 2h), the  $N_{150}$  and  $N_{240}$  treatments significantly decreased SWC at 100 cm and 120 cm soil layers compared to the  $N_0$  treatment. Under FW condition, compared with  $N_0$  treatment, the  $N_{240}$  and  $N_{330}$  treatments decreased SWC at 20 cm soil layer at the jointing stage (Fig. 2c), and increased SWC at 80 cm and 100 cm soil layers in the silking stage (Fig. 2f). At maturity (Fig. 2i), SWC of the  $N_{240}$  and  $N_{330}$  treatments at 20 cm soil layer were higher than that of the  $N_0$  treatment, while SWC of the  $N_{240}$  and  $N_{330}$  treatments at 60 cm, 80 cm, and 100 cm soil layers decreased significantly compared to those of the  $N_0$  treatment.



**Figure 2:** SWC of different N treatments at the jointing (a, b, and c), silking (d, e, and f), and maturity (g, h, and i) under three irrigation levels. Note: a, d, and g under SD condition; b, e, and h under MD condition; and c, f, and i under FW condition

### 3.2 Physiological Traits

The leaf relative water content, root bleeding sap, and  $P_n$  were significantly affected by water (Tab. 3). Analysis across N treatments illustrated that all these physiological parameters were increased significantly with the improvement of irrigation levels. From the silking to filling stage,  $P_n$  was decreased 52.0%, 24.3%, and 23.1%, respectively, under SD, MD, and FW conditions. The results addressed that N had a significant effect on  $P_n$  (Tab. 3).  $P_n$  reached a peak value at N application rates of 240 kg ha<sup>-1</sup> (N<sub>240</sub>) which was 11.1%–18.9% higher than that of the N<sub>0</sub> treatment, while there was no significant difference compared with that of the N<sub>150</sub> or N<sub>330</sub> treatment. The physiological traits of maize were more sensitive to the improvement of soil water status.

**Table 3:** Analysis of variance (*F* value) for leaf relative water content, root bleeding sap, and  $P_n$  under different growth stages of summer maize

Treatment	d.f.	Leaf relative water content (%)		Root bleeding sap (g h <sup>-1</sup> root <sup>-1</sup> )			$P_n$ (umol m <sup>-2</sup> s <sup>-1</sup> )	
		Jointing stage	Silking stage	Jointing stage	Silking stage	Filling stage	Silking stage	Filling stage
2014								
Water	2	23.2**	55.0**	209.1**	178.7**	143.4**	–	–
N	3	1.2	1.7	13.0**	0.5	4.6*	–	–
Water × N	6	4.26**	11.4**	30.7**	7.93**	7.48**	–	–
2015								
Water	2	53.4**	184.0**	54.9**	38.6**	62.2**	79.9**	195.6**
N	3	1.7	14.4**	3.0	8.4**	1.5	25.0**	22.7**
Water × N	6	19.8**	22.4**	14.4**	10.0**	1.9	11.67**	14.1**

Note: \* and \*\* refers to significant difference at the 0.05 and 0.01 probability levels, respectively.

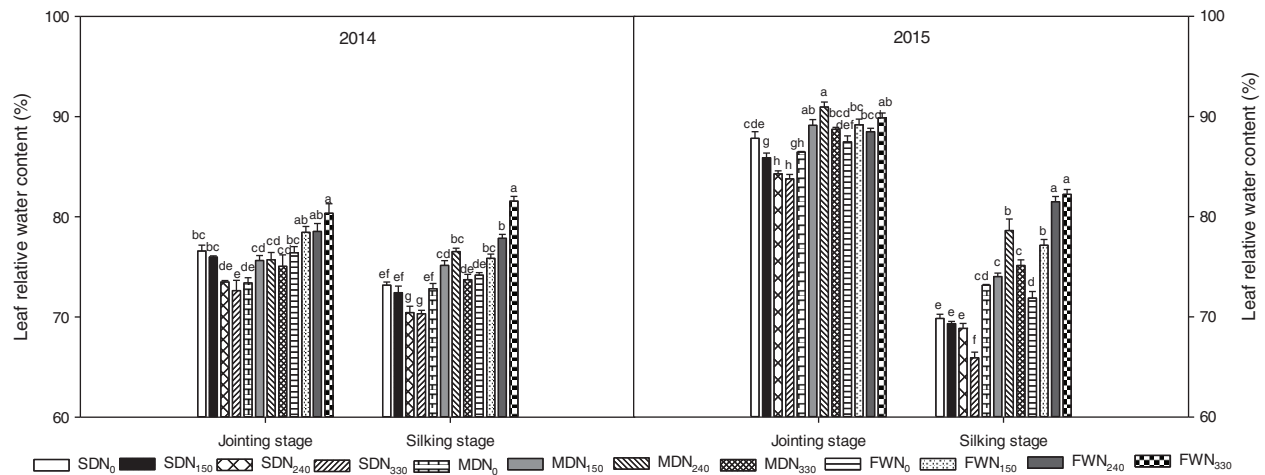
Water and N had significant interactive effects on all these physiological parameters of maize (except for root bleeding sap of filling stage in 2015). Under SD condition, leaf relative water content and root bleeding sap were decreased along with the increasing of N application rates (Figs. 3 and 4). The leaf relative water content, root bleeding sap, and  $P_n$  of the N<sub>0</sub> and N<sub>150</sub> treatments under SD condition were similar and both of them significantly higher than those of the N<sub>330</sub> treatment. These parameters were first increased and then decreased as the N application rates increased under MD condition, and they reached their maximum in the N<sub>150</sub> or N<sub>240</sub> treatment (Figs. 3–5). Under FW condition, leaf relative water content, root bleeding sap, and  $P_n$  were increased with the increasing of N application rates. The N<sub>330</sub> treatment significantly increased these physiological parameters compared to the N<sub>0</sub> treatment, while the differences between the N<sub>240</sub> and N<sub>330</sub> treatments were not significant in most growth stages.

### 3.3 Dry Matter Accumulation after Silking and Grain Yield

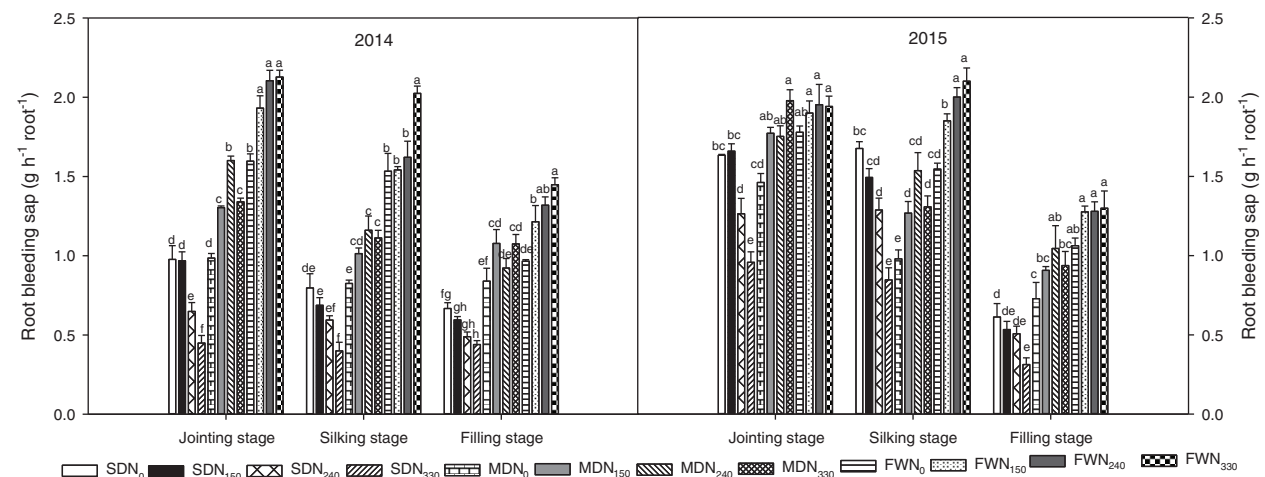
Water and N had significant influences on dry matter remobilization into grain, dry matter accumulation after silking and their contribution to grain, and grain yield (except for dry matter remobilization into grain in 2014) (Tab. 4). In two growing seasons, dry matter accumulation after silking and grain yield were increased gradually as the improvement of irrigation levels with the order of SD < MD < FW, whereas dry matter remobilization into grain and its contribution to grain showed a reverse tendency. On the average, dry matter accumulation after silking and grain yield under FW condition were increased by 18.7% and 8.2%



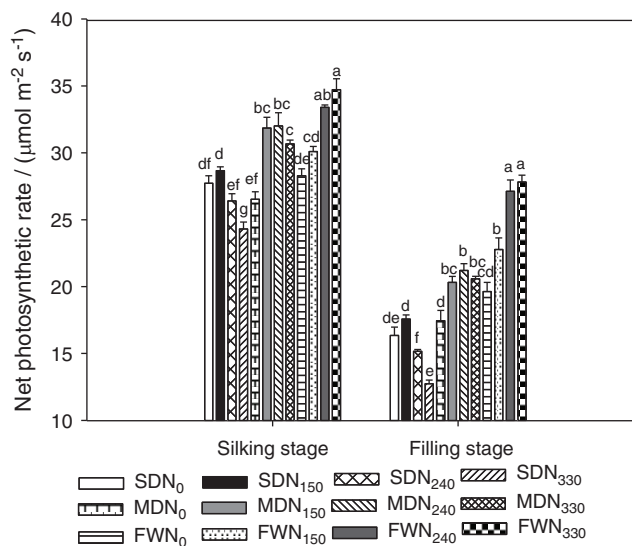
than those under MD condition, and by 72.3% and 51.7% than those under SD condition, respectively (Tabs. 5 and 6). N supply significantly improved maize yield without significant difference among three different N application rates ( $N_{150}$ ,  $N_{240}$ , and  $N_{330}$ ). Maximum grain yield was obtained from the FW and 330 kg N  $ha^{-1}$  for both growing seasons. The yield was more sensitive to the improvement of soil water status.



**Figure 3:** Leaf relative water content of different treatments at the jointing and silking stage of maize in 2014 and 2015. Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC.  $N_0$ : N application rate 0 kg  $ha^{-1}$ ,  $N_{150}$ : N application rate 150 kg  $ha^{-1}$ ,  $N_{240}$ : N application rate 240 kg  $ha^{-1}$ ,  $N_{330}$ : N application rate 330 kg  $ha^{-1}$ . Vertical bars represented standard errors of three replicates. Means within the same growth stage followed by a different small case are significantly different at  $P \leq 0.05$



**Figure 4:** Root bleeding sap of different treatments at the jointing, silking, and filling stage of maize in 2014 and 2015. Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC.  $N_0$ : N application rate 0 kg  $ha^{-1}$ ,  $N_{150}$ : N application rate 150 kg  $ha^{-1}$ ,  $N_{240}$ : N application rate 240 kg  $ha^{-1}$ ,  $N_{330}$ : N application rate 330 kg  $ha^{-1}$ . Vertical bars represented standard errors of three replicates. Means within the same growth stage followed by a different small case are significantly different at  $P \leq 0.05$



**Figure 5:**  $P_n$  under different treatments in the silking and filling stage of maize in 2015. Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC.  $N_0$ : N application rate 0 kg ha<sup>-1</sup>,  $N_{150}$ : N application rate 150 kg ha<sup>-1</sup>,  $N_{240}$ : N application rate 240 kg ha<sup>-1</sup>,  $N_{330}$ : N application rate 330 kg ha<sup>-1</sup>. Vertical bars represented standard errors of three replicates. Means within the same growth stage followed by a different small case are significantly different at  $P \leq 0.05$

**Table 4:** Analysis of variance ( $F$  value) for dry matter accumulation after silking, grain yield, WUE, N uptake, and NUE of summer maize in 2014 and 2015

Treatments	d.f.	Dry matter remobilization into grain (g m <sup>-2</sup> )	Contribution of remobilization to grain (%)	Dry matter accumulation after silking (g m <sup>-2</sup> )	Contribution of dry matter accumulation after silking to grain (%)	Grain yield (g m <sup>-2</sup> )	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	N uptake (g m <sup>-2</sup> )	NUE (g g <sup>-1</sup> )
2014									
Water	2	58.0**	255.2**	101.8**	255.2**	97.4**	52.0**	94.6**	8.4**
N	3	1.8	4.2*	4.4*	4.2*	4.1*	1.6	13.1**	12.5**
Water × N	6	6.0**	35.2**	11.9**	35.2**	9.5**	3.4**	4.2**	8.6**
2015									
Water	2	16.8**	123.9**	180.6**	123.9**	225.5**	54.5**	3238.7**	20.3**
N	3	15.2**	30.5**	21.6**	30.5**	16.3**	2.08	443.3**	29.8**
Water × N	6	17.6**	35.1**	18.9**	35.1**	14.1**	4.6**	95.1**	20.7**

Note: \* and \*\* refers to significant difference at the 0.05 and 0.01 probability levels, respectively.

Water and N had significant interactive effects on the parameters of dry matter transport and yield (Tab. 4). The  $N_0$  or  $N_{150}$  treatment obtained the highest dry matter accumulation after silking and grain yield under SD condition (Tabs. 5 and 6). Dry matter accumulation after silking and yield of the  $N_0$  treatment were increased by 35.3%–56.1% and 19.3%–25.0%. These parameters of the  $N_{150}$  treatment were increased by 33.6%–36.0% and 14.9%–16.6% than those of the  $N_{330}$  treatment, respectively. Under MD condition, dry matter accumulation after silking and grain yield of the  $N_{240}$  treatment were the highest, which were higher than the  $N_0$  treatment for 23.7%–34.1% and 12.9%–23.0%, respectively. The  $N_0$  treatment significant difference observed among the  $N_{150}$ ,  $N_{240}$ , and  $N_{330}$  treatments in grain yield,

and the N<sub>150</sub> treatment achieved similar yield compared to the N<sub>240</sub> treatment. Increasing N application rates increased dry matter accumulation after silking and grain yield under FW condition. Dry matter accumulation after silking and grain yield of the N<sub>330</sub> treatment were increased by 45.4%–71.9% and 27.1%–34.7% than those of the N<sub>0</sub> treatment, and by 16.5%–27.5% and 7.2%–17.2% than those of the N<sub>150</sub> treatment, respectively. While there was no significant difference in dry matter accumulation after silking and grain yield between the N<sub>240</sub> and N<sub>330</sub> treatments in 2015.

### 3.4 Water and Nitrogen Use Efficiency

The N uptake and NUE were significantly affected by water and N, while only water had a significant effect on WUE (Tab. 4). N uptake was increased significantly with improving irrigation levels, whereas WUE and NUE showed a reverse tendency as SD > MD > FW. Increasing of N application rates increased N uptake but reduced NUE for both growing seasons.

The results indicated that water and N had significant interactive effects on WUE, N uptake, and NUE (Tab. 4). The N<sub>0</sub> treatment obtained the highest WUE and NUE (except for WUE in 2014) under SD condition, which was 10.9%–12.5% and 34.6% higher than the N<sub>330</sub> treatment, respectively (Tab. 6). Under MD condition, the N<sub>240</sub> treatment yielded the highest N uptake, with no significant different from the N<sub>150</sub> treatment, while was 20.1% and 6.6% higher than the N<sub>0</sub> and N<sub>330</sub> treatments in 2015, respectively. Under FW condition, the N<sub>330</sub> treatment obtained the highest WUE and N uptake, but its NUE was 7.8%–7.9% lower than that of the N<sub>0</sub> treatment. There was no significant difference in WUE and NUE between the N<sub>240</sub> and N<sub>330</sub> treatments.

**Table 5:** Interaction of water and N on dry matter remobilization into grain and dry matter accumulation after silking of maize under drip fertigation in 2014 and 2015

Treatments	2014				2015				
	Dry matter remobilization into grain (g m <sup>-2</sup> )	Contribution of remobilization to grain (%)	Dry matter accumulation after silking (g m <sup>-2</sup> )	Contribution of dry matter accumulation after silking to grain (%)	Dry matter remobilization into grain (g m <sup>-2</sup> )	Contribution of remobilization to grain (%)	Dry matter accumulation after silking (g m <sup>-2</sup> )	Contribution of dry matter accumulation after silking to grain (%)	
SD	N <sub>0</sub>	172.0 bcd	28.9 de	423.0 ef	71.1 de	180.0 bcd	24.6 bc	552.3 ef	75.4 de
	N <sub>150</sub>	192.0 ab	34.7 b	362.1 f	65.3 g	150.0 de	21.3 cd	555.4 ef	78.8 cd
	N <sub>240</sub>	182.0 abc	32.6 bc	377.0 f	67.4 fg	177.1 bcd	26.2 b	499.2 f	73.8 e
	N <sub>330</sub>	205.0 a	43.1 a	271.0 g	56.9 h	205.0 ab	33.5 a	408.3 g	66.5 f
MD	N <sub>0</sub>	205.0 a	31.1cd	452.0 de	68.9 ef	163.3 cde	20.1 d	649.0 de	79.9 c
	N <sub>150</sub>	208.1 a	29.0 de	510.2 cd	71.1 de	164.0 cde	19.0 d	700.0 c	81.0 c
	N <sub>240</sub>	183.2 ab	24.6 f	559.3 bc	75.4 c	129.0 e	12.9 e	870.1 b	87.1 b
	N <sub>330</sub>	189.1 ab	26.1 ef	537.2 bc	73.95 cd	202.1 abc	21.7 cd	728.0 cd	78.3 cd
FW	N <sub>0</sub>	158.2 cde	24.5 f	485.1 cde	75.5 c	227.2 a	26.9 b	617.2 e	73.2 e
	N <sub>150</sub>	144.2 e	20.6 g	553.1 bc	79.4 b	150.0 de	14.1 e	911.0 b	85.9 b
	N <sub>240</sub>	154.1 de	20.4 g	603.2 b	79.6 b	83.0 f	7.6 f	1008.1 a	92.4 a
	N <sub>330</sub>	111.2 f	13.6 h	705.1 a	86.4 a	77.1 f	6.8 f	1061.1 a	93.2 a

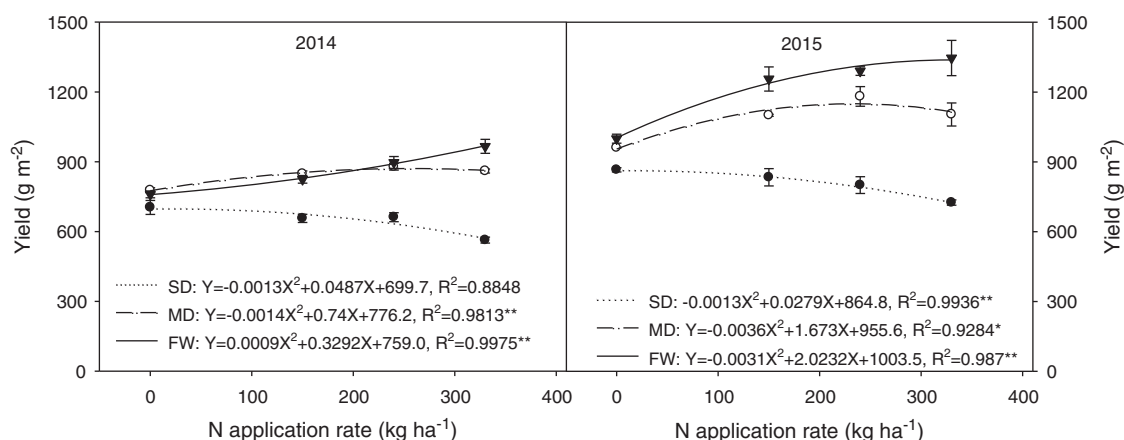
Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC. N<sub>0</sub>: N application rate 0 kg ha<sup>-1</sup>, N<sub>150</sub>: N application rate 150 kg ha<sup>-1</sup>, N<sub>240</sub>: N application rate 240 kg ha<sup>-1</sup>, N<sub>330</sub>: N application rate 330 kg ha<sup>-1</sup>. Mean values within a column by a different letter are significantly different at  $P \leq 0.05$ .

The relationship between N application rates and grain yield and between N application rates and N uptake suggested that the N application rates for optimal yield or N uptake was variable under different irrigation levels (Figs. 6 and 7).

**Table 6:** Interaction of water and N on grain yield, WUE, and NUE of maize under drip fertigation in 2014 and 2015

Treatment		2014				2015			
Water	N	Grain yield (g m <sup>-2</sup> )	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	N uptake (g m <sup>-2</sup> )	NUE (g g <sup>-1</sup> )	Grain yield (g m <sup>-2</sup> )	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	N uptake (g m <sup>-2</sup> )	NUE (g g <sup>-1</sup> )
SD	N <sub>0</sub>	703.9 de	33.3 ab	10.6 g	56.0 a	865.8 f	36.6 a	12.7 j	58.4 a
	N <sub>150</sub>	656.6 e	34.6 a	11.4 fg	48.9 b	833.8 f	34.4 abc	14.0 i	51.0 b
	N <sub>240</sub>	661.7 e	34.7 a	12.7 ef	44.3 cd	800.4 fg	34.5 ab	14.8 h	46.2 cd
	N <sub>330</sub>	563.3 f	29.6 cde	11.4 fg	41.6 d	725.5 g	33.0 bc	14.3 i	43.4 e
MD	N <sub>0</sub>	777.5 c	30.5 bcd	14.5 cd	45.4 bcd	960.8 e	29.0 de	17.4 g	47.3 c
	N <sub>150</sub>	848.5 b	32.3 abc	16.1 bc	44.7 cd	1100.1 d	31.5 bcd	20.2 de	46.8 c
	N <sub>240</sub>	878.4 b	33.0 ab	16.1 bc	46.2 bc	1181.4 cd	31.4 cd	20.9 d	48.2 c
	N <sub>330</sub>	860.3 b	33.1 ab	15.8 bc	46.2 bc	1103.9 d	31.6 bcd	19.6 ef	48.2 c
FW	N <sub>0</sub>	760.0 cd	24.8 g	13.9 de	46.1 bc	999.8 e	24.2 f	17.8 g	48.1 c
	N <sub>150</sub>	824.6 bc	26.6 efg	15.7 bc	44.3 cd	1256.3 bc	29.3 de	23.2 c	46.2 cd
	N <sub>240</sub>	897.1 b	25.1 fg	17.2 b	44.1 cd	1290.4 ab	27.8 e	24.0 b	46.0 cd
	N <sub>330</sub>	966.3 a	28.0 def	19.3 a	42.5 d	1346.5 a	29.4 de	26.0 a	44.3 de

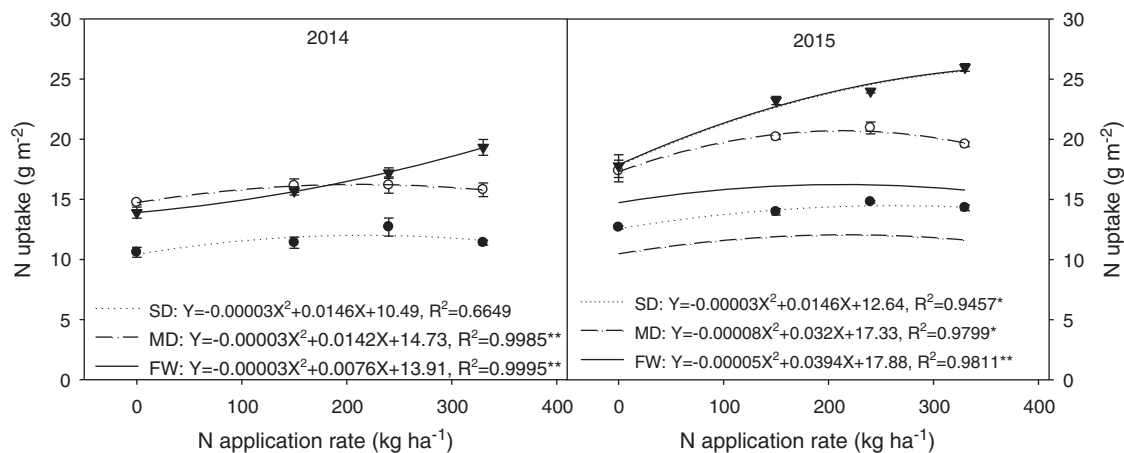
Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC. N<sub>0</sub>: N application rate 0 kg ha<sup>-1</sup>, N<sub>150</sub>: N application rate 150 kg ha<sup>-1</sup>, N<sub>240</sub>: N application rate 240 kg ha<sup>-1</sup>, N<sub>330</sub>: N application rate 330 kg ha<sup>-1</sup>. Mean values within a column by a different letter are significantly different at  $P \leq 0.05$ .

**Figure 6:** The relationships between N application rates and grain yield under three irrigation levels. Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC

#### 4 Discussions

Judicious management of water and N are very important aspect in maize production, since the requirement of water and nutrient, especially N was large in the whole growing season [34]. A comprehensive understanding of the interactive response of water and N on physiological traits and yield improvement will help the farmer in water and N management in field maize productions [35]. The current study may give some insight into the complicated water and N interactive effects on crop growth

and resource utilization, and also might provide an agronomic strategy to cope with the problems of water and fertilizer application in maize production.



**Figure 7:** The relation between N application rates and N uptake under three irrigation levels. Note: SD: 45%–55% FC, MD: 65%–75% FC, FW: 85%–95% FC

#### 4.1 The Physiological Response of Maize Leaf and Root under Integrative Regulation of Water and N with Drip Fertigation

Increasing N application rates showed a negative effect on leaf relative water content and root bleeding sap under SD condition, while appropriate N application under MD and FW conditions exhibited a positive effect. The leaf relative water content was decreased rapidly under water stress conditions, which was mainly contributed to the root function of water and nitrate absorption [36]. N application can alleviate the adverse effects of water stress on physiological activity of plants [37]. However, under SD condition, the physiological activity of maize (leaf relative water content and root bleeding sap) was inhibited by N application, especially at high N application ( $N_{240}$  or  $N_{330}$ ) rates. Soil water status is the main factor limiting the effectiveness of N fertilizer under water-limited condition [38]. Maybe higher N application rates resulted in higher soil solution concentration under SD condition, which aggravated osmotic stress and inhibited absorptive capacity of roots. The SWC of the  $N_{330}$  treatment in root zone was higher than that of the  $N_0$  treatment under SD condition, which could indirectly support the above hypothesis. The result of Wang et al. [39] illustrated that low N enhanced root growth of maize in subsoil, and leaf relative water content was increased compared to that in high N treatment under water stress conditions ( $SWC \leq 50\%$  FC). Favorable soil water status is conducive to the conversion and absorption of N [40]. Increasing N application rates can promote crop growth and enhance root physiological activity under well-watered conditions [35,37], thereby enhancing root water uptake. Accordingly, the SWC of the  $N_{330}$  treatment under FW condition in middle and deeper soil layers was lower than those of the  $N_0$  treatment. Therefore, the compensation effect of N is effectively exerted and the synergistic interaction promotes crop physiological activity by improving soil water status [37]. The inhibition of physiological activity in leaves and roots were offset by increasing N application rates to a reasonable range under MD and FW conditions. The  $N_{150}$  and  $N_{240}$  treatments under MD condition and the  $N_{240}$  and  $N_{330}$  treatments under FW condition can obtain higher leaf relative water content and root bleeding sap, while the  $N_{330}$  treatment under MD condition showed a negative effect on these parameters in the current study. Therefore, appropriate N application under MD (150–240 kg N ha<sup>-1</sup>) and FW (240–330 kg N ha<sup>-1</sup>) could improve the water status of plants and enhance the physiological activity of plants, which would promote the absorption of water and N.

Higher leaf photosynthetic capacity and longer leaf functional duration during the grain filling period are important for crop yield improvement [41]. The  $P_n$  of ear leaf is the key factor determining maize yield [42]. The largest decline of  $P_n$  of ear leaf during the filling period was observed under SD condition. This result noted that plant senescence was accelerated due to water stress and  $P_n$  was decreased significantly, which affected dry matter production during filling period. Li et al. [37] also illustrated that the leaf area index, chlorophyll content, and  $P_n$  of maize were gravely restricted under severe drought condition. Under drought conditions, proper N supply can increase the leaf area index and leaf photosynthetic pigments content to improve photosynthetic capacity and alleviate the damage to photosystem II [43], whereas excessive N application restricts photosynthesis under water stress [35,37]. In the current study, the  $P_n$  of maize was decreased when N application rates over than 150 kg ha<sup>-1</sup> under SD condition. The stomatal conductance of plants with high N was lower than that with low N under water stress conditions [44]. The reduction of stomatal conductance would reduce intercellular CO<sub>2</sub> concentration [45], thus inhibiting CO<sub>2</sub> assimilation [45]. Premature senescence due to reduction  $P_n$  in high N application rates would increase soil evaporation in the later growth period under SD condition. Accordingly, SWC of the N<sub>330</sub> treatment was reduced in upper soil layers (0–40 cm) at maturity when compared to that of the N<sub>0</sub> treatment under SD condition. Reasonable water and N supply can enhance photosynthetic capacity [35]. There was no significant difference in  $P_n$  between the N<sub>150</sub> and N<sub>240</sub> treatments under MD condition and between the N<sub>240</sub> and N<sub>330</sub> treatments under FW condition, whereas the N<sub>330</sub> treatment still showed negative effects on  $P_n$  under MD condition. The  $P_n$  did not be continuously improved when supplying N exceeded the reasonable level. The  $P_n$  of maize decreases when N application rate exceeds 270 kg ha<sup>-1</sup> under well-watered conditions [46]. Wang et al. [23] studied mulched drip-fertigated maize in Xinjiang of China exhibited that yield showed a decreasing trend when N application rate exceeded 240 kg ha<sup>-1</sup> under no water stress conditions. Therefore, the N<sub>150</sub> treatment with MD and the N<sub>240</sub> treatment with FW synergistically enhanced photosynthetic ability during filling period, helping to achieve high dry matter accumulation after silking.

#### **4.2 The Yield Response of Maize under Integrative Regulation of Water and N with Drip Fertigation**

The present results indicated that adjusting N application rates in accordance with soil water availability was essential to obtain better yield by regulating the physiological traits of maize plant. The basis of grain yield is high dry matter accumulation, and the greater potential of yield is achieved by the higher dry matter accumulation after anthesis [47]. Therefore, maintaining high physiological activity during the filling period is an important physiological basis for obtaining high dry matter accumulation after silking. Wu et al. [6] showed that compared with drip irrigation, drip fertigation increased dry matter accumulation after silking of maize due to longer leaf longevity. In the current study, a marked reduction in grain yield under SD condition, which was attributed to reduction in physiological activity during the filling period, thereby decreasing dry matter accumulation after silking. Significant interactive effects of water and N on dry matter accumulation and yield were observed in the current study, which was consistent with previous researches on maize under drip fertigation [4,22]. The physiological traits were inhibited by supplying high N (N<sub>240</sub> or N<sub>330</sub>) under SD condition, resulting in low dry matter accumulation after silking and yield.  $P_n$  and RuBP carboxylase activity in leaves were reduced due to high N application rates under water stress condition [48]. Under MD and FW conditions, increasing N application rates to an appropriate level showed better synergistic interaction to enhance physiological activity of plants during the filling period, facilitating dry matter accumulation after silking. Under MD condition, the yield of the N<sub>150</sub> treatment was similar to that of the N<sub>240</sub> treatment because they had similar effects on physiological traits, whereas the N<sub>330</sub> treatment did not further increase dry matter production and ultimately yield. Under FW condition, compared with the N<sub>330</sub> treatment, the yield of the N<sub>240</sub> treatment was similar in 2015, while was lower in 2014. These results indicated that the N<sub>150</sub> treatment was sufficient to meet normal growth of maize under MD condition.

### ***4.3 The WUE and NUE Response of Maize under Integrative Regulation of Water and N with Drip Fertigation***

Many scholars reported that N uptake generally was increased with the improvement of soil water status or N application levels [4,33], while WUE was reduced with increasing irrigation amount [39]. These results were further confirmed in the current study [39]. Previous experiments on interactive effects of water and N on maize were mainly focuses surface irrigation conditions (e.g., sprinkler and flooding irrigation) [20,24,33,49]. Wu et al. [6] exhibited that compared with drip irrigation, drip fertigation reduced inter-plant competition for water and N because of the synchronous improvement of water and N management, which was conducive to increase WUE and partial N productivity. Their results indicated that water and N had significantly interactive effects on resource utilization efficiency under drip fertigation, which was also confirmed by our study. Under FW condition, WUE and N uptake were increased significantly with the increasing N application rate. N supply can increase transpiration efficiency of crops by increasing the proportion of water loss by transpiration and reducing the proportion of water loss by evaporation [50], which is beneficial to increase WUE and production under suitable water and N conditions. While under MD condition, the difference of WUE among different N treatments was not significant. These results suggested N application showed better effects in promoting plant water use under fully water supply than that under drought conditions. Ning et al. [4] studied summer maize under drip fertigation in 3HP also exhibited the similar results, under full irrigation, supplying N was favorable to improve WUE; while different N application rates had no significant effect on WUE under limited-water irrigation. WUE and N uptake reduced when the N application rates over 240 kg ha<sup>-1</sup> under SD condition, which principally because of lower dry matter production and grain yield in the N<sub>330</sub> treatment. Wang et al. [24] showed that the plant growth and dry biomass of maize were reduced significantly when excessive N was supplied under drought conditions, thus decreasing WUE and N uptake. NUE was declined with any increase of N application rates under SD and FW conditions. Under severe drought condition, higher N application rates would cause less N uptake [38], and greater losses from ammoniation and denitrification [51]. While high N application rates reduces NUE under fully irrigation and over irrigation mainly due to NO<sub>3</sub>-N leaching [33]. Under MD condition, N<sub>240</sub> achieved the highest NUE, with no significant different from N<sub>150</sub>. Therefore, reasonable water and N management can promote N uptake and utilization.

Under the same irrigation level, fertilizer and yield are the most important input and output parts of farmland management, respectively. Optimizing water and N management in farmland requires not only maximizing output and resource utilization efficiency, but also reducing the risk of environmental pollution. In the current study, the yield, WUE, and NUE of the N<sub>150</sub> treatment were similar to those of the N<sub>240</sub> treatment under MD condition; although the N<sub>240</sub> treatment reduced yield by 5.4% in average compared to the N<sub>330</sub> treatment under FW condition, it saved 27.3% N. There is significant positive linear correlation between N application rate and soil available N concentration in deep soil (100–140 cm soil layers) [52]. More NO<sub>3</sub>-N leaching may be caused by increasing N application rate. Therefore, considering environmental risks and cost, the N<sub>330</sub> treatment with FW might be excessive, and the N<sub>150</sub> treatment with MD or N<sub>240</sub> treatment with FW was recommended. Additionally, to develop a more sustainable agricultural considering food security and environmental protection, quantifying N leaching under drip fertigation should be further explored.

## **5 Conclusions**

Water and N had significant influences on physiological traits of summer maize under drip fertigation. The response of physiology to increasing N application rates was varied under different soil water status. Adjusting N application rates in accordance with soil water availability synergistic enhanced leaf relative water content, root bleeding sap, and  $P_n$ , promoted dry matter accumulation after silking, ultimately increased yield and N uptake. High N application rates (>240 kg ha<sup>-1</sup>) inhibited the plant physiological

under drought conditions, and yield, WUE, and NUE of the N<sub>330</sub> treatment were significantly lower than those of the N<sub>0</sub> or N<sub>150</sub> treatment under severe drought condition. Comprehensive consideration of output, cost and possible environmental risks, 150 kg N ha<sup>-1</sup> under moderate drought and 240 kg N ha<sup>-1</sup> under fully water supply are recommended under drip fertigation for maize production.

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**Conflicts of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

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