Effects of water, fertilizer, dissolved oxygen and temperature coupling on the photosynthesis, quality and yield of lettuce

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Abstract: With the increased demand for vegetables of nutritional value, enhancing vegetable quality while pursuing high yield has become a goal of many investigators. In the present study, the effects of irrigation quota (*A*), fertilizer amount (*B*), dissolved oxygen (DO, *C*), and temperature in the climate chamber (*D*) applied in combination with the quality and yield of lettuce were investigated. Four-factor and three-level orthogonal designs were used to determine the single-factor trend, the sequence of primary and secondary influencing factors, and optimal regulatory measures concerning the lettuce. The order of the primary and secondary effects on the plant height, net photosynthesis rate (P_n), transpiration rate (*E*), vitamin C content (Vc), soluble protein content (*Sp*), dry matter accumulation (D_m), yield, irrigation water use efficiency (IWUE) of the lettuce plants followed the order *A*>*B*>*D*>*C*. In addition, the order of the primary and secondary effects on the intercellular carbon dioxide concentration (C_i), chlorophyll content, and nitrate content followed the order *A*>*D*>*B*>*C*. The optimum scheme for lettuce was $A_3B_2C_1D_3$ (irrigation quota of 69 mm; fertilizer amount of 1.30 g/pot; DO of 6.5 mg/L; and temperature in the climate chamber of 20°C). Under the coordinated regulation of water, fertilizer, air, and temperature, the quality and yield of lettuce have been significantly improved. This study had important reference significance for the comprehensive regulation of water, fertilizer, air, and temperature facilities for vegetables.

Keywords: Aerated irrigation, Increased-temperature irrigation, Growth, Photosynthesis, Dry matter accumulation, Principal component analysis
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1 Introduction

Water, fertilizer, air, and temperature are essential for crop growth, and maintaining appropriate soil moisture, fertility, air permeability, and temperature is essential for any period of crop growth. Water-fertilizer coupling technology is used extensively worldwide. In recent years, researchers have shown interest in the impact of water-fertilizer coupling on the quality of lettuce[1]. By controlling irrigation[2] and fertilization[3], crop growth has been regulated to increase production and conserve water, but the impact of the soil environment on crops has been ignored. Soil aeration and temperature are additional important factors affecting crop growth. Long-term submembrane drip irrigation results in poor soil aeration in the root zone of greenhouse crops and low soil temperature, both of which negatively affect crop growth.

The plant root system needs sufficient oxygen for aerobic respiration to maintain metabolism and the growth and development of the whole plant. Hypoxia leads to weakened root respiration; as respiration shifts from aerobic to anaerobic, root growth stops, ion migration slows, and root fluid loss is controlled, thereby affecting the growth of the whole plant[4]. Aerated irrigation can improve the oxygen environment in the root zone, increase the length of fine roots, improve root activity, improve the physiological function of plants[5], and enhance soil microbial respiration[6]. Compared with non-aerated irrigation, aerated irrigation has been shown to improve the quality of fruit crops[7-9] and their yield and water use efficiency (WUE)[10-13]. Furthermore, aerated irrigation has been shown to improve the soil environment and soil microbial activity[14,15].

In Northwest China, large temperature differences between day and night occur, and winter and early spring temperatures are extremely low, restricting the yield and quality of greenhouse vegetables[16]. Therefore, to meet the temperature requirements for plant growth, the temperature is increased to allow the crop root system to fully absorb fertilizer[17] and to increase efficiency[18-20].

Studies on lettuce have focused mainly on the effects of light intensity[21], quality[22], duration and cycle[23,24] on yield and quality. The research team of Professor Tian Juncang from Ningxia University has conducted systematic studies on the effects of water-fertilizer and gas-heat coupling on tomato[25], cucumber[26], watermelon[27], melon[28] and pepper[29]. The appropriate regulation of water-fertilizer-gas-heat coupling is of great significance for the efficient development of greenhouse vegetables and ecological agriculture.

To reduce interference from uncontrolled factors (such as climatic conditions) in the experiment, this study aimed to identify

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the combined influences of water, fertilizer, DO, and temperature on lettuce and identify the optimum combination scheme of water, fertilizer, DO, and temperature. Based on previous research[9,30,31], this study adopted an orthogonal design to study the effects of water, fertilizer, DO, and temperature in combination on the growth, photosynthesis, quality, D_m, WUE and yield of lettuce in a climate chamber. The effects of water, fertilizer, DO and temperature in combination on the quality and growth efficiency of lettuce were elucidated, and the water, fertilizer, DO and temperature requirements of lettuce were revealed. The present study provides a new irrigation scheme to improve the quality and growth efficiency of leafy vegetables or similar vegetables.

2 Materials and methods

2.1 Experimental site

The experiment was conducted from December 25, 2018, to January 22, 2019, at the Engineering Research Center for Efficient Utilization of Modern Agricultural Water Resources in Arid Regions, Ministry of Education, Ningxia University, China (38°30'N, 106°08'E). The center is located at an altitude of 1111.5 m in the temperate continental climate zone.

2.2 Experimental design

An orthogonal design is a design that allows the investigation of multiple factors and levels. Representative points from the overall test are selected according to the orthogonality of the test. These representative points have the characteristics of "uniform dispersion and neat comparability". The method offers an efficient and economical experimental design. The experiment in the present study involved four factors and three levels in an orthogonal design[29,32,33]. The four factors were irrigation quota (*A*), fertilizer amount (*B*), dissolved oxygen (DO, *C*), and temperature in the climate chamber (*D*). Each factor had 3 levels and was applied in 9 treatments, and each treatment was repeated 3 times, a CK treatment was set as a control treatment. The orthogonal test factors and horizontal coding tables are shown in Table 1.

Table 1 Four-factor three-level orthogonal experiment design

Treatments	Factors				Combination plan
	Irrigation quota (*A*)/mm	Fertilization amount (*B*)/(g·pot^{-1})	Dissolved oxygen (*C*)/(mg·L^{-1})	Temperature in the climate chamber (*D*)/°C	
1	1 (27)	1 (0.65)	1 (6.5)	1 (16)	$A_1B_1C_1D_1$
2	1 (27)	2 (1.30)	2 (7.5)	2 (18)	$A_1B_2C_2D_2$
3	1 (27)	3 (1.95)	3 (8.5)	3 (20)	$A_1B_3C_3D_3$
4	2 (48)	1 (0.65)	2 (7.5)	3 (20)	$A_2B_1C_2D_3$
5	2 (48)	2 (1.30)	3 (8.5)	1 (16)	$A_2B_2C_3D_1$
6	2 (48)	3 (1.95)	1 (6.5)	2 (18)	$A_2B_3C_1D_2$
7	3 (69)	1 (0.65)	3 (8.5)	2 (18)	$A_3B_1C_3D_2$
8	3 (69)	2 (1.30)	1 (6.5)	3 (20)	$A_3B_2C_1D_3$
9	3 (69)	3 (1.95)	2 (7.5)	1 (16)	$A_3B_3C_2D_1$
CK	2 (48)	2 (1.30)	2 (7.5)	2 (18)	$A_2B_2C_2D_2$

2.3 Experimental materials and implementation

The dimensions of the tri-color-light climate chamber were as follows: width, 0.50 m; depth, 0.47 m; and height, 1.145 m (Figure 1). Irrigation water was applied as ultrapure water produced by the Heal Force laboratory. According to the experimental design, planted pots were subjected to three temperatures (16°C, 18°C, and 20°C) in the climate chamber. The tri-color-light climate chamber (white: red: blue ratio=5:4:2, light intensity: 210 μmol/m^2·s) used light-emitting diodes as light sources, and the humidity of the climate chamber was controlled at 60%-70%. The temperature and light were automatically controlled for 30 d.

The test lettuce variety was "*Lactuca sativa* L. var. Grand Rapids Tbr", and each plant had four leaves and one heart when it was transplanted. After transplantation, the pot experiment was carried out. The pots (height, 10.5 cm; lower diameter, 9 cm; and upper diameter, 12 cm) were round and made of plastic. The bottom of each pot was perforated, and 2 plants were placed in each pot, which was then covered with plastic film. Before the experiment, original noncultivated greenhouse soil was used to fill the pots, and the soil texture was a sandy loam. The initial physical and chemical properties of the soil are listed in Table 2.

Table 2 Initial physical and chemical properties of soil

Depth (cm)	PH	Total salt/(g·kg^{-1})	Soil field capacity/%	Bulk density/(g·cm^{-3})
0-20	8.53	0.47	18.88%	1.413
	Organic matter/(g·kg^{-1})	Alkali N/(mg·kg^{-1})	Available P/(mg·kg^{-1})	Available K/(mg·kg^{-1})
	20.15	60.78	85.56	423.68

At the time of transplanting, irrigation at 6 mm was performed, and the conductivity of the irrigation water was 7.0 μS/cm. The pots were irrigated 7 times during the growth period, and the three horizontal irrigation quotas were 27 mm, 48 mm, and 69 mm. The amount of fertilizer applied was determined according to the nutrient balance method, i.e. the amount of fertilizer applied corresponding to the target yield minus the base soil fertility, the amount of topdressing was 30 g of bio-organic fertilizer (organic matter>45%, N+P_2O_5+K_2O>5%) per pot, which was applied before planting. The amount of topdressing was converted according to the pure N content, and fertilizer (19-19-19+TE; N+P_2O_5+K_2O>50%) was applied 6 times during the test period. The irrigation water was aerated by multiple micro/nano foamers before irrigation. The water was aerated 7 times during the experiment period.

2.4 Measurements and methods

The amount of irrigation water was measured by using a measurement cylinder. After planting, at the end of the seedling period, one plant with normal growth was randomly selected in each pot. The height of the plant was measured from the base of the stem to the top using a steel tape measure. The stem diameter was measured with a digital Vernier caliper at the base of the main vine. The output was the fresh weight of the aboveground part. At the end of the experiment, each part of the aboveground part was weighed with a balance (Sartorius, Germany) with an accuracy of 0.01 g. A portable photosynthesis measurement system (LI-6800, LI-COR, USA) was used to determine the photosynthesis of lettuce leaves, and a chlorophyll meter (SPAD-502, Konica Minolta, Japan) was used to determine the chlorophyll content. Three plants per treatment were measured. Base measurements and calculation methods for the leaf area index (LAI), quality, bioaccumulation, and lettuce water consumption were based on previous work by Ouyang et al.[30] A DO (*C*) analyzer (JPB-607A, INESA, China) was used to measure the DO in the aerated irrigation water. The soil nutrient determination method was based on the "Soil Agricultural Chemical Analysis Method".

Lettuce irrigation water use efficiency (IWUE) was calculated as IWUE=Y/I, where Y is yield (kg) and I is irrigation quota (m^3). Lettuce WUE was calculated as WUE=Y/ET, where Y is yield (kg) and ET is water consumption (m^3). The instantaneous water use efficiency (WUEint) in lettuce leaves was calculated as WUEint=P_n/E, where P_n is the net photosynthetic rate (μmol/m^2·s) and E is the transpiration rate (mmol/m^2·s). Data Processing System 18.10

software (Hangzhou Ruifeng Information Technology Co., Ltd., China) was used for the range analysis, analysis of variance, and Duncan multiple comparisons, and Origin 2021 software (OriginLab Corporation, USA) was used to prepare figures.

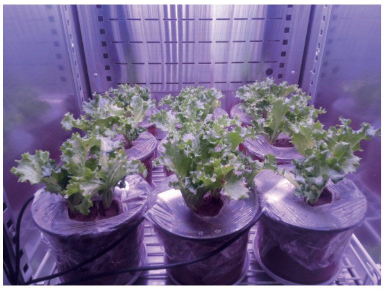


Figure 1 Test of water-fertilizer-dissolved oxygen-temperature coupling on the lettuce

3 Results and analysis

3.1 Lettuce growth

Table 3 lists the effects of water, fertilizer, DO, and temperature coupling on the plant height, stem diameter, and LAI of lettuce. The plant height, stem diameter, and leaf area index of T8 were the highest, increased by 22.64%, 21.73%, and 66.64% respectively compared with CK.

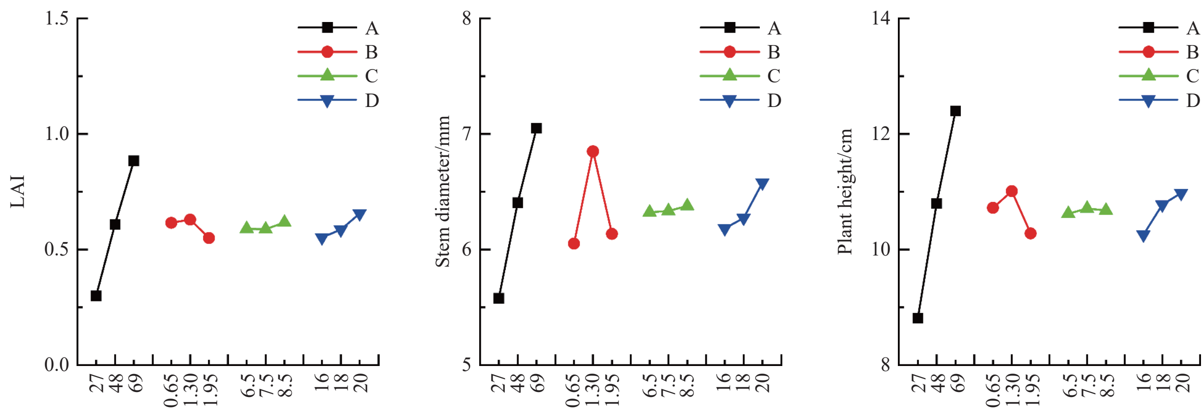
Analysis of the significance of differences in LAI, stem diameter, and plant height among the lettuce treatments revealed that the four factors exhibited the following order: $A>B>D>C$ (for plant height and stem diameter) and $A>D>B>C$ (for LAI). The analysis of variance results revealed that the effects of irrigation quota on LAI, stem diameter, and plant height were extremely significant ($p<0.01$). In addition, fertilizer amount had a significant effect on plant height ($p<0.05$), and the effect on stem diameter was extremely significant ($p<0.01$). Temperature significantly affected LAI and plant height ($p<0.05$) and strongly significantly affected stem diameter ($p<0.01$). The effects of the other factors were not significant.

As shown in Figure 2, the LAI, stem diameter, and plant height of the lettuce plants increased with increasing irrigation quota, DO, and temperature. The values initially increased but then decreased with increasing fertilizer amount. Increasing the irrigation quota, fertilizer amount, DO and temperature promoted the growth of lettuce, but the lettuce plants were easily burned by excessive amounts of fertilizer, which was not conducive to their growth. The reference combination was $A_3B_2C_1D_3$, and considering DO as a secondary factor, the optimal combination of factors for the LAI, stem diameter, and plant height of lettuce was $A_3B_2C_1D_3$; the corresponding LAI, stem diameter, and plant height were 0.96, 7.77 mm and 13.0 cm, respectively.

Table 3 Changes in the growth in the different lettuce treatments

Treatment	Plant height/cm	Stem diameter/mm	LAI
T1	8.4d	5.10f	0.26e
T2	9.3d	6.00d	0.31e
T3	8.7d	5.64e	0.33e
T4	11.2bc	6.34c	0.67cd
T5	10.7bc	6.78b	0.61d
T6	10.5c	6.1cd	0.54d
T7	12.6a	6.72b	0.91ab
T8	13.0a	7.77a	0.96a
T9	11.6b	6.67b	0.78bc
CK	10.6	6.38	0.62
	F-value		
A	115.01**	218.45**	116.46**
B	4.86*	77.20**	2.48ns
C	0.07ns	0.34ns	0.38ns
D	4.95*	17.46**	3.83*

Note: Data are given as the means, and different letters within each column indicate significant differences according to Duncan's multiple range tests; * and ** represent significant differences at the $p<0.05$ and $p<0.01$ levels, respectively; and ns represents no significant difference ($p>0.05$).



Note: 27, 48, and 69 indicate that the irrigation quotas for lettuce are 27 mm, 48 mm, and 69 mm, respectively. 0.65, 1.3, and 1.95 indicate that the fertilization amount is 0.65 g/pot, 1.3 g/pot, and 1.95 g/pot, respectively. 6.5, 7.5 and 8.5 indicate that the dissolved oxygen in the irrigation water is 6.5 mg/L, 7.5 mg/L and 8.5 mg/L, respectively. 16, 18 and 20 indicate that the temperature in the climate chamber is 16°C, 18°C and 20°C, respectively. The same as below.

Figure 2 Changes in growth indicators and four factors

3.2 Lettuce photosynthesis

Table 4 lists the effects of water, fertilizer, DO, and temperature coupling on the P_n, E, intercellular carbon dioxide concentration (C_i), stomatal conductance (g_{sw}), leaf temperature (T_{leaf}) and chlorophyll content of lettuce. Compared with CK, P_n, E, C_i, and g_{sw} of T8 increased by 76.54%, 94.77%, 12.51%, and 49.22%, respectively.

According to the analysis of the lettuce photosynthesis data, the effects of the four factors exhibited the following order: $A>B>C>D$ (for Ci and chlorophyll), $A>B>D>C$ (for P_n, E, and g_{sw}), and $D>C>A>B$ (for T_{leaf}). The analysis of variance revealed that the effects of irrigation quota on P_n, E, C_i, g_{sw}, T_{leaf} and chlorophyll were significant ($p<0.01$). The effects of fertilizer amount on P_n, E, and g_{sw} were extremely significant ($p<0.01$), and the effects of fertilizer amount on Ci and chlorophyll were significant (p<0.05). The effect of DO on T_{leaf} was extremely significant (p<0.01). The effect of temperature on E, g_{sw}, and T_{leaf} was extremely significant (p<0.05), and the effect of temperature on P_n was significant (p<0.01).

As shown in Figure 3, the P_n, E, C_i, and g_{sw} of lettuce increased as the irrigation quota increased, whereas T_{leaf} and chlorophyll content decreased with increasing irrigation quota. P_n, E, C_i, g_{sw}, chlorophyll, and T_{leaf} first increased but then decreased as the fertilizer amount increased, and P_n, E, C_i, g_{sw}, chlorophyll, and T_{leaf} increased with increasing DO and temperature.

Considering DO as a secondary, nonsignificant influencing factor, the optimal combination for enhancing the P_n, E, C_i, and g_{sw} of lettuce was determined to be $A_3B_2C_1D_3$. The corresponding P_n, E, C_i, and g_{sw} were 1.12 μmol/m²·s, 0.292 mmol/m²·s, 346 μmol/mol, and 0.029 mol/m²·s, respectively. Considering fertilizer amount as a secondary, nonsignificant influencing factor, the optimal combination of the various factors for enhancing the T_{leaf} of lettuce was $A_1B_3C_3D_3$, which yielded a T_{leaf} of 21.1°C. Considering DO and temperature as secondary, nonsignificant factors, the optimal combination of factors for enhancing lettuce chlorophyll content was $A_1B_2C_2D_2$, which yielded a chlorophyll content of 50.2 (SPAD value).

Table 4 Changes in photosynthesis in the different lettuce treatments

Treat-ment	P_n/ (μmol·m^{-2}·s^{-1})	E/ (mmol·m^{-2}·s^{-1})	C_i/ (μmol·mol^{-1})	g_{sw}/ (mol·m^{-2}·s^{-1})	T_{leaf}/ °C	Chlorophyll (SPAD)
T1	0.373f	0.046g	274d	0.011e	19.2e	44.8bcd
T2	0.491e	0.083f	296c	0.019d	20.4b	50.2a
T3	0.395ef	0.085f	297c	0.018d	21.1a	49.9a
T4	0.823c	0.184d	308bc	0.023cd	20.4b	45.5bc
T5	0.826c	0.195d	316b	0.025bc	19.7c	47.6ab
T6	0.642d	0.108e	308bc	0.021cd	19.2e	45.0bc
T7	1.369a	0.335b	350a	0.030b	19.8c	40.6d
T8	1.382a	0.350a	354a	0.042a	19.7cd	42.4cd
T9	1.112b	0.292c	346a	0.029b	19.2de	41.3cd
CK	0.873	0.180	315	0.028	20.0	44.9
			F-value			
A	439.13**	283.38**	166.26**	74.18**	13.64**	20.54**
B	21.05**	14.17**	5.51*	12.22**	0.73ns	4.12*
C	2.78ns	3.23ns	3.28ns	0.16ns	25.98**	1.84ns
D	5.54*	6.12**	2.76	9.80**	31.48**	0.80ns

Note: Data are given as the means, and different letters within each column indicate significant differences according to Duncan's multiple range tests; * and ** represent significant differences at the p<0.05 and p<0.01 levels, respectively; and ns represents no significant difference (p>0.05).

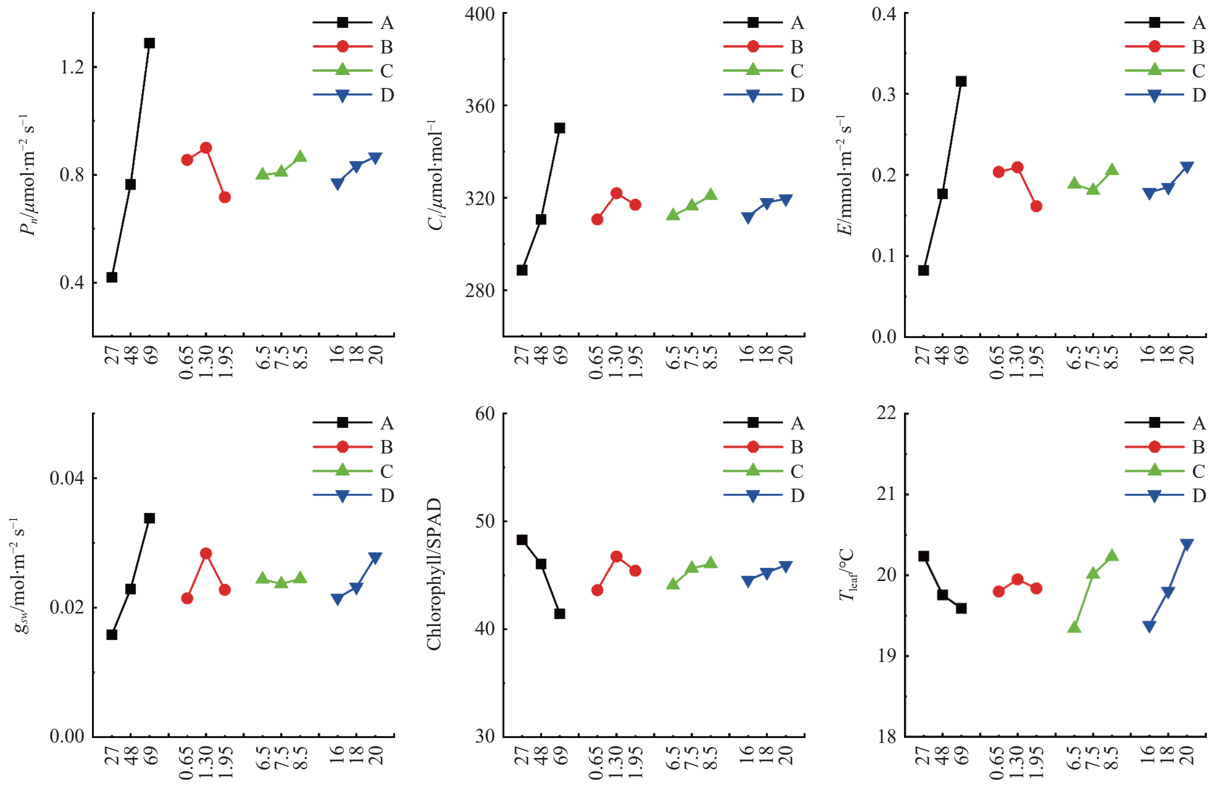


Figure 3 Changes in photosynthesis indicators and four factors

3.3 Lettuce quality

Table 5 lists the effects of water, fertilizer, DO and temperature coupling on the vitamin C content (Vc), soluble protein content (*Sp*) and nitrate content of lettuce. Compared with CK, Vc and *Sp* of T3 increased by 30.31% and 44.21%, respectively, and the nitrate content of T7 decreased by 42.08%.

Analysis of the significance of differences in Vc, *Sp* and nitrate in lettuce revealed that the four factors exhibited the following order: *A*>*B*>*D*>*C* (for Vc and Sp) and *A*>*B*>*C*>*D* (for nitrate). The analysis of variance results indicated that the effects of irrigation quota and fertilizer amount on Vc, *Sp*, and the nitrate mass fraction were extremely significant (p<0.01). The effect of DO on the Sp fraction was extremely significant (p<0.01), and significant effects of DO were detected for Vc and the nitrate mass fraction (p<0.05). In addition, temperature had a significant effect on both Vc and *Sp* (p<0.01).

As shown in Figure 4, the Vc, *Sp*, and nitrate mass fraction of lettuce increased as the irrigation quota and fertilizer amount increased. Vc and *Sp* decreased with increasing DO and temperature, whereas the nitrate mass fraction decreased with

Table 5 Changes in the quality of different lettuce treatments

Treatment	Vc/(mg·100g^{-1})	*Sp*/(g·100g^{-1})	Nitrate/(mg·kg^{-1})
T1	19.50c	0.27c	3740b
T2	21.63b	0.41b	3954ab
T3	23.93a	0.46a	4006a
T4	15.87d	0.24c	2583e
T5	14.94def	0.28c	2945d
T6	15.49de	0.28c	3228c
T7	13.10g	0.25c	1680g
T8	13.97fg	0.26c	2267f
T9	14.35efg	0.23c	2440ef
CK	18.37	0.32	2900
F value			
A	297.03**	61.24**	327.31**
B	13.60**	19.14**	33.91**
C	5.55*	11.47**	4.25*
D	12.52**	12.37**	1.08ns

Note: Data are given as the means, and different letters within each column indicate significant differences according to Duncan's multiple range tests; * and ** represent significant differences at the p<0.05 and p<0.01 levels, respectively; and ns represents no significant difference (p>0.05).

increasing DO and temperature. When factors such as costs and benefits were considered, the optimal combination for enhancing the Vc and Sp of lettuce was $A_1B_3C_3D_3$, and the corresponding Vc and *Sp* were 23.93 mg/100 g and 0.46 g/100 g, respectively. When factors such as costs and benefits were considered, the optimal combination for enhancing nitrate quality in lettuce was $A_3B_1C_3D_3$, and the corresponding nitrate mass fraction was 1680 mg/kg.

3.4 Lettuce biomass accumulation

Table 6 shows the effects of water, fertilizer, DO, and temperature coupling on the tissue water content (D_w), dry matter accumulation (D_m), dry-fresh ratio (D_f), and root-shoot ratio (R_s) of lettuce. Compared with CK, the f_a, f_u, d_a, d_u, and D_m of *T*8 increased by 31.08%, 1.01%, 34.53%, 11.73% and 29.56%, respectively.

According to the range analysis of biomass in each lettuce treatment, the influence of the four factors exhibited the following order: A>B>D>C (for f_a, f_u, d_a, d_u, D_m, T_w, and D_f) and *A*>*C*>*D*>*B* (for R_s). The analysis of variance results showed that the effects of irrigation quota on the D_m, T_w, D_f, and R_s of lettuce were significant (p<0.01) and that the effects of fertilizer amount on D_m, T_w, and D_f were extremely significant (p<0.01). The analysis of variance results also showed that temperature had a significant effect on D_m (p<0.01) and a significant effect on R_s (p<0.05). The effects of the other factors were not significant.

As shown in Figure 5, as the irrigation quota increased, the D_m and T_w of lettuce increased, and the D_f: R_s ratio decreased. The D_m of lettuce first increased and then decreased as the fertilizer amount increased. As the fertilizer amount increased, T_w decreased, and the D_f: R_s ratio increased. D_m and D_f increased as DO and temperature

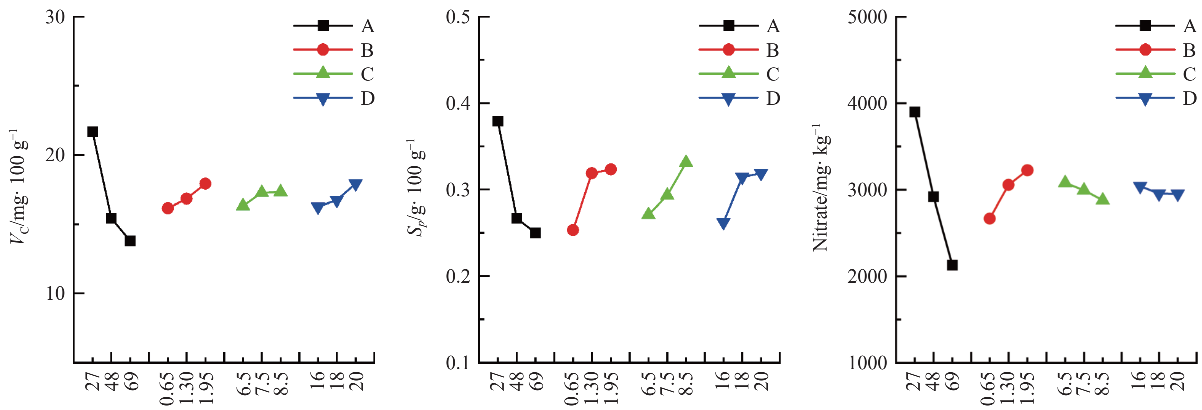


Figure 4 Changes in quality indicators and four factors

Table 6 Changes in biomass accumulation of lettuce in the different treatments

Treatment	f_a/g	f_u/g	d_a/g	d_u/g	D_m/g	T_w/%	D_f/%	R_s/%
T1	9.34e	4.45f	1.00e	0.38d	1.38e	89.24b	10.76b	48.02a
T2	10.72e	5.08e	1.50d	0.49bc	1.98d	86.03c	13.97a	47.36a
T3	11.21e	4.91e	1.58d	0.43cd	2.01d	85.91c	14.09a	43.89a
T4	36.53b	7.51c	2.06bc	0.44cd	2.51c	93.35a	5.65d	20.58c
T5	32.12c	7.66bc	2.29b	0.49bc	2.78b	92.87a	7.13cd	23.87bc
T6	24.7d	6.98d	2.02c	0.41d	2.43c	91.83a	8.17c	28.29b
T7	37.04b	8.02b	2.24bc	0.62a	2.87b	93.94a	6.06d	21.68c
T8	40.77a	8.64a	2.88a	0.67a	3.55a	92.9a	7.10cd	21.27c
T9	30.67c	7.98b	2.14bc	0.52b	2.66bc	92.98a	7.02cd	26.19bc
CK	31.10	8.55	2.14	0.60	2.74	93.10	6.90	27.53
				F-value				
A	586.06**	535.78**	140.79**	56.22**	169.82**	86.84**	98.69**	151.91**
B	32.52**	13.30**	25.22**	14.51**	32.54**	7.82**	11.87**	1.70ns
C	2.72ns	1.63ns	2.23ns	2.10ns	3.19ns	0.59ns	0.34ns	1.63ns
D	30.68**	5.99*	16.69**	4.98*	19.68**	2.67	2.42	4.67*

Note: Data are given as the means, and different letters within each column indicate significant differences according to Duncan's multiple range tests; * and ** represent significant differences at the p<0.05 and p<0.01 levels, respectively; and ns represents no significant difference (p>0.05).

in the climate chamber increased, whereas T_w and R_s decreased as DO and temperature in the chamber increased. When DO was considered a secondary, nonsignificant influencing factor, the optimal combination of factors for enhancing the D_m of lettuce was $A_3B_2C_1D_3$, and the corresponding D_m was 3.55 g. When DO and temperature were considered secondary, nonsignificant influencing factors, the optimal combination of the various factors for determining the T_w of lettuce was $A_3B_1C_3D_2$; the corresponding T_w was 93.94%. When DO was considered a secondary, nonsignificant influencing factor, the optimal combination for enhancing the D_f and R_s of lettuce was determined to be $A_1B_3C_3D_3$; the corresponding D_f and R_s were 14.09% and 48.02%, respectively.

3.5 Lettuce WUE and yield

Table 7 shows the effects of water, fertilizer, DO, and temperature in combination on the IWUE, WUE, WUE_{int}, and yield of lettuce. Compared with CK, the yield of T8 increased by 31.08%, the IWUE and WUE of T4 increased by 19.69% and 12.95%, respectively, and the WUE_{int} of T2 increased by 36.43%.

Analysis of lettuce WUE_{int}, WUE, IWUE, and yield revealed that the primary and secondary effects of the four factors exhibited the following order: *A*>*B*>*D*>*C* (for yield, IWUE, and WUE) and

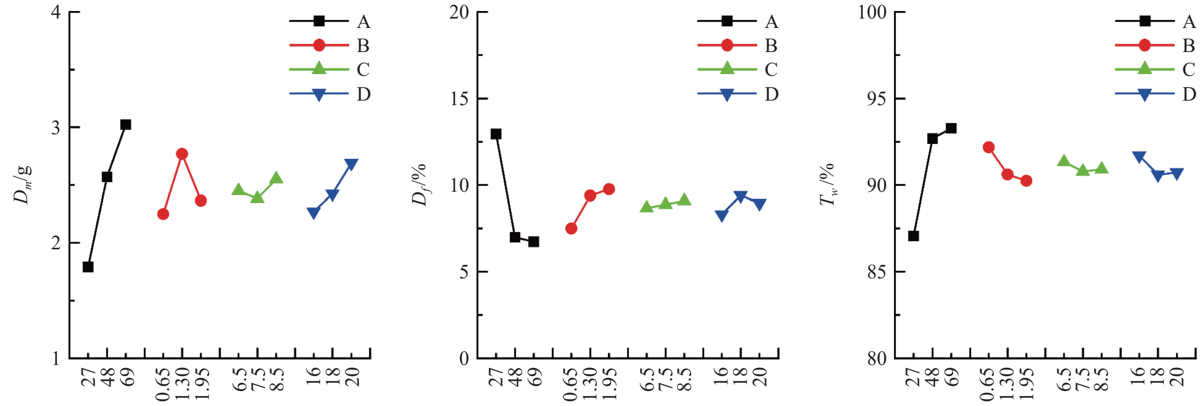


Figure 5 Changes in biomass indicators and four factors

Table 7 Changes in water use efficiency and the yield of lettuce in the different treatments

Treatment	I/mm	ET/mm	Yield/ (g·pot^{-1})	IWUE/ (kg·m^{-3})	WUE/ (kg·m^{-3})	WUE_{int}/ (μmol·mmol^{-1})
T1	27	17.90	9.34e	39.97f	60.38f	4.76bc
T2	27	19.36	10.72e	45.88e	63.94f	5.94a
T3	27	21.17	11.21e	44.26ef	61.19f	4.64bc
T4	48	31.39	36.53b	89.6a	134.43a	4.19bc
T5	48	30.89	32.12c	77.31b	120.22b	4.23bc
T6	48	25.89	24.7d	60.12d	110.17c	4.78b
T7	69	42.67	37.04b	60.45d	100.27d	4.09bc
T8	69	44.97	40.77a	66.6c	104.70cd	3.95c
T9	69	38.07	30.67c	48.03e	92.90e	4.29bc
CK	48	30.19	31.10	74.87	119	4.36
			F-value			
A			586.06**	363.51**	459.73**	13.60**
B			32.52**	72.43**	14.88**	1.70ns
C			2.72ns	13.40**	3.63*	3.06ns
D			30.68**	61.65**	13.00**	6.34**

Note: Data are given as the means, and different letters within each column indicate significant differences according to Duncan's multiple range tests; * and ** represent significant differences at the $p<0.05$ and $p<0.01$ levels, respectively; and ns represents no significant difference ($p>0.05$).

$A>D>C>B$ (for WUE_{int}). The analysis of variance results showed that the effects of irrigation quota and temperature on WUE_{int}, WUE, IWUE, and yield were significant ($p<0.01$). Moreover, fertilizer amount had significant effects on IWUE, WUE, and yield ($p<0.01$), and DO had significant effects on WUE ($p<0.05$) and IWUE ($p<0.01$). The remaining factors were not significant.

Figure 6 shows that lettuce yield increased as the irrigation quota increased. IWUE and WUE first increased but then decreased as the irrigation quota increased, and WUE_{int} increased with increasing irrigation quota. Yield and WUE_{int} first increased but then decreased as fertilizer amount increased, and IWUE and WUE decreased with increasing fertilizer amount. Yield increased with increasing DO level, whereas IWUE, WUE, and WUE_{int} first increased but then decreased with increasing DO. IWUE, WUE, and yield increased with increasing temperature, whereas WUE_{int} first increased but then decreased with increasing temperature.

Regarding lettuce yield, when DO was considered a secondary, nonsignificant factor, the optimal combination encompassing all factors was $A_3B_2C_1D_3$, and the maximum yield was 40.77 g/pot. With respect to WUE and IWUE during lettuce irrigation, the optimal combination of factors was $A_2B_1C_2D_3$, which yielded a WUE and IWUE of 89.60 kg/m^3 and 134.43 kg/m^3, respectively. Regarding the WUE_{int} of lettuce, the optimal combination encompassing all factors was $A_1B_2C_2D_2$; this combination yielded a WUE_{int} of 5.94 μmol/mmol.

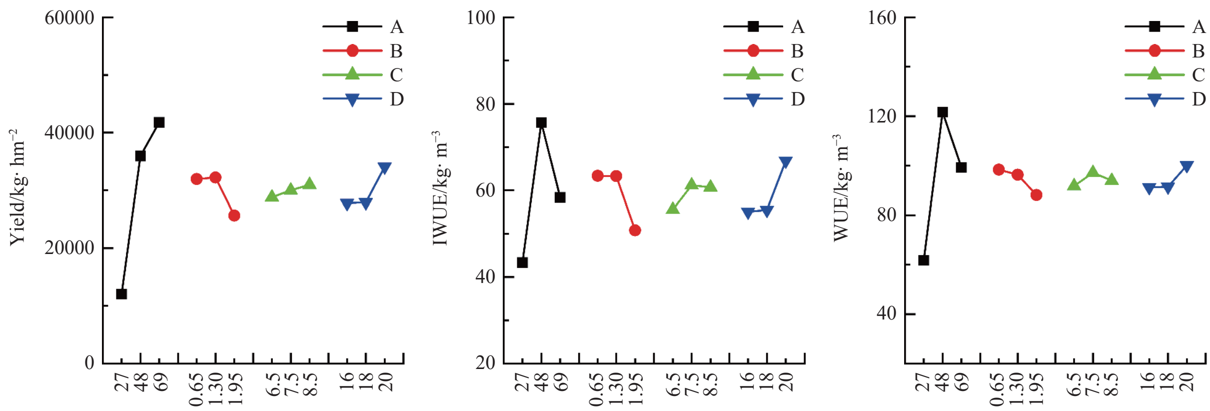
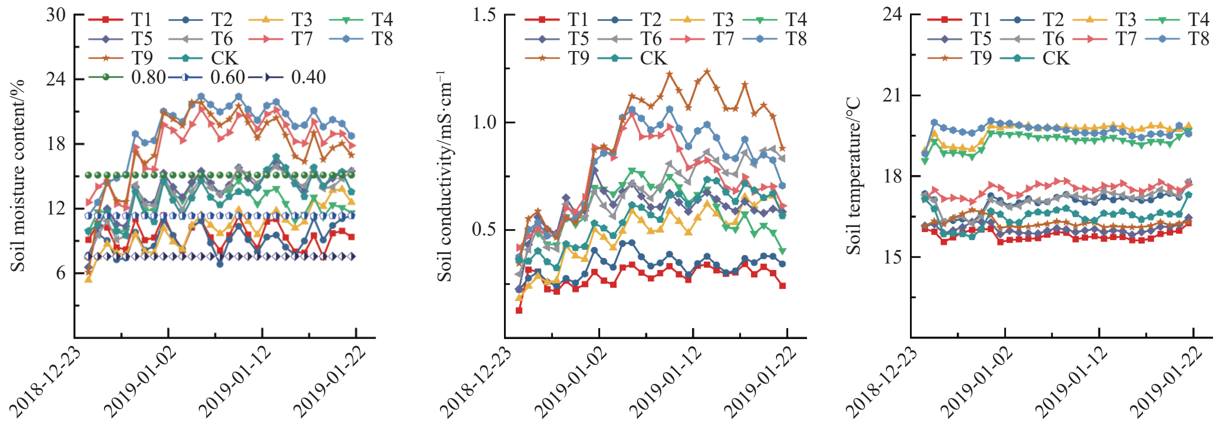


Figure 6 Changes in yield, WUE, and four factors

3.6 Soil moisture content, temperature and electrical conductivity

Figure 7a shows that during the test period, the average soil moisture content of the low water treatment (T1-T3) was 9.20%-10.16%, and the basic dynamic stability was 0.4-0.6 θ (θ, soil field capacity). The average soil moisture content of the medium water treatment (T4-T6) and CK was 12.60%-13.72%, and the basic dynamic stability was 0.6-0.8 θ. The average soil moisture content of the high water treatment (T7-T9) was 17.71%-19.33%, and the basic dynamic stability was 0.8-1.0 θ. Figure 7b shows that the average soil electrical conductivity of each treatment was 0.29-0.92 mS/cm. Under the same irrigation quota, the soil electrical conductivity increased with increasing fertilizer application rate. Under the experimental conditions, T9 corresponded to high water and high fertilizer, and the maximum average soil electrical conductivity was 0.92 mS/cm. Compared with CK (0.56 mS/cm), the average soil electrical conductivity of T9 increased by 64.73%. The average electrical conductivity of soil in the whole growth period increased with increasing irrigation quota and fertilizer application rate. Figure 7c shows that the average soil temperatures of the low-temperature treatments (T1, T5, and T9), middle-temperature treatments (T1, T5 and T9), high-temperature treatments (T3, T4 and T8) and control treatment (CK) were 15.80°C-16.26°C, 17.04°C-17.50°C, 19.29°C-19.69°C and 16.49°C, respectively.



a. Soil moisture content b. Soil electrical conductivity c. Soil temperature

Figure 7 Changes of soil moisture content, electrical conductivity and temperature

3.7 Comprehensive evaluation

Since the optimal combination of lettuce growth, photosynthesis, quality, biomass, and yield was not completely consistent, it was necessary to comprehensively evaluate the yield, quality, and other indicators for each treatment. At present, the comprehensive evaluation of water, fertilizer, DO and temperature coupling of lettuce based on principal component analysis has not been reported. Principal component analysis and comprehensive evaluation were performed using the main indexes of lettuce growth (plant height and LAI), photosynthesis (P_n and chlorophyll), fruit quality (Vc, *Sp* and nitrate), biomass (D_m), yield and WUE to provide a theoretical basis for improving the quality and efficiency of lettuce.

Data processing software (v18.10) was used to calculate the correlation coefficient matrix of lettuce plant height (X_1), LAI (X_2), P_n (X_3), chlorophyll (X_4), Vc (X_5), *Sp* (X_6), nitrate (X_7), D_m (X_8), yield (X_9) and WUE (X_{10}). Because the correlation coefficient matrix was used to eliminate the difference between the different orders of magnitude and dimensions of the original index variables, there was no need for standardization of the original data, and the calculation results are listed in Table 8. According to the comprehensive evaluation of the correlation coefficient matrix of lettuce, the yield was significantly positively correlated with growth indexes (plant height and LAI), P_n, D_m, and WUE but significantly negatively correlated with chlorophyll and quality indexes (Vc, Sp, and nitrate). Due to the different degrees of correlation among the indexes of lettuce, a direct comprehensive evaluation would have produced duplicated information, thereby affecting the evaluation results. Therefore, the principal component analysis method was used to combine the relevant indexes into a new set of unrelated comprehensive indexes for comprehensive evaluation to improve the reliability of the evaluation.

Calculation of the principal component contribution rate and cumulative contribution rate: Table 9 shows that the cumulative contribution rate of the first two principal components was 90.16% (>85%), indicating that the first two principal components accounted for most of the variation information of the original data. Selecting the first two principal components as the main principal components can reduce the number of variables and retain most of the original information. The first principal component (f_1) accounted for 80.92% of the original variation, mainly including the variation in plant height (X_1), LAI (X_2), P_n (X_3), Vc (X_5), D_m (X_8), yield (X_9) and other indicators. The second principal component (f_2) accounted for 9.24% of the original variation, mainly including the variation in chlorophyll (X_4), *Sp* (X_6), D_m (X_8), and other indicators.

Table 8 Correlation coefficient matrix

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
X_1	1.00									
X_2	0.99**	1.00								
X_3	0.98**	0.98**	1.00							
X_4	–0.74*	–0.78**	–0.79**	1.00						
X_5	–0.87**	–0.86**	–0.84**	0.81**	1.00					
X_6	–0.64*	–0.65*	–0.61	0.82**	0.89**	1.00				
X_7	–0.94**	–0.96**	–0.95**	0.85**	0.89**	0.75*	1.00			
X_8	0.92**	0.91**	0.87**	–0.51	–0.71*	–0.41	–0.79**	1.00		
X_9	0.94**	0.94**	0.88**	–0.66*	–0.86**	–0.69*	–0.92**	0.90**	1.00	
X_{10}	0.64*	0.64*	0.50	–0.35	–0.69*	–0.61	–0.64*	0.67*	0.85**	1.00

Note : * Represents a significant correlation between row and column variables ($p < 0.05$), * * represents a highly significant correlation between row and column variables ($p < 0.01$).

Table 9 Contribution rate and the cumulative contribution rate of principal components

Principal component	Eigenvalues	Contribution rate/%	Cumulative contribution rate/%
f_1	8.09	80.92	80.92
f_2	0.92	9.24	90.16

Note: The f_3 -f_{10} principal components in spring and summer and autumn and winter only contain 9.84% of the original variation information, so the table is not listed.

Principal component analysis and comprehensive score: Table 10 shows that the order of comprehensive scores of lettuce treatments was T8>T7>T9>T4>T5>CK>T6>T1>T2>T3. Therefore, the effects of water, fertilizer, DO and temperature coupling on the growth, photosynthesis, quality, D_m, yield, and WUE of lettuce were comprehensively analyzed. The T8 treatment ($A_3B_2C_1D_3$) was optimal for lettuce. The T8 treatment consisted of an irrigation quota of 69 mm, fertilization amount of 1.30 g/pot, DO of 6.5 mg/L, climate box temperature of 20°C and soil temperature of 19.01°C, which resulted in a yield of 40.77 g/pot, and exhibited an IWUE of 89.60 kg/m³ and WUE of 134.43 kg/m³.

Table 10 Principal component analysis score

Treatment	F_1	F_2	F	Comprehensive rank
T1	–3.20	–2.09	–2.78	8
T2	–3.71	0.61	–2.95	9
T3	–4.28	1.01	–3.37	10
T4	1.39	0.11	1.13	4
T5	0.64	0.69	0.58	5
T6	–0.11	–0.28	–0.12	7
T7	3.40	–0.48	2.71	2
T8	3.55	0.71	2.94	1
T9	1.98	–0.92	1.52	3
CK	0.34	0.64	0.33	6

4 Discussion

4.1 Effects of water, fertilizer, DO and temperature coupling on the growth of lettuce

The plant height, stem thickness, and LAI of the lettuce increased with increasing *A* (Figure 2). Previous studies have shown that the root system of plants is the main organ for water absorption and that its development is affected by many aspects, primarily soil moisture status and aeration status. When the soil water content is high, root diffusion is reduced by the resistance of the soil. A high soil water content is conducive to the development of new roots and root systems. Increasing A increases soil moisture accelerates lettuce metabolism, promotes the absorption of soil moisture by the root system, enhances lettuce growth, enhances development, and increases yield. An increase in irrigation volume has been shown to significantly promote the growth of lettuce[34, 35]. Increasing *B* improves the electrical conductivity of the soil and soil nutrient capacity. However, excessive fertilization can “burn” the seedlings, which is not conducive to crop growth. The growth indexes of this experiment first increased and then decreased with increasing B, indicating that an appropriate B is beneficial to the growth of crops.

O_2 is essential for crop growth. Oxygen is the electron acceptor of the mitochondrial electron transport chain during aerobic respiration, and it is one of the necessary conditions for ATP generation in aerobic respiration[36]. Increases in *C* in irrigation water occur mainly due to increases in soil oxygen content, a prolonged residence time of oxygen in soil, and enhanced micro/nano bubble mass transfer ability[12]. To a certain extent, the soil permeability is improved, and the soil microbial community plays an active role in decomposing organic matter, improving soil structure and the soil environment[37]. Aeration also promotes the absorption of fertilizer by crop roots[38, 39], which is beneficial to crop growth and production[40]. Increases in leaf growth rate are related to cell swelling or other changes in cell wall characteristics[41], and aerated irrigation enhances leaf water potential[42]. Increasing *D* indirectly increases soil temperature, improves soil enzyme activity, and improves the plant utilization rate of soil fertilizers. Lee et al.[43] reported that temperature had a significant effect on the growth rate of lettuce, and Okazaki et al.[44] concluded that an increase in temperature could promote the growth of lettuce. The main results of the present study are consistent with previous studies.

4.2 Effects of water, fertilizer, DO, and temperature coupling on the photosynthesis and Dm of lettuce

In this study, P_n, E, C_i, g_{sw}, and D_m increased with increasing *A*, *C*, and *D*, whereas T_{leaf} and chlorophyll content decreased with increasing *A* (Figure 2, Figure 4). The P_n and D_m of plants, which are affected by water supply, directly reflect dynamic changes in plant height, stem thickness, LAI, and yield formation[45]. Crop yield is the result of the accumulation of solar energy converted into chemical energy in the crop. Soil moisture status affects plant root water absorption and leaf transpiration, which in turn affects D_m and ultimately affects crop yield. The photosynthesis of crops is very sensitive to water stress, and a reduction in the photosynthetic rate of crops due to water stress is an important reason for reductions in crop yield during drought[46].

Previous studies have reported that aerated irrigation improves crop photosynthetic productivity and increases D_m[47]. Aerated irrigation can improve rhizosphere ventilation and increase DO saturation[48]. Aerated irrigation improves soil aeration conditions by affecting the air-to-water ratio of the soil, thereby increasing rhizospheric soil microbial abundance and soil enzyme activity[49], promoting soil respiration[40], and improving the microenvironment of the soil-crop root zone. Furthermore, aerated irrigation affects stem flow[42], g_{sw}[50], leaf water potential, and chlorophyll content[51], thereby promoting crop transpiration and light. In this study, P_n, E, C_i, g_{sw}, T_{leaf}, and chlorophyll increased with increasing *D*. This result indicated that under the temperature conditions of this experiment, a temperature increase not only enhanced P_n but also promoted D_m[52]. The main results of this study are consistent with previous studies.

4.3 Effects of water, fertilizer, DO, and temperature coupling on the yield, quality, and WUE of lettuce

In this study, the yield, V_c, S_p, and nitrate mass fraction of lettuce increased with increasing *A*, and V_c and S_p increased with increasing *A* and *B*. The mass fraction of nitrate decreased as *C* and *D* increased. Previous studies have shown that the combination of water and fertilizer is significantly associated with the quality and yield of lettuce[53]. In the present study, IWUE and WUE first increased and then decreased with increasing *A*, whereas WUE_{int} increased as *A* increased.

Root zone ventilation improves the rhizosphere oxygen environment, guarantees the normal operation of plant physiological functions, and promotes fruit quality. The quality of the graduate’s dishes (Figure 3), yield, and IWUE (Figure 5) increased with increasing *C*. Previous studies have shown that aerated irrigation promotes plant growth, promotes plant development and increases tomato yield while improving fruit quality, fruit flavor, and WUE[8,50,54,55]. Studies have shown that root hypoxia reduces the induction of most genes in the biosynthetic pathways of fruits, limiting the accumulation of V_C in fruits[56]. The main conclusions of this study are largely consistent with those of previous studies. An increase in yield is strongly dependent on an increase in fruit size[57]. A decrease in fruit size can occur due to a reduction in water potential, which limits the growth rate of the fruit[51, 58]. The main results of this study are consistent with previous studies. In the present study, IWUE, WUE and WUE_{int} first increased and then decreased with increasing *C*.

Within the appropriate temperature range, an increase in the temperature of the root zone promotes the function of the plant root cells, enhancing root vitality. The vitality of the roots directly affects the plant’s absorption of mineral nutrients and water, which influences the growth and development of the plant. A previous study showed that lettuce yield and nitrate content show decreasing trends with increasing temperature[52]; these decreasing trends may be due to temperature affecting the activity of nitrate reductase, thereby weakening the absorption of NO_3^- by the root system, which reduces the leaf nitrate content of lettuce[9,59]. The main results of the present study are consistent with previous studies. In the present study, IWUE and WUE both increased with increasing *D*.

The four factors soil water, fertilizer, DO and temperature are controlled within the range of optimal values[29]. Water-soluble fertilizers are used to enhance the absorption of moisture and nutrients by the root systems of crops. At the same time, appropriate DO and temperature are supplied so that water, fertilizer, gas, and temperature work together to promote crop metabolism, growth, development, and photosynthesis, thereby achieving the goal of high-quality production. The results of the present study show that the proper and comprehensive regulation of water, fertilizer, gas, and temperature can help improve lettuce quality and growth efficiency.

5 Conclusions

This study comprehensively considered the effects of water, fertilizer, DO, and temperature on lettuce quality and yield. According to the range analysis, the primary and secondary influences of the four factors exhibited the following order of importance: irrigation quota >fertilization amount >climate chamber temperature > DO. The water, fertilizer, DO, and temperature had a significant impact on the main indicators of lettuce growth (p<0.01).

Based on the principal component analysis, the optimal treatment for birth vegetables was T8 ($A_3B_2C_1D_3$), irrigation quota of 69 mm, fertilizer amount of 1.30 g/pot, DO of 6.5 mg/L; and temperature in the climate chamber of 20°C. Compared with CK, the yield of T8 increased by 31.08%. The coordinated regulation of water, fertilizer, DO, and temperature had improved the yield and quality of lettuce.

This optimum combination scheme provides a theoretical reference for the combined application of water, fertilizer, DO, and temperature to achieve improvements in quality, yield, and growth efficiency in lettuce.

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