Coupling effects of water and nitrogen on photosynthetic characteristics, nitrogen uptake, and yield of sunflower under drip irrigation in an oasis

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Abstract: An experiment was conducted in an oasis area in northwest China to assess the coupling effects of water and nitrogen supply through drip irrigation on photosynthetic characteristics, nitrogen uptake, and yield of sunflower (*Helianthus annuus* L.), as well as the nitrate distribution in the root-zone soil. The experiment included three irrigation levels (210 [W1], 300 [W2], and 390 [W3] mm), three nitrogen levels (162 [N1], 232 [N2], and 302 [N3] kg/hm^2), and control treatment (no fertilization during whole growth period and only irrigated at the budding stage). A nitrogen content over-accumulation in the soil was observed under the low irrigation amount with high fertilizer supply, which cannot enhance the sunflowers' absorption of nitrogen. Excessive irrigation caused leach of the soil nitrogen, reduced nitrogen content in the root-zone soil (0-40 cm) and limited nitrogen uptake by the crop. Although low irrigation amount with high fertilizer supply can increase the nitrogen content in the soil, it cannot enhance the sunflowers' absorption of nitrogen. At the vegetative stage of sunflower growth, the accumulation of nitrogen in the organs was mainly concentrated in the leaves, while it was transferred to the flower disk at the reproductive growth stage. Reasonable coupling of water and nitrogen improved the transport of nitrogen from leaves and stems to the flower disk and promoted the formation of yield components. Six regression equations were established with irrigation and fertilization amount as independent variables, and seed rate, seed weight per flowerdisk, 1000 grain weight, yield, water use efficiency and nitrogen partial factor productivity as dependent variables, respectively. Multiple regression and spatial analysis suggested that the irrigation amount of 241.62-253.35 mm and the fertilization application of 202.02-209.40 N kg/hm^2 was a good irrigation strategy, under which all six factors exceed 75% of their maxima, and the yield of sunflower reached 3 229.3 kg/hm^2.
Keywords: fertilization, *Helianthus annuus* L., irrigation management, nitrogen transformation, nutrient uptake, Xinjiang
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1 Introduction

Sunflower (*Helianthus annuus* L.) is second only to soybeans in terms of global importance as an oil crop. Given its ability to adapt to various environments and climates, sunflower is widely planted even in many semi-arid and arid areas, such as the midwestern USA[1], the Mediterranean region[2], northeastern India[3], and northwestern China[4,5]. Sunflower is often supplied with large amounts of water and fertilizer to yield high-quality oil[6]. However, excessive application of chemical fertilizer will cause water pollution and increase production costs. Thus, increasing the efficiency of water and fertilizer application is essential for the regional environment and sunflower cultivation in arid areas[7].

Sunflower is the most important oil-bearing crops in Xinjiang, China, which accounts for more than 60% of the total oil-bearing crop planting area. Irrigation management and nitrogen supply, including the rate, timing, and method of supply, strongly affect the yield and quality of sunflower[8,9]. In China, most sunflowers are watered with flood irrigation[10]. However, drip irrigation has proven superior to furrow irrigation because of its ability to save water and reduce nutrient leaching, especially in arid environments[11-13]. Xinjiang has an arid desert climate, the average annual rainfall in this area is only 150-200 mm, but the evaporation is as high as 1000-4500 mm[14]. This leads to a shortage of fresh water resources in the region, especially during the crop growing season (May-October). It is necessary to popularize drip irrigation to sunflower planting. Sahoo et al.[15] showed that compared with furrow irrigation, drip irrigation could improve the water use efficiency (WUE) and seed yield of sunflower. Improved irrigation and nitrogen use efficiency can be achieved through drip irrigation by adjusting the irrigation schemes according to the local environment[16]. Therefore, exploring the

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suitable drip irrigation and fertilization scheme for sunflower planting in Xinjiang will help to guide the local agricultural production.

Previous papers have studied the impact of irrigation strategies and fertilization schedules on sunflower under drip irrigation in various regions. Sezen et al.[17] studied the effects of different irrigation strategies on sunflower under a drip system and found that the oil contents of sunflower increased with increasing irrigation amounts. Elnaz et al.[18] also found that the seed yield and oil yield of sunflower decreased by 16.2% and 22.3% when irrigation water was lacking. The duration of water stress and the timing during different growth stages of sunflower influence the growth and yield of the crop. For example, if no irrigation was applied in the early growth stage but started when miniature floral head appeared, the sunflower yield was only reduced by 6%[10]. Karam et al.[19] showed that irrigation deficits in the early- and mid-flowering stages reduced seed yield, but irrigation deficit during early seed formation could raise seed yield.

Nitrogen (N) fertilizer is an important yield limiting factor for sunflower production[20]. Gul and Kara[21] indicated that although the effects of nitrogen on sunflower oil content were irregular, nitrogen had a positive effect on sunflower seed yield. Kara[22] found that nitrogen fertilizers applied at the time of sowing had a stronger effect on the agronomic traits of sunflower than fertilizer application at the time of flowering and plate formation. Achieving a beneficial balance of water and nitrogen application is essential to make the best use of drip irrigation as well as crop growth and yield[23-25]. Careful regulation of nitrogen and water under drip irrigation not only improves crop yields and oil quality in sunflowers but also decreases the leaching of nitrogen into groundwater[26-28].

However, the impact of the interaction between water and nitrogen under drip irrigation on sunflower growth remains largely uninvestigated, and the nitrogen distribution in soil and crop has rarely been monitored. The accumulation and translocation of nitrogen in sunflower have not been analyzed. An irrigation scheme based on the comprehensive consideration of quality, yield, and water and fertilizer utilization efficiency of sunflower is still lacking. A field experiment was conducted in an oasis agroecosystem in Xinjiang, China to evaluate the effects of practicing drip irrigation on photosynthesis, WUE_{ins}, production, and total nitrogen uptake of sunflower under three irrigation levels and three nitrogen supply levels. In addition, the distribution of soil nitrates under these different combinations in the root zone was examined. This study clarifies the optimal management protocols for sunflower production under drip irrigation in arid environments, thus providing valuable information to boost yields while reducing production costs and fertilizer pollution in similar areas.

2 Methods and materials

2.1 Experiment site

A field experiment was conducted in 2014 and 2015 at the Key Laboratory of Modern Water-Saving Irrigation of Xinjiang Production and Construction Corps of Shihezi University (85°59′E, 44°19′N, 412 m a.s.l.). The area of the experimental plot is 0.06 hm^2, and the regional groundwater level is deeper than 8 m. The average annual sunshine duration at the study site is 2865 h, and the accumulated air temperature above 10 ℃ and above 15 ℃ is 3463.5 ℃ and 2960.0 ℃, respectively, with an average frost-free period of 170 d. The average annual rainfall and potential evaporation are 207 mm and 1660 mm, respectively. Before the experiment, we randomly selected five points in the test area and measured soil particle composition, soil type, bulk density, pH, total nitrogen, nitrate-nitrogen, and soil organic matter at each depth (Table 1).

Table 1 Physical and chemical properties of 0-100 cm soil before the experiment

Soil depth/cm	Soil particle composition/g kg^{-1}			Texture	Bulk density /g cm^{-3}	pH	Total N /mg kg^{-1}	Nitrate-N /mg kg^{-1}	Soil organic matter /g kg^{-1}
	<0.002 mm	0.002-0.02 mm	0.02-2 mm						
0-20	113	683	212	Silty loam	1.455	7.71	352.2	15.602	1.49
20-40	126	716	186	Silty loam	1.525	7.96	215.9	16.738	2.59
40-60	193	581	53	Silty loam	1.565	7.83	150.15	11.201	2.19
60-80	220	653	50	Silty loam	1.650	7.18	116.00	9.097	1.47
80-100	207	705	51	Silty loam	1.645	7.74	183.64	8.250	1.91

2.2 Experimental design

The experiment was established in a randomized complete block design in a factorial approach with different combinations of irrigation (W) and nitrogen (N) treatments delivered through a drip irrigation system. A common local sunflower variety, the early-maturing cultivar "Xinkuizi V", was planted in both two years. Sowing dates were 12/7/2014 and 14/7/2015, and the harvest dates were 10/10/2014 and 12/10/2015. According to the sunflower planting habits of local farmers, the irrigation amount is usually more than 350 mm in a growth period, and the amount of nitrogen fertilizer is generally about 280 kg/hm^2. It's generally high, which may lead to a waste of water and fertilizer. In addition, in our previous work, by separately studying the water and nitrogen requirement of the same variety of sunflower, it was found that the suitable irrigation amount and nitrogen supply of sunflower with drip irrigation in this area are 285.2-287.7 mm and 187.9-243.6 kg/hm^2, respectively[29,30]. To comprehensively analyze the interaction effects of water and nitrogen on sunflower, find the most suitable irrigation and fertilization strategy, and improve the utilization efficiency of water and fertilizer, a range based on previous research and local habits was designed. Therefore, three irrigation levels were designed: 210 (W1), 300 (W2), and 390 (W3) mm. The nitrogen supply also had three levels: 162 (N1), 232 (N2), and 302 (N3) kg/hm^2. With each irrigation level being paired with each nitrogen level, a total of nine treatments (3 × 3) were applied. In addition, we chose a rain-fed sunflower field as the control check (CK), which was irrigated only at the budding stage, while no irrigation and fertilization were applied for the rest of the sunflower growth period. Urea [$CO(NH_2)_2$; nitrogen content of 46.4% by mass] was applied as the nitrogen fertilizer at the determined levels at the same time of irrigation. The irrigation and fertilizer date, and the meteorological data of the experimental area during the sunflower growth periods are shown in Figure 1. Climatic conditions of both experimental years were close. According to our previous research on crop water requirement[29], 6 times of irrigation was carried out during the whole growth period of sunflower, the water-soluble fertilizer was added into irrigation system and entered the field in each irrigation. A detailed record of water and nitrogen applications, grouped by growth stage and treatment, is shown in Table 2. 165 kg/hm^2 of Potassium dihydrogen phosphate [KH_2PO_4] as the phosphate and potash fertilizer for all treatments, was applied in the first irrigation at one time.

Irrigation was performed with drip tapes that were 16 mm in external diameter with a 0.3 mm wall thickness and the dripper spacing of 30 cm, with no plastic film mulched, capable of delivering 1.2 L/hm^2 at the operating pressure of 60 kPa. The non-pressure compensating, inline labyrinth-channel dripper, was used. Prior to the planting of sunflower, the drip tape was laid on the midline of the north–south-oriented soil beds with a spacing of 0.6 m. Sunflower seeds were hand-planted in early July at a density of approximately 83 000 plants/hm^2. All sunflowers were planted in rows along the drip lines with 25 cm between plants. Each treatment was replicated in three plots (4.5 m wide×18.6 m long). A schematic representation of the experimental setup is shown in Figure 2.

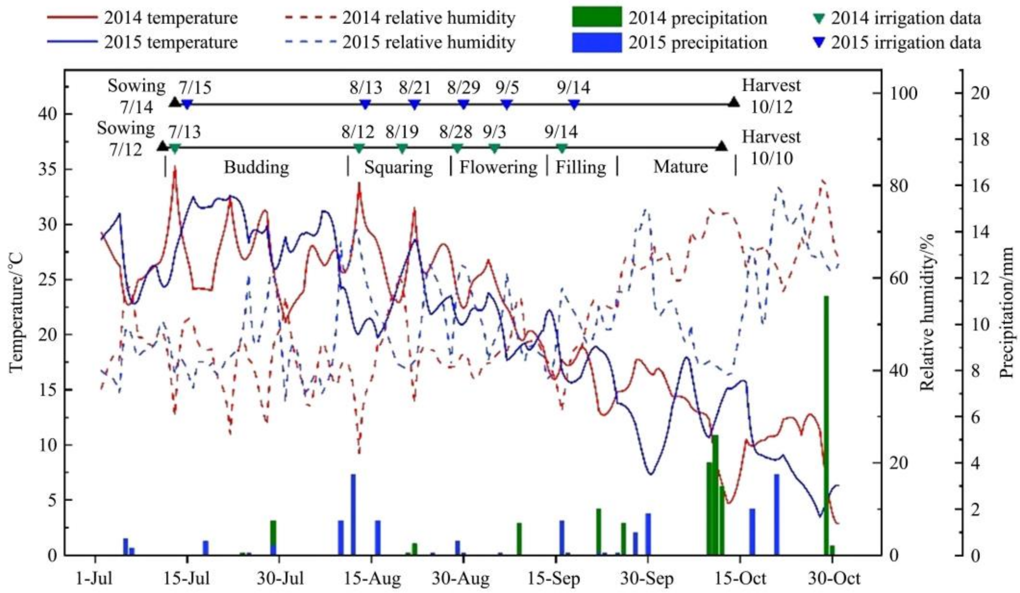
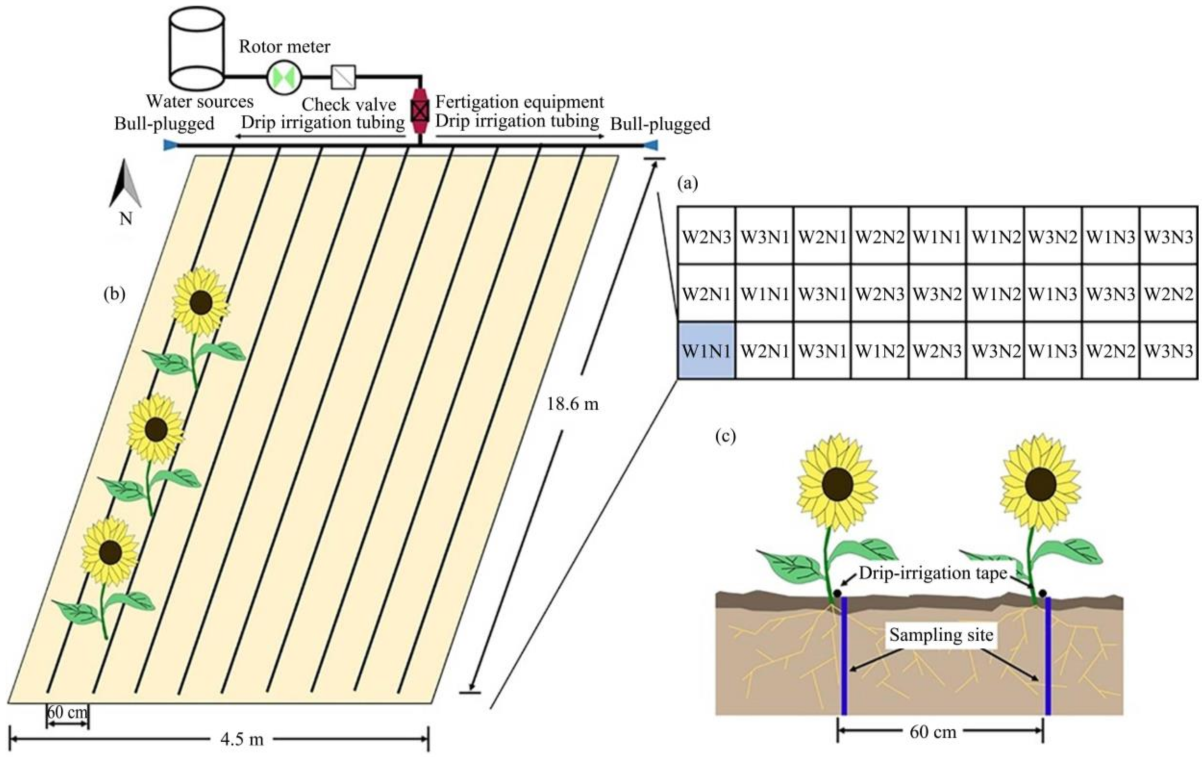


Figure 1 Daily meteorological variation and field management information during sunflower growth periods (July to October) in 2014 and 2015

Table 2 Irrigation and fertilizer treatments in the experiment

Treatments	Growth stages	Budding stage	Squaring stage	Flowering	Filling stage	Mature stage	Growth stage
	Periods	Jul. 11 to Aug. 10	Aug. 11 to Aug. 27	Aug. 28 to Sept. 10	Sept. 11 to Sept. 27	Sept. 28 to Oct. 12	Jul. 11 to Oct. 12
Distribution of irrigation and fertilization	Irrigation times	1	2	2	1	-	6
	Fertilization times	-	2	2	1	-	5
	Nitrogen ratio/%	-	50	25	25	-	100
CK	Irrigation/mm	50	-	-	-	-	-
	Nitrogen/kg hm^{-2}	-	-	-	-	-	-
W1N1	Irrigation/mm	35	70	70	35	-	210
	Nitrogen/kg hm^{-2}	-	81	40.5	40.5	-	162
W1N2	Irrigation/mm	35	70	70	35	-	210
	Nitrogen/kg hm^{-2}	-	116	58	58	-	232
W1N3	Irrigation/mm	35	70	70	35	-	210
	Nitrogen/kg hm^{-2}	-	151	75.5	75.5	-	302
W2N1	Irrigation/mm	50	100	100	50	-	300
	Nitrogen/kg hm^{-2}	-	81	40.5	40.5	-	162
W2N2	Irrigation/mm	50	100	100	50	-	300
	Nitrogen/kg hm^{-2}	-	116	58	58	-	232
W2N3	Irrigation/mm	50	100	100	50	-	300
	Nitrogen/kg hm^{-2}	-	151	75.5	75.5	-	302
W3N1	Irrigation/mm	65	130	130	65	-	390
	Nitrogen/kg hm^{-2}	-	81	40.5	40.5	-	162
W3N2	Irrigation/mm	65	130	130	65	-	390
	Nitrogen/kg hm^{-2}	-	116	58	58	-	232
W3N3	Irrigation/mm	65	130	130	65	-	390
	Nitrogen/kg hm^{-2}	-	151	75.5	75.5	-	302



Note: (a) shows the experimental plot distribution under three levels of irrigation (W1, W2, W3) and nitrogen (N1, N2, N3), with three replicates. (b) is a diagram of the W1N1 treatment under drip irrigation, showing the system and its size. (c) provides a profile view of the cultivation pattern of the drip-irrigated sunflower used in this study, with sampling locations depicted.

Figure 2 Depiction of the water–nitrogen coupling experimental design

2.3 Sampling and measurements

Soil samples were taken at 1 m depth before sowing and again at the end of seedling, squaring, flowering, filling, and mature stages for the analysis of total nitrogen and nitrate nitrogen. The soil cores were taken with a 3 cm diameter semi-cylindrical auger. Three duplicate cores were taken from random positions within each plot under the drip tape and directly between two plants. The soil samples were split into four depth intervals: 0-20, 20-40, 40-60, and 60-80 cm. The total nitrogen contents were extracted by shaking 40 g of fresh soil with 100 mL of KCl solution for 2 h. The resulting slurry was settled for 30 min and was then filtered (sieve pore diameter 1 mm). The solution was analyzed for nitrate-nitrogen with an AMS SmartChem140 Analyzer (Alliance, Italy). Concentrations (mg/L) in the extracted solution were converted to field units of kg/hm^2 by the value of soil bulk density measured in advance (Table 1).

Five random plants per treatment were sampled at 7-10 day intervals to measure dry matter during different sunflower growth stages (squaring, flowering, filling, and mature stages). Dry matter was weighted after the samples were oven-dried at 105℃ for 1 h and then at 80℃ to a constant weight. Total nitrogen and nitrate-nitrogen in the sunflower leaves, stem, and flower disk were determined from oven-dried, milled samples by using the Dumas combustion method with a Leco Nitrogen Analyzer[31].

After the seedling stage, the photosynthetic characteristics of sunflower under natural atmospheric condition were measured every ten days between 9:00 and 11:00 (local time) by using a handheld photosynthesis system (CI-340, CID, USA). The air temperature was 27-31℃, light intensity was 800-1000 μmol/m^2 s, and CO_2 concentration was 350-360 μmol/mol. Three readings were taken for each parameter on the fully expanded leaves of the plants. These readings of photosynthetic physiological characteristics included net photosynthetic rate (*Pn*) and transpiration rate (*Tr*), as well as environmental factors such as photosynthetically active radiation (PAR), air temperature (*Ta*), and CO_2 concentration in the air. On the basis of these measurements, WUE_{ins} was calculated as:

$$\text{WUEins} = Pn/Tr \quad (1)$$

At harvest, sunflower plants from the inner 1 m×1 m in the middle row of each plot were collected for analysis. Aboveground biomass, seed yield, thousand-grain weight, and seed weight per flower disk (including empty seed husks) were recorded. The setting percentage was calculated as the ratio of the number of available seeds to the total number of seeds per flower disk. Then, the sunflower yield in the whole plot was recorded, which was converted into yield per hectare.

2.4 Quantification of N in crop and soil

The absolute accumulation of soil N (S_A, kg/hm^2) was calculated as:

$$S_A = h\gamma S/10 \quad (2)$$

where, h is the depth of soil, cm; γ is the soil bulk density, g/cm^3; and S is the contents of soil nitrogen, mg/kg.

The accumulation of total nitrogen (M_N, g/plant) in different plant organs (leaves, stem, or flower disk) was calculated as:

$$M_N = DC/100 \quad (3)$$

where, D is the quantity of dry matter in a specific organ, g/plant; and C is the total nitrogen concentration in that organ, %. The transportation of total nitrogen (T, g/plant) was calculated as:

$$T = M_{FN} - M_{MN} \quad (4)$$

where, M_{FN} is the accumulation of the total nitrogen in the different organs in the flowering stage, g/plant; while M_{MN} is the accumulation of total nitrogen in the mature stage, g/plant. The ratio of transportation at mature stage (P_T, %) was calculated as:

$$P_T = M_T/M_{FN} \times 100\% \quad (5)$$

where, M_T is the mass of the total nitrogen transportation per organ, g/plant. The total nitrogen distribution ratio at mature stage (P_D, %) was calculated as:

$$P_D = M_{SN}/M_{PN} \times 100\% \quad (6)$$

where, M_{SN} is the accumulation of total nitrogen in one organ, g/plant; and M_{PN} is the accumulation of total nitrogen in a plant, g/plant. The amount of total nitrogen accumulated in the flower disks (M_{FIN}, g/plant) was calculated as:

$$M_{FIN} = M_{FMN} - M_{FFN} \quad (7)$$

where M_{FMN} is the accumulation of total nitrogen in the flower disk at maturity, g/plant; and M_{FFN} is the accumulation of total nitrogen in the flower disk at flowering, g/plant.

2.5 Irrigation water, fertilizer utilization efficiency and regression equation

The irrigation water utilization efficiency ($iWUE$) (kg/m^3) was calculated as[32]:

$$iWUE = Y/I \quad (8)$$

where, Y is the sunflower yield, kg/hm^2; and I is the irrigation water amount, m^3/hm^2. The nitrogen partial factor productivity (N_{PFP}) (kg/kg) was calculated as:

$$N_{PFP} = Y/N_T \quad (9)$$

where, Y is the sunflower yield, kg/hm^2; and N_T is the nitrogen application rate.

Some studies have demonstrated that the effect of water fertilizer binary factors on yield can be fitted by binary quadratic regression equation[25,33,34].

$$z = z_0 + ax + by + cx^2 + dy^2 + fxy \quad (10)$$

In order to further analyze the relationship between different factors, the fitting results were normalized by

$$X_{Norm} = (X - X_{\min})/(X_{\max} - Xmin) \quad (11)$$

where, X_{norm} is the normalized value (range 0-1); X is the raw data that needs to be normalized; and $X_{\min}$ and $X_{\max}$ denote the minimum and maximum value of the original data.

2.6 Statistical analysis

The value of each indicator (nitrate distribution and accumulation, net photosynthesis, transpiration rate, WUE$_{ins}$, nitrogen transportation and accumulation in sunflower organs and yield components) has passed the Shapiro-Wilk normality test and Homogeneity of variance test, and there is no significant difference between the two years (p>0.05). The value of each indicator is the average of the data for 2014 and 2015. Given that the numerical results of each measurement item of CK are significantly lower than those of other treatments, to prevent our judgment of water nitrogen coupling effect from being affected, the analysis of variance (ANOVA) was performed only for the other nine treatments except CK. The IBM SPSS 19 (IBM, USA) package was used to conduct the normality test, Homogeneity of variance test, and the ANOVA tests on the experimental results. The Duncan test was performed to conduct multiple comparisons to examine the significant difference between the means of different treatments. Differences were considered statistically significant when p<0.05.

3 Results

3.1 Effects of irrigation and N levels on soil nitrogen change

3.1.1 Dynamic distribution of nutrients in the soil profile

The dynamic distribution of soil nitrate content during the whole growth period is shown in Figure 3. The variation of nitrate content in different soil depths was basically the same, which increased at first, reached the maximum at the flowering stage, and then decreased. This is because the nitrogen fertilizer was applied mainly in the squaring and flowering stages. For all treatments, the lowest concentration range is 4.99 mg/kg and the highest concentration range is 85.58 mg/kg. The content of nitrate-nitrogen showed a downward trend with soil depth, and the content of nitrate nitrogen in 0-40 cm soil depth was higher than that in 40-80 cm in each treatment. At the soil depth of 20 cm and 40 cm, W1N3 was basically always at a high level. This was due to the minimum leaching effect of low irrigation on nitrate-nitrogen under high nitrogen application, which led to the accumulation of nitrate-nitrogen in the surface soil. At the soil depth of 60 and 80 cm, W3N3 was basically always at a high level. This was due to the leaching effect of high irrigation amount under high nitrogen application, which made the nitrate-nitrogen move to deep soil. A comparison of three irrigation levels showed that under the same irrigation condition, the distribution of nitrate-nitrogen in the 0-40 cm and 40-80 cm soil layers increased with the increase in nitrogen application. However, compared with the three fertilization levels, under the same nitrogen application, as the irrigation amount increased, the distribution of nitrate-nitrogen at only 0-40 cm showed a downward trend during the sunflower growth period, whereas that at 40-80 cm showed no obvious downward trend, which was less affected by irrigation amount and was relatively stable.

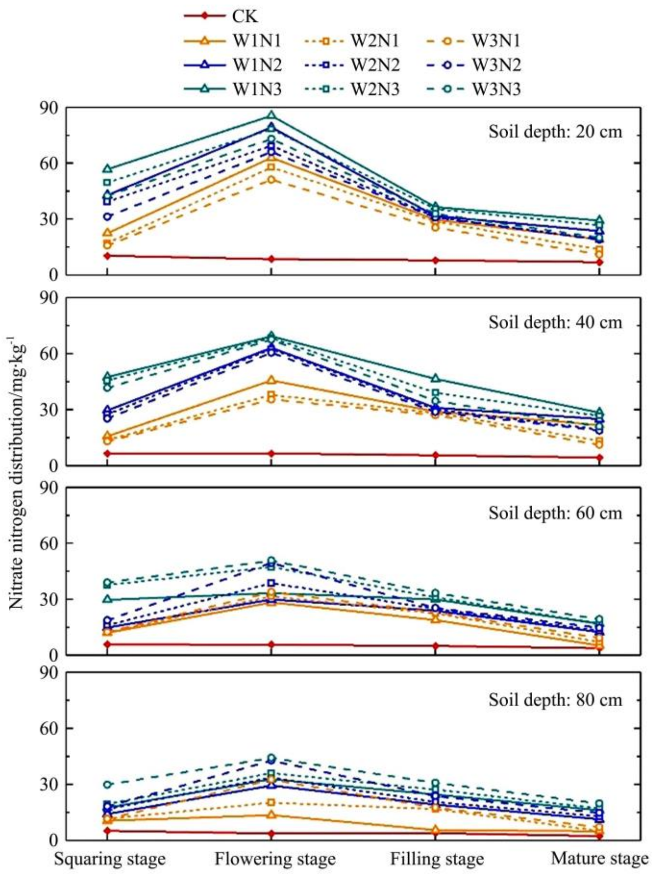
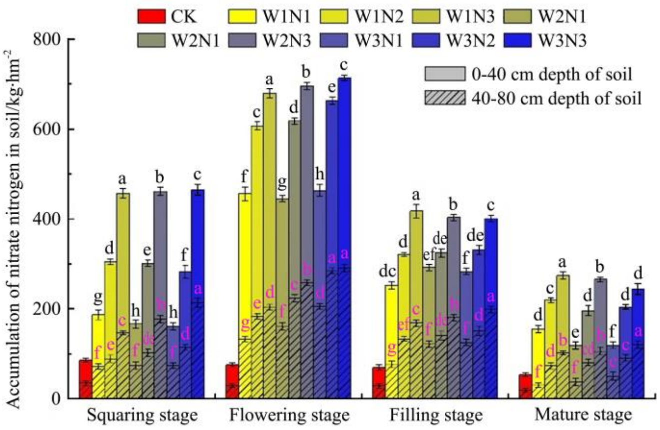


Figure 3 Nitrate nitrogen distribution in 0-80 cm soil profiles

3.1.2 Accumulation of soil nitrate-nitrogen

The tillage layer in the experimental location was 0-40 cm and was also the primary reservoir for nutrition absorption by crop roots. At different levels of irrigation and nitrogen fertilization, soil nitrate-nitrogen accumulation at 0-40 cm and 40-80 cm (Figure 4) increased at first and then decreased as the crops grew. Soil nitrate-nitrogen accumulation peaked during the flowering stage, reaching maximum values of 474.38 kg/hm^2 at 0-40 cm and 290.62 kg/hm^2 below the tillage layer at 40-80 cm. Nitrate-nitrogen declined in subsequent growth phases, dropping to minimum values of 67.78 kg/hm^2 at 0-40 cm and 31.12 kg/hm^2 at 40-80 cm during the mature stage. Throughout growth, the accumulation of nitrate-nitrogen was improved by larger nitrogen application rates under a given irrigation condition. With nitrogen application being held constant, increased irrigation was associated with less soil nitrate-nitrogen accumulation. With increased water and nitrogen application, the relative accumulation of nitrate-nitrogen in 40-80 cm soil increased, but the relative accumulation of nitrate-nitrogen in 0-40 cm soil decreased.



Note: The data is a bar stacked chart of 0-80cm, the error bars represent Standard Deviation (STD), and the significant difference groups of the mean values (n=3) are indicated by lowercase letters (p<0.05) for each soil layer.

Figure 4 Effects of different irrigation and nitrogen levels on nitrate-nitrogen accumulation in soil profiles

3.2 Effects of irrigation and N level on photosynthesis, transpiration, and WUE_{ins} of sunflower

During the seedling stage, the net photosynthetic rates (*Pn*) of the top functional leaves were relatively small, with an average value of 26.16 μmol/m^2 s across the different treatments (Table 3), but they then peaked during the squaring stage, reaching an average value of 43.68 μmol/m^2 s across the different treatments. Then, *Pn* decreased rapidly during the flowering and filling stages, reaching their lowest values at the end of the filling stage, with an average of 23.99 μmol/m^2 s. The average value of *Pn* reduced by 34.32% over the growth period from squaring to flowering and by 16.38% from flowering to filling. The patterns in the transpiration rate (*Tr*) were similar to *Pn* across the different treatments throughout the sunflower growing season. Over the whole growth period, at the end of the seedling stage, the average *Tr* value was relatively small (5.01 μmol/m^2 s), but it reached its highest at the end of the squaring stage (5.24 μmol/m^2 s). Then, *Tr* rates declined rapidly at the end of the filling stage, registering average values of just 3.34 μmol/m^2 s. The results of ANOVA showed that the coupling effect of water and nitrogen on photosynthesis was highly significant throughout sunflower growth, but only had a significant effect on transpiration during the filling stage.

Under the same irrigation water amount, the maximum *Pn* often appeared at the N2 level, and a low or high fertilization level reduced *Pn*. Similarly, under the same fertilization level, the maximum of *Pn* often appeared in the W2 irrigation level. *Pn* of W2N2 was basically at a high level in the whole growth period, even being significantly higher than other treatments in the flowering period. WUE_{ins} at different levels of water and nitrogen supply increased first, then decreased, and then rebounded slightly. In the squaring stage, all treatments reached the maximum value of WUE_{ins} except W3N1.With a value in the W2N2 treatment of 8.00 mol/mmol, which was 15.3% lower than the highest treatment (W3N3) during this stage, the value of WUE_{ins} in the W2N2 treatment was at a medium level among the different treatments for the entire growth period.

Table 3 Effects of different irrigation and nitrogen levels on leaf net photosynthesis rate (*Pn*), transpiration rate (*Tr*), and WUE_{ins}

Treatments	Seedling stage			Squaring stage			Flowering stage			Filling stage		
	Pn /μmol m^{-2} s^{-1}	*Tr* /μmol m^{-2} s^{-1}	WUE_{ins}	*Pn* /μmol m^{-2} s^{-1}	*Tr* /μmol m^{-2} s^{-1}	WUE_{ins}	*Pn* /μmol m^{-2} s^{-1}	*Tr* /μmol m^{-2} s^{-1}	WUE_{ins}	*Pn* /μmol m^{-2} s^{-1}	*Tr* /μmol m^{-2} s^{-1}	WUE_{ins}
CK	19.36	3.91	4.95	35.26	4.51	7.82	23.26	4.33	5.37	16.25	2.11	7.70
W1N1	26.12^{ab}	4.60^{bc}	5.68^{a}	42.99^{abc}	5.09^{ab}	8.49^{ab}	27.81^{def}	4.41	6.53	19.41^{e}	3.31^{ab}	5.90^{c}
W1N2	27.62^{abc}	5.33^{a}	5.18^{ab}	43.63^{ab}	5.30^{ab}	8.24^{ab}	28.32^{cde}	4.75	5.93	24.41^{bc}	3.42^{ab}	7.48^{bc}
W1N3	22.64^{d}	5.03^{abc}	4.51^{b}	41.20^{bc}	5.21^{ab}	7.95^{b}	27.41^{f}	4.40	6.23	21.70^{d}	3.20^{ab}	6.85^{bc}
W2N1	27.30^{ab}	5.17^{abc}	5.29^{ab}	44.31^{a}	5.24^{ab}	8.59^{ab}	27.10^{f}	5.10	5.34	24.02^{bc}	3.30^{ab}	7.30^{bc}
W2N2	28.03^{a}	5.33^{a}	5.26^{ab}	45.55^{a}	5.71^{a}	8.00^{b}	32.00^{a}	5.46	6.28	26.85^{a}	3.80^{a}	7.08^{bc}
W2N3	27.70^{ab}	5.07^{abc}	5.46^{a}	44.11^{a}	5.41^{ab}	8.21^{ab}	27.61^{ef}	5.33	5.32	25.11^{ab}	3.81^{a}	6.60^{bc}
W3N1	23.75^{cd}	4.90^{abc}	4.86^{ab}	40.91^{c}	5.04^{ab}	8.20^{ab}	30.53^{b}	4.61	6.64	25.62^{ab}	2.82^{b}	9.17^{a}
W3N2	26.94^{ab}	5.20^{ab}	5.30^{ab}	45.14^{a}	5.36^{ab}	8.38^{ab}	28.91^{c}	5.21	5.60	26.03^{ab}	3.62^{a}	7.23^{bc}
W3N3	25.33^{bc}	4.50^{c}	5.64^{a}	45.30^{a}	4.81^{b}	9.45^{a}	28.61^{cd}	4.52	6.36	22.80^{cd}	2.82^{b}	8.14^{ab}
Average	26.16	5.01	5.24	43.68	5.24	8.39	28.7	4.87	6.03	23.99	3.34	7.31
							P values					
W	<0.05	0.252	0.700	0.089	0.138	0.346	0.118	0.075	0.401	<0.01	<0.05	<0.01
N	<0.05	<0.05	0.963	0.090	0.090	0.629	<0.05	0.343	0.875	<0.05	0.079	0.890
W*N	<0.01	0.086	0.113	<0.01	0.231	0.299	<0.01	0.512	0.710	<0.01	<0.05	<0.01

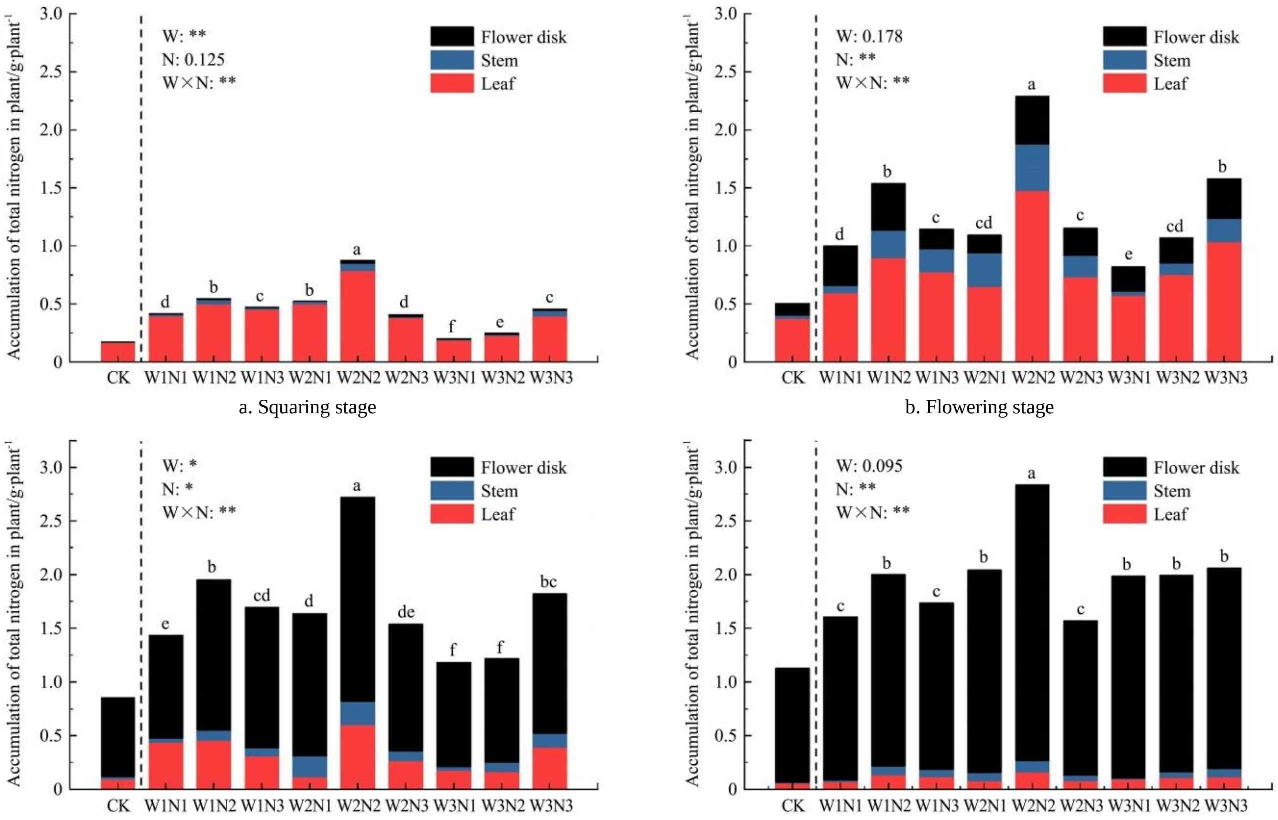
Note: The significant difference groups of the mean values (n=3) are denoted with lowercase letters (p<0.05).

3.3 Interaction effects of irrigation and N levels on nutrient accumulation of sunflower

3.3.1 Total-N content by organ

The total nitrogen contents in different organs (leaves, stem, and flower disk) of sunflower are provided in Figure 5. The accumulation of total nitrogen in sunflower plants increased throughout the growth period, and reached the maximum at the mature stage. We also observed that when the irrigation amount was at W1 and W2, the total nitrogen of each treatment increased first and then decreased with the increase in nitrogen application. When the irrigation amount was at W3 during the squaring and flowering stage, the total nitrogen of each treatment increased with the increase in nitrogen application. Nonetheless, this relationship did not reach a significant level at the filling and mature stage. Overall, the total nitrogen accumulation in plants with the W2N2 treatment was the highest in all growth periods, i.e., 0.88, 2.29, 2.72, and 2.84 g/plant, respectively.

The accumulation of total nitrogen in sunflower leaves increased initially and reached the maximum value at the flowering stage, accounting for 58.39%-70.72% of the total nitrogen content in the aboveground part of the plant. Then, the total nitrogen content began to decline. During the maturity stage, the nitrogen accumulation in leaves decreased to the lowest level of only 0.07-0.16 g/plant. The accumulation of nitrogen in the stem was small, reaching the maximum value in the flowering period, accounting for 4.06%-26.24% of the total nitrogen content in the aboveground part of the plant. Although the sunflower stems contained significantly less total nitrogen than the leaves, the general trend was basically the same, that is, it increased first, and then began to decline after flowering stage. The nitrogen accumulation in the flower disk was always on the rise. At maturity, the total nitrogen accumulation in the flower disks accounted for 89.07%-94.57% of the total nitrogen accumulation in the aboveground part of the plant.



a. Squaring stage b. Flowering stage

c. Filling stage d. Mature stage

Note: Significant difference group for the mean values (n=3) is indicated by lowercase letters (p<0.05). The p values of the ANOVA tests for water regimes (W), nitrogen treatments (N), and their interactions (W×N) are indicated: * denotes p<0.05 and ** denotes p<0.01.

Figure 5 Nitrogen accumulation in leaves, stems, and flower disks at different growth stages

3.3.2 Nitrogen transport, uptake, and utilization in plants

At mature stage, the nitrogen transportation quantity and rate in the leaves and stem, and the amount of total nitrogen in the disk are presented in Table 4. The transportation quantity of leaves was 0.48-1.32 g/plant, with transportation rates ranging from 82.97% to 89.16%. The transportation quantity and rates were considerably smaller in the stems at 0.02-0.29 g/plant and 50.68%-73.85%, respectively. The interaction effects of irrigation and nitrogen fertilizer on total nitrogen transportation quantity and rate, total nitrogen yield, and the yield of leaves and stems were significant (p<0.05). Moreover, nitrogen had a significant effect on the nitrogen transport of leaves, while the nitrogen transport of stems was mainly affected by the irrigation amount. Under the W2N2 treatment, the transport quantity of leaf and stem reached the maximum, which also led to a significantly higher disk nitrogen accumulation quantity of W2N2 than other treatments. The effect of water–nitrogen coupling on the accumulation of nitrogen in flower disk was highly significant.

3.4 Sunflower yield components

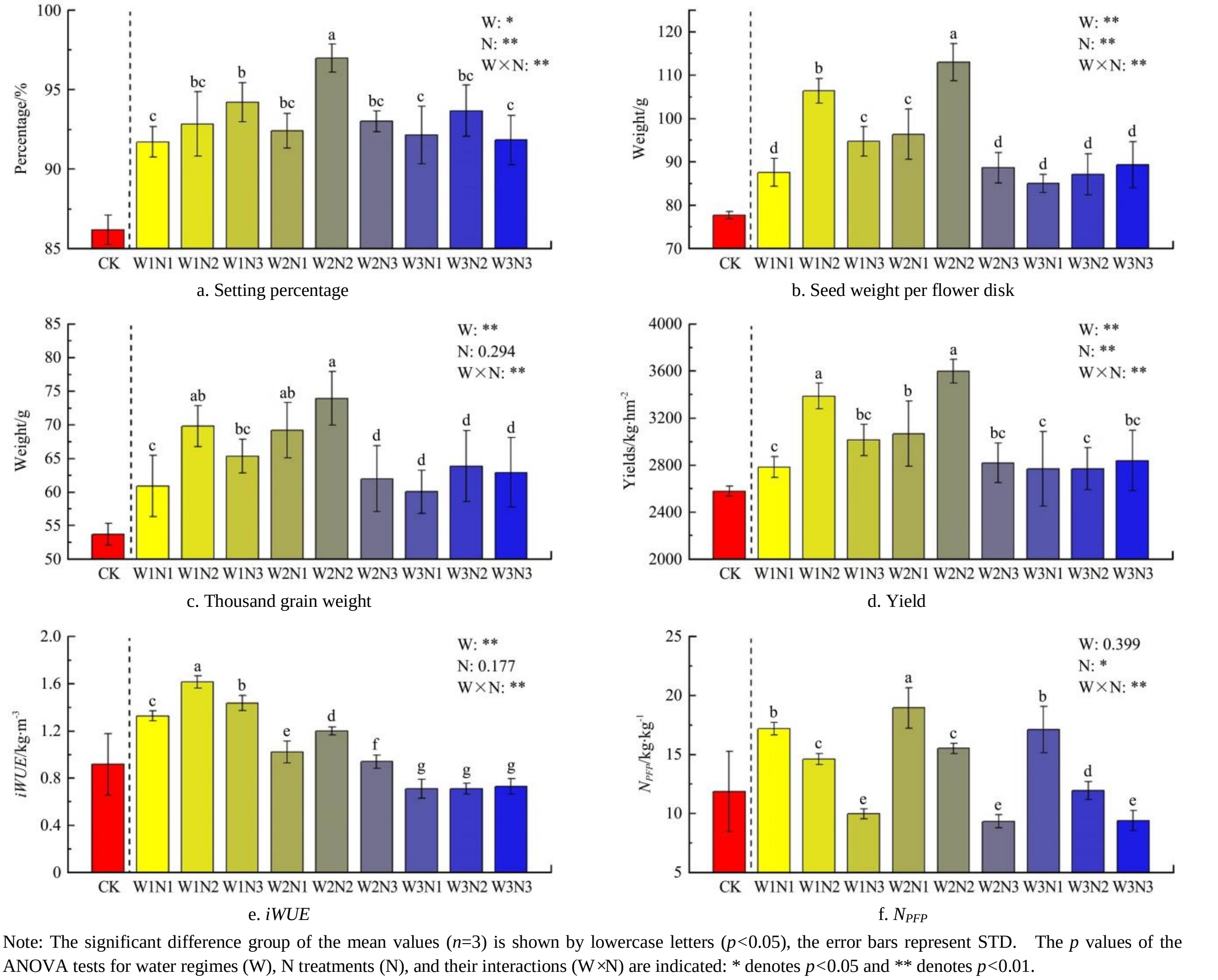
Figure 6 shows the yield components of sunflower. The interaction of water and fertilizer reached a highly significant level at the setting percentage, seed weight per flower disk, thousand-grain weight, yields, *iWUE* and N_{PFP}. We conducted ANOVA on the data of two years. The p values of setting percentage, seed weight per flower disk, thousand-grain weight, and yield were 0.69, 0.444, 0.116, and 0.566, respectively. Therefore, the planting years had no significant effect on the yield components of sunflower. However, the effect of water or nitrogen single factor on yield components was not the same in two years, which may be affected by climate factors. W2N2 had the highest setting percentage and seed weight per flower disk, which was significantly higher than the other treatments, reaching 96.97% and 112.96 g on average. The yield of W2N2 (3 597 kg/hm^2) was also higher than that of the other treatments; in terms of sunflower yield, the optimal water–nitrogen combination was W2N2, but the *iWUE* and N_{PFP} of W2N2 were not the highest. The *iWUE* was highest in W1N2 (1.61 kg/m^3), N_{PFP} was highest in W2N1 (18.93 kg/kg).

In order to determine a reasonable irrigation strategy, taking irrigation and fertilization amount as independent variables, and setting percentage, seed weight per flower disk, thousand-grain weight, yield, *iWUE*, and N_{PFP} as dependent variables, six regression equations were established (Table 5). After data normalization, the contour lines of the maximum value of each factor in different intervals are extracted. When the yield reaches the maximum value (Figure 7a), setting percentage, seed weight per flower disk, and thousand-grain weight can also be in the range of 95% of the maximum. In the case of only considering the yield components, it is concluded that the irrigation amount of 272.45 mm and the fertilization application of 233.43 kg/hm^2 is a good irrigation strategy, thus ensuring that the yield of sunflower can reach 3426.7 kg/hm^2. When *iWUE* and N_{PFP} were considered, the intersection occurs at 75% level of each index (Figure 7b), the irrigation water in the range of 241.62-253.35 mm and the amount of fertilizer in the range of 202.02-209.40 kg/hm^2 is a reasonable irrigation strategy, the yield of sunflower can reach 3229.3 kg/hm^2.

Table 4 Effects of different irrigation and nitrogen levels on nitrogen transportation in leaves and stems, and nitrogen increment in flower disks

Treatments	Leaf translocation		Steam translocation		Disk translocation quantity/g plant^{-1}
	Quantity/g plant^{-1}	Rate/%	Quantity/g plant^{-1}	Rate/%	
CK	0.33	78.26	0.004	45.36	0.93
W1N1	0.53^{f}	88.28^{ab}	0.05^{e}	72.64^{ab}	1.18^{f}
W1N2	0.76^{c}	84.77^{abc}	0.16^{c}	65.42^{bc}	1.38^{e}
W1N3	0.66^{d}	84.47^{bc}	0.13^{d}	65.67^{abc}	1.37^{e}
W2N1	0.57^{e}	87.80^{ab}	0.21^{b}	72.50^{ab}	1.73^{b}
W2N2	1.32^{a}	89.16^{a}	0.29^{a}	73.85^{a}	2.16^{a}
W2N3	0.65^{d}	89.06^{ab}	0.14^{cd}	72.72^{ab}	1.20^{f}
W3N1	0.48^{g}	82.97^{c}	0.02^{f}	70.70^{ab}	1.67^{bc}
W3N2	0.64^{d}	84.91^{abc}	0.05^{e}	50.68^{d}	1.61^{cd}
W3N3	0.92^{b}	88.78^{ab}	0.12^{d}	61.33^{c}	1.53^{d}
			P values		
W	0.191	<0.05	<0.01	<0.01	<0.01
N	<0.01	0.663	0.177	0.072	<0.05
W*N	<0.01	<0.05	<0.01	<0.01	<0.01

Note: The significant difference groups of the mean values (n=3) are denoted with lowercase letters (p<0.05). The p values of the ANOVA tests for water regimes (W), nitrogen treatments (N), and their interactions (W×N) are indicated.



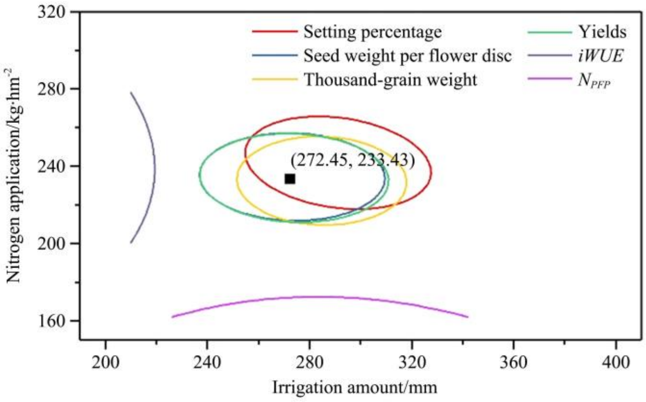
Note: The significant difference group of the mean values (n=3) is shown by lowercase letters (p<0.05), the error bars represent STD. The p values of the ANOVA tests for water regimes (W), N treatments (N), and their interactions (W×N) are indicated: * denotes p<0.05 and ** denotes p<0.01.

Figure 6 Effects of different irrigation and nitrogen levels on (a) setting percentage, (b) seed weight per flower disk, (c) thousand-grain weight, (d) sunflower yield, (e) *iWUE*, and (f) N_{PFP}

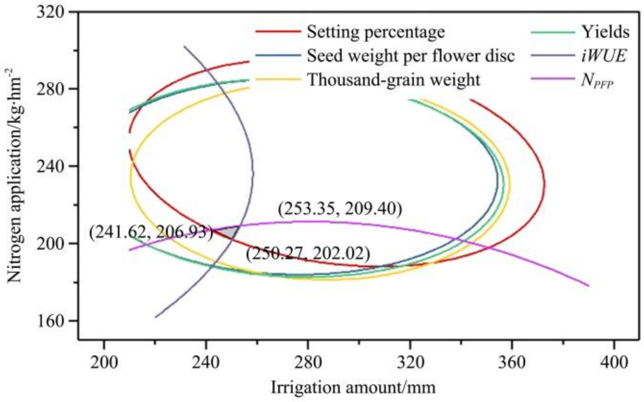
Table 5 Regression equations between irrigation amount and nitrogen application rate and setting percentage, seed weight per flower disk thousand-grain weight, and yields

Response variable Y	Regression equation	R^2	P Value
Setting percentage	Y=50.05958+0.1264W+0.22333N–1.71605*10$^{-4}W^2$–3.95918*10$^{-4}N^2$–1.09524*10^{-4}WN	0.69	<0.01
Seed weight per flower disk	Y=-103.42471+0.5428W+1.17022N–9.43747*10$^{-4}W^2$–2.43*10$^{-3}N^2$–1.15789*10^{-4}WN	0.71	<0.01
Thousand-grain weight	Y=-41.16747+0.3343W+0.56699N-5.61728*10$^{-4}W^2$–0.00118N^2–6.19048*10^{-5}WN	0.70	<0.01
Yields	Y=-3279.29103+17.3495W+37.0183N–2.89564*10$^{-2}W^2$–0.07542N^2–6.34011*10^{-3}WN	0.69	<0.01
$iWUE$	Y=0.86079-5.72914*10^{-3}W+0.01514N+4.08356*10$^{-6}W^2$–3.00533*10$^{-5}N^2$–3.62802*10^{-6}WN	0.97	<0.01
N_{PFP}	Y=11.26972+0.0892W–0.01833N-1.5149*10$^{-4}W^2$–7.37309*10$^{-5}N^2$–1.92312*10^{-5}WN	0.96	<0.01

Note: W is irrigation amount, mm; N is nitrogen application, kg/hm^2.



a. 95%



b. 75%

Note: After data normalization, the grid area in the (a) and (b) is a reasonable acceptable range for each response indicator to be greater than the 0.95 and 0.75 levels, respectively.

Figure 7 Comprehensive analysis of optimal irrigation strategy based on yield components and water and nitrogen use efficiency

4 Discussion

Sunflower roots mainly absorb nutrients in the tillage soil layer (0-40 cm). The nitrogen accumulation in tillage and deeper layers (40-80 cm soil depth) first increased, reached the maximum in the flowering stage, and then decreased with the sunflower growth. The quantity of soil nitrate-nitrogen in the shallow soil decreased noticeably, but it remained higher in the deeper soil because the formation of sunflower seeds required large amounts of soil nitrogen in the shallow soil. Some studies showed that the reduction of nitrate accumulation in the tillage soil layer under heavy irrigation was related not only to the crop's consumption of nitrate but also to leaching triggered by the irrigation[35].

The common drip irrigation system in Xinjiang usually needs a fertilizer equipment (also called pressure tank) to apply water-soluble fertilizer, which leads to a gradual decrease of fertilizer concentration in water with the increase of irrigation amount and irrigation time[36,37]. Under this condition, the interaction of irrigation and fertilization will affect the accumulation of nitrate-nitrogen in the soil. In this study, a small irrigation amount (210 mm) causes the increased accumulation of nitrate-nitrogen in the surface soil (0-40 cm) because it has less leaching effect; this finding was also obtained by Zhang et al.[38] However, if the nitrate-nitrogen content in the soil is higher, does it mean that crops will grow better? The result indicates otherwise. Moraes et al.[39] showed that the application amount of nitrogen fertilizer had a significant effect on the physiological growth of sunflower. But this does not mean that blindly increasing the application of nitrogen fertilizer will promote sunflower growth.

In general, the photosynthesis of sunflower reaches the maximum at squaring stage (or so-called heading stage), and then decreases with the plant age[40]. In arid areas, the effect of irrigation and fertilization on crop's photosynthesis is very obvious[41-44]. The increase in irrigation and fertilization will promote the photosynthesis and transpiration of crops[45,46]. However, once a threshold value is exceeded, excessive fertilization and irrigation will inhibit the photosynthetic characteristics of crops[47]. In this study, when the irrigation level reaches W3, the nitrate-nitrogen content in the soil under W3 level was the lowest under the same fertilization level (N1, N2, N3). The excessive irrigation amount may have an obvious leaching effect on soil nitrogen, which also weakens the promotion of fertilization in the photosynthetic characteristics of sunflowers. On the other hand, it is not feasible to apply large amounts of fertilizer to promote photosynthesis, because a high soil nitrate-nitrogen content will lead to potential toxicity[48]. The growth of many crops under a high concentration of nitrate has been inhibited, and the decline of photosynthesis is one of the main manifestations[49-51]. In our case, the sunflower growth appeared a similar phenomenon. This phenomenon was most obvious during the flowering stage when the average soil nitrate-nitrogen reached 51.1 mg/kg.

Pascual et al.[52] showed that WUE_{ins} is determined by plant water status and nitrogen application, which could reflect the response of plants to the coupling of water and fertilizer to a certain extent. In our experiment, the WUE_{ins} of each treatment did not show an obvious change rule, but overall, WUE_{ins} increased with the increase in irrigation amount, especially at the squaring and filling stages. Meanwhile, fertilization had a minimal effect on WUE_{ins}. Guizani et al.[53] found that irrigation deficit can improve the WUE_{ins} of crops. The reason for this result is that irrigation deficit will reduce the Pn and Tr of crops, but their ratio will increase, as reflected in the increase of WUE_{ins}. In our experiment, under the condition of fertilization, the Pn and Tr of sunflowers will increase, and the WUE_{ins} will also increase with the increase in the irrigation amount, thereby indicating that sunflower grows with improved efficiency of water and fertilizer utilization.

Soil nutrients are absorbed, accumulated, transported, and distributed to support the reproductive crop organs, which are typically the basis of crop yield[54]. It is feasible to adjust the nitrogen absorption of crops by changing the amount of water and fertilizer[55,56]. As for sunflower, in the reproductive growth stage (late growth period), nitrogen was mainly accumulated in the flower disk, as it was transferred from leaves and stems to seeds and promoted the formation of yield[57]. At low or moderate levels of nitrogen fertilization, a certain range of irrigation increase (below 300 mm) could accelerate nitrogen accumulation and transportation in leaves and stems, thus significantly increasing the yield of sunflower. With low or moderate irrigation levels, an increase in nitrogen application (below 232 kg/hm^2) would improve nitrogen accumulation in organs and boost the amount of transportation from leaves and stems to flower disks, leading to a higher yield of sunflower. This result is similar to the findings of Wang et al.[58]

In addition, The nitrogen required for seed formation of sunflower mainly depends on the accumulated nitrogen absorbed by crops before flowering, which is supported by nitrogen redistribution in vegetative tissues[59]. Seeds began to form at the later stage of reproductive development, and the transfer of nitrogen to seed was related to seed growth. Compared with stem, leaf is the main source of nitrogen for seeds[60]. The accumulation of total nitrogen in leaves sharply declined after flowering, just as total nitrogen began accumulating rapidly in the flower disks. The moderate rate (W2N2: 300 mm & 232 kg/hm^2) of water and nitrogen supply used in this study was the most beneficial for flower disk nitrogen accumulation. Through the redistribution of nitrogen, increasing the nitrogen content in the flower disk has a positive significance to improve the nitrogen use efficiency and yield of sunflower.

The crop yield is the most concerned point for farmers. If we take the increase of yield as the main evaluation basis, the theoretical maximum yield can be achieved when the irrigation amount is 272.45 mm, and the fertilizer amount is 233.43 kg/hm^2. But in this case, *iWUE* and N_{PFP} will reduce, which means a waste of water resources and fertilizer. In arid areas like Xinjiang, water resources are very scarce[61]. Kiani et al.[62] indicated that increasing the amount of nitrogen fertilizer was not a suitable strategy under severe deficit irrigation. With the N application from 0 to 93 kg/hm^2, the seed yield of two hybrids sunflower reduced 25% and 32%, respectively. Therefore, it is necessary to consider irrigation strategy more comprehensively. Finally, in combination with yield components and irrigation water and fertilizer use efficiency, the results showed that the irrigation amount should be 241.62-253.35 mm, and the fertilization rate should be 202.02-209.40 kg/hm^2, which is a reasonable irrigation strategy. Its yield is lower than the maximum theoretical yield, but it is still within the acceptable range. In fact, there are some differences in the amount of irrigation and nitrogen applied for sunflower in different areas. Wan et al.[63] planted sunflower on alkaline soil and found that the irrigation efficiency was the highest when the irrigation amount reached 750 mm. Rafiei et al.[64] indicated that the application of 150 kg/hm^2 could meet the demand for the growth of sunflower. Nasim and Bano[65] believed that 240 kg/hm^2 was a reasonable amount of fertilizer, and the yield of sunflower could reach the maximum. The reason for this difference is the different irrigation methods and soil quality in the planting area. The soil fertility in Xinjiang is poor because the cultivated land mostly comes from wasteland reclamation[66]. At the same time, because of high evaporation, the irrigation and fertilizer amount in this area are generally higher. However, the application of drip irrigation system improves the efficiency of water and fertilizer utilization to a certain extent, the irrigation amount and fertilizer application amount in Xinjiang are still within a reasonable range. Other areas should adjust the amount of irrigation and fertilization according to the soil quality, climate conditions, irrigation technology, and other conditions.

5 Conclusions

Different coupling of water and fertilizer application will change the accumulation of nitrogen in soil, which will affect the growth of sunflower. The photosynthesis of sunflower was significantly affected by the coupling of water and nitrogen and reached the maximum at the squaring stage, while the transpiration was less affected by the water-nitrogen coupling. The accumulation of nitrogen was concentrated in the sunflower leaves during the early growth stage, whereas this focus shifted to the disk in the late growth stage. Under W2N2 (300 mm and 232 kg/hm^2), the nitrogen transfer from leaf and stem to flower disks was the largest, and the accumulation of nitrogen in the flower disk was the largest. Reasonable coupling of water and nitrogen will be beneficial to the transfer of nitrogen to the flower disk of sunflower and will increase yield. Through the fitting analysis of yield components and water and fertilizer utilization efficiency, including the setting percentage, seed weight per flower disk, thousand-grain weights, yield, *iWUE* and N_{PFP}, it is concluded that the irrigation amount of 241.62-253.35 mm and the fertilization application of 202.02-209.40 kg/hm^2 is a good irrigation strategy, which ensures that the yield of sunflower can reach 3229.3 kg/hm^2. This irrigation strategy may provide some reference for drip irrigation of sunflower in some similar arid areas.

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