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Photophysiological Mechanism of Dense Planting to Increase the Grain Yield of Intercropped Maize with Nitrogen-Reduction Application in Arid Conditions

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Abstract: Leaf photophysiological characteristics are the main indexes that determine crop yield formation. However, it remains unclear whether photosynthesis is systematically regulated via the cropping pattern and nitrogen supply when maize crops are planted with a high density. So, a field experiment that had a three-factor split-plot arrangement of treatments was conducted from 2020 to 2021. The main plot was two cropping patterns that included the sole cropping of maize and wheat–maize intercropping. The split plot had two nitrogen application rates: a traditional nitrogen application rate (N2, 360 kg ha^{-1}) and one reduced by 25% (N1, 270 kg ha^{-1}) for maize. The split–split plot had three planting densities: a traditional density (M1, 78,000 plant ha^{-1}), a medium density (M2, 10,400 plant ha^{-1}), and a high density (M3, 129,000 plant ha^{-1}) for sole maize; the corresponding densities of intercropped maize were 45,000, 60,000, and 75,000 plant ha^{-1}, respectively. The grain yield, the photosynthetic traits, and chlorophyll a fluorescence of the maize were assessed. The results showed that a 25% nitrogen reduction and dense planting had a negative impact on the individual maize's photosynthesis. However, intercropping could alleviate these drawbacks. When the maize was grown in the intercropping system at a lower nitrogen level and a medium planting density (IN1M2), the photosynthetic traits were better or similar to those of the traditional treatment (SN2M1) at the reproductive growth stage. Moreover, IN1M2 improved the light energy distribution among photochemistry, photo-protective and heat dissipation process of maize compared with SN2M1. A grey relation analysis demonstrated that the Pn and Tr of the individual maize played the most significant role in the group's productivity. Thus, the IN1M2 treatment achieved the highest grain yield and can be recommended as a feasible agronomic practice in oasis-irrigated regions.

Keywords: intercropping; nitrogen level; photosynthetic traits; yield performance; oasis-irrigated regions

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1. Introduction

It is estimated that the production of major food crops will need to be increased by 70% to 100% by 2050 to meet the increasing population of the world [1,2]. Reclamation of new land for crop production is not a viable option because there is little high-quality land available, and therefore increased inputs of nutrients are required to achieve desired yields. A large number of fertilizer inputs have a negative impact on the farmland ecosystem that damages the soil's biological habitat and leads to reductions in biological activity and diversity [3]. Meanwhile, unreasonable land reclamation and extensive agricultural production management have caused a large amount of greenhouse gas emissions and soil micro-ecological environment damage, thus reducing soil productivity [4]. To alleviate these issues, global food demand must be met without increasing the amount of arable land and with a focus on increasing the food production per unit area.

Studies have shown that crop-diversification configurations can enhance the stability of farmland ecosystems [5]. So, a feasible way to improve crop productivity and enhance the yield stability would be through multiple cropping systems such as strip intercropping, which has been confirmed to maintain higher and more stable grain yields than sole cropping [6,7]. Strip intercropping has played a significant role in ensuring grain production [7,8], especially in the arid irrigated areas of northwestern China where annual total solar radiation is abundant but water resource is scarce. Wheat and maize are two of the major grain crops that are popularly planted in intercropping systems [9,10]. This intercropping system, which achieves a high yield due to the simultaneous increase in production in component crops, possibly requires more fertilizer than sole crops [10]. Therefore, it is urgent to develop a promising agronomic practice that easy to operate in intercropping to boost the crop productivity while decreasing the dependence on fertilizers, especially the input of nitrogen fertilizer.

A moderate increase in the planting density is the most effective and simple way to achieve a high grain yield in an intercropping system [11]. Generally, increasing the planting density will enhance the degree of mutual shading among plants, limit the efficiency of individual plants in interception and utilization of light energy [12], reduce the photosynthetic rate of leaves, and affect crop production [13,14]. Previous studies have found that changes in the light-interception environment of mature leaves in the lower layer of crops will affect the structure and photosynthetic characteristics of new leaves in the upper canopy [15]. It was reported that when crops were planted at high densities, photosynthesis was limited and yield formation was affected [16].

Photosynthesis is not only affected by planting density, but is also regulated by nitrogen fertilizer [17,18]. It is one of the important measures to achieve high crop yields and to regulate photosynthetic characteristics and the accumulation and distribution of photosynthetic products through reasonable nitrogen operations [19]. A moderate reduction in the nitrogen fertilizer input for crop production leads to changes associated with acclimation such as promoting root growth, slowing leaf growth, rolling leaves, decreasing osmotic activity and synthesis of protective compounds, and causing stomatal closure to preserve normal water content of green leaves [20,21]. It can promote the transfer of photosynthetic products to grains and enhance crop yield [22]. With an excess reduction in nitrogen, the balance between light interception and energy utilization is disturbed, resulting in an impaired photosynthetic system II (PSII) [23]. In addition, a moderate reduction in the nitrogen-application rate can restrain photosynthesis in various growth stages of crops, while increasing density can readjust the effect of nitrogen fertilizer on the photosynthetic rate, increase the stomatal conductance and transpiration rate, and enhance the adaptability to stress [24]. Therefore, reasonable nitrogen management and population size can regulate the photosynthetic characteristics of crops.

Collectively, increasing the seeding density altered the light-interception intensity and light-utilization efficiency of crops and enhanced the root competition for soil resources [25,26]. Therefore, changes in photosynthetic characteristics, including the pigment content and photosynthetic rate, can occur when the crops are planted at a high density. So far, most studies on the systemic regulation of photosynthesis have been conducted under artificially regulated light conditions. In addition, the research focused on the photosynthetic traits of the entire plant under low- or high-light-intensity conditions [27]. Except for the newly developed leaves, the rest of the leaves were placed in a uniform low-light environment [14]. However, for field crop production, the light conditions under a densely planted population were more complicated, and the ambient light-interception intensity of each green leaf gradually improved from the bottom to the top of the plant [8]. Few studies have confirmed whether photosynthetic systems are regulated by a reduction in nitrogen in densely planted crops grown using intercropping in the field, so the related regulation mechanism remains unclear. The main mechanism by which this reduction in nitrogen application regulates the competition of roots for soil resources in densely planted crops of intercropping system and thus affects the photosynthetic performance has not been thoroughly investigated. Further study of this mechanism will provide a practical and theoretical basis for optimizing nitrogen management to increase the planting density to boost the grain yield per unit area in intercropping systems.

Maize is a typical C_4 plant with a high photosynthetic intensity. It is widely planted around the world as a major grain and fodder crop. In this study, the systemic response to different nitrogen conditions in the photosynthetic performance of densely planted maize in a wheat–maize intercropping was studied. The results of this study further explained the photosynthetic physiological basis of intercropped maize with nitrogen reduction to enhance the high-density tolerance and laid a foundation for enhancing the potential of nitrogen reduction in the dense planting of maize in arid irrigated areas of northwest China.

2. Materials and Methods

2.1. Test Area Description

The field experiment was carried out from 2020 to 2021 at the Oasis Agricultural Experiment Station of Gansu Agricultural University in Wuwei City. The station is located on the eastern side of the Hexi Corridor, which is in a temperate arid region with a continental climate. The annual mean rainfall in the past 20 years was less than 180 mm, which obviously could not meet the water demand of crop production, so crop production in this region was completely dependent on irrigation. In the last 20 years, the annual mean air temperature was less than 7.2 °C, and the accumulated temperature above 10 °C was about 2900 °C, which was in line with the thermal requirements of the wheat–maize intercrop. The research site is representative of an irrigated agroecosystem in an arid area of land. The precipitation levels in the two-year maize-growth period were 154.5 mm and 167.4 mm, respectively.

2.2. Experimental Design

A three-factor split-plot design was used in this experiment; the main plot was the cropping pattern, the split plot was the nitrogen application rate, and the split–split plot was the maize planting density. The two cropping patterns were sole maize and wheat–maize intercropping. The two nitrogen application rates were a traditional nitrogen application rate (N2, 360 kg ha^{-1}) and one reduced by 25% (N1, 270 kg ha^{-1}) for maize in the same base area for both sole cropping and intercropping. The three maize planting densities were a traditional density (M1, 78,000 plant ha^{-1}), a medium density (M2, 10,400 plant ha^{-1}), and a high density (M3, 129,000 plant ha^{-1}) for sole maize; the corresponding densities of the intercropped maize were 45,000, 60,000, and 75,000 plant ha^{-1}, respectively. Overall, this experiment comprised 12 treatments, each with three replicates. Among these treatments, SN2M1 was considered CK. Each plot was 51.3 m^2 (9.0 m × 5.7 m) with a ridge that measured 0.5 m wide by 0.3 m high between two neighboring plots to eliminate the potential movement of water and nutrition. The treatment abbreviations are described in Table 1.

Table 1. Codes of treatment.

Cropping Pattern	Nitrogen Level (kg ha^{-1})	Maize Planting Density (Plant ha^{-1})	Treatment Codes
Sole cropping (S)	N1, 270	M1, 78,000	SN1M1
		M2, 103,500	SN1M2
		M3, 129,000	SN1M3
	N2, 360	M1, 78,000	SN2M1 (CK)
		M2, 103,500	SN2M2
		M3, 129,000	SN2M3
Intercropping (I)	N1, 270	M1, 78,000	IN1M1
		M2,103,500	IN1M2
		M3, 129,000	IN1M3
	N2, 360	M1, 78,000	IN2M1
		M2, 103,500	IN2M2
		M3, 129,000	IN2M3

Field maize (cv. Xian-yu 335), which is widely planted in China, was applied in this experiment. It is an erectophile maize hybrid that is suitable for dense planting. It belongs to the medium ripening group and its growth period was about 140–145 d in the test area under an irrigated condition.

In the wheat–maize intercropping plots, wheat and maize were alternated in 1.9 m wide strips; three wheat–maize intercropped strips constituted one intercropped plot. In each intercropped plot, 11/19 were occupied by maize, and the remaining 8/19 were occupied by wheat. Each intercropped plot consisted of three pairs of wheat and maize strips, each wheat strip consisted of six rows of wheat, and each maize strip consisted of three rows of maize. The row spacing of both the sole and intercropped maize was 0.4 m. The row spacing of the wheat and maize in the intercropping system were 0.12 m and 0.44 m, respectively. Three maize planting densities were regulated by plant spacing; the plant spacings of the traditional density, medium density, and high density were 0.32 m, 0.24 m, and 0.19 m, respectively.

In the sole cropping and intercropping systems, the nitrogen and phosphorus application ratios for the wheat and maize were consistent under the same land area. The phosphorus amount (P_2O_5) was 90 kg ha^{-1} and 180 kg ha^{-1} for the wheat and maize, respectively. All pure nitrogen and P_2O_5 were used as the base fertilizer for the intercropped wheat. All P_2O_5 was used as the base fertilizer for the intercropped maize, but 30% pure nitrogen was used as the base fertilizer, 50% pure nitrogen was used at the jointing stage, and 20% pure nitrogen was used at the grain-filling stage for the maize in the sole cropping and intercropping. The irrigation amount was 4800 and 4050 m^3 for wheat–maize intercropping and sole maize, respectively. In 2020–2021, both crops were planted in each experimental year with sowing and harvesting dates consistent with all treatments: wheat was sown on 20 March and 22 March and harvested on 22 and 21 July in 2020 and 2021, respectively; and maize was sown on 19 and 20 April and harvested on 28 and 27 September in 2020 and 2021, respectively.

2.3. Data Collection

Dry-matter accumulation (DMA): The sole and intercropped plots were sampled for the aboveground biomass accumulation of maize at 15 d intervals starting at 45 days after emergence (DAE) of the maize (Table 2). For the sole and intercropped plots, 2/3 of the maize strips were used to measure the aboveground biomass accumulation, and the remaining 1/3 of the maize strips were used to measure the grain yield at physiological maturity. At each sampling time, the aboveground biomass accumulation was measured from five maize plants in the same row that were randomly sampled and cut at the soil surface; to reduce sampling errors, the next sampling occurred away from the open holes caused by the previous sampling. All biomass samples were oven-dried at 105 °C for 1 h for desiccation and then at 80 °C until they reached a constant mass. The biomass samples were weighed after drying using an electronic balance.

Table 2. Phenological stages of maize at sampling times in 2020 and 2021.

Intercropping Relation	Sampling Time (Days after Emergence)	Maize Growth Stage
Co-growth period with wheat	15	Fourth-leaf stage (V4)
	30	Eighth-leaf stage (V8)
	45	Fourteenth-leaf stage (V14)
	60	Tasseling stage (VT)
	75	Blister kernel stage (R3)
After the wheat harvest (maize's independent growth period)	90	Milking stage (R3)
	105	Doughing stage (R4)
	120	Denting stage (R5)
	145	Maturing Stage (R6)

Grain yield (GY): At physiological maturity, three rows that were 5 m long were harvested manually from the remaining 1/3 area of each plot. The grain moisture was measured using a portable seed moisture meter (PM-8188-A; KETT, Tokyo, Japan). The grain yield was determined at a 14% moisture content.

Chlorophyll content (SPAD): The chlorophyll content of the maize leaves was detected with a plant chlorophyll meter (SPAD-502 Plus Chlorophyll Meter, Konica Minolta, Japan) at an interval of 15 d starting at 45 DAE of the maize. The top fully expanded leaves were selected to measure before the maize's tasseling stage, and three ear leaves were selected to measure after the maize's tasseling stage. The SPAD value was determined in the center of the selected leaves avoiding the main vein; the mean value of three measurements was calculated to represent the SPAD value of each plot.

Photosynthetic gas-exchange parameters: The leaf gas-exchange parameters, including the net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr), were measured with a portable photosynthesis system (LI-6800XT; LI-COR, Lincoln, OR, United States) equipped with 2 cm^2 chamber. The reference CO_2 concentration was maintained at 400 μmol mol^{-1} by applying a CO_2 cylinder. The flow rate for all measurements was 500 μmol s^{-1}. The photosynthetic photon flux density (PPFD) used was 1200 μmol m^{-2} s^{-1}. During the measurements, the leaf temperature was maintained at air temperature and the relative humidity was maintained at 55%. Measurements were taken on a sunny day in the morning (9:00–11:00 a.m.) to avoid potential stomatal closure around noontime. The measurements of the time, date, and position of the leaves were consistent with the values of SPAD obtained for maize.

Chlorophyll a fluorescence parameters: The parameters for chlorophyll a fluorescence in the center part of the ear leaves were determined with a portable fluorescence measuring system (PAM-2500; Heinz Walz, Effeltrich, Germany) using the saturation pulse method after 11:00 p.m. on the leaves that were well adapted to the dark. The duration of the dark adaptation was 2 h to achieve a full dark-adapted state. The three maize leaves from each treatment were measured at 75, 90, and 105 DAE. A PAM chlorophyll fluorescence induction kinetic curve was recorded under darkness. The measuring light was set at 1 μmol (photon) m^{-2} s^{-1} to determine the minimum fluorescence (Fo); the light intensity was set at 600 μmol m^{-2} s^{-1} to determine the maximum fluorescence (Fm), maximal fluorescence in the light-adapted state (Fm′), and steady-state fluorescence (F). Using these data, the effective quantum yield of photosynthetic system II (PSII) in light (Y(II) or ΦPSII), the quantum yield of regulated non-photochemical energy dissipation (Y(NPQ)), and the quantum yield of non-regulated non-photochemical energy dissipation (Y(NO)) were calculated using the following equations [28]:

$$Y(II) = (Fm' - F)/Fm' \tag{1}$$

$$Y(NPQ) = F/Fm' - F/Fm \tag{2}$$

$$Y(NO) = F/Fm \tag{3}$$

Nitrogen nutrition index (NNI): The nitrogen nutrition index is the ratio between the actual crop nitrogen uptake (Na) and the critical nitrogen uptake (Nc) (Equation (4)) [29,30] The Na of the maize plants was measured using a fully automatic azotometer (Primacs SNC100; SKALAR, Breda, Netherlands). The Nc (the minimum nitrogen uptake for maximum shoot dry-matter accumulation (DMA)) corresponded to the actual crop dry-matter accumulation (Equation (5)) [29]. For the maize, the coefficients a = 34 and b = 0.63 were determined [29,30].

$$NII = Na/Nc \tag{4}$$

$$Nc = a(DMA)^b \tag{5}$$

2.4. Statistical Analysis

A permutational multivariate ANOVA was conducted using the "lme4" and "lmerTest" packages in R (version 4.2.1) to test the effects of the year, seedling emergence time, cropping pattern, nitrogen level, and maize planting density on the maize's yield, NNI, and photosynthetic performance. Duncan's multiple-range test was applied to verify the significant differences among the variables ($p < 0.05$) for the GY and chlorophyll a fluorescence parameters. The means among the treatments for the DMA, NNI, SPAD, and gas-exchange parameters were compared using a least significant difference test (LSD 0.05). A grey relational analysis (GRA) was used to evaluate the importance of each parameter to the grain yield. The grey relational grade was calculated according to Zhou L [31] to evaluate the importance of each indicator for the grain yield. A Pearson correlation analysis was conducted using the SPSS 22.0 software (SPSS 22.0; IBM, New York, NY, USA) to analyze the relationships between the photosynthetic characteristics of the maize leaves.

3. Results

3.1. Analysis of Variance of the Experimental Factors

The results for the year (Y), seedling emergence time (T), cropping pattern (P), nitrogen level (N), and maize planting density (M), and their interactions that affected the maize's DMA, GY, NNI, SPAD, gas-exchange parameters, and chlorophyll a fluorescence parameters are summarized in Table 3.

Table 3. Analysis of variance table.

	DMA	GY	NNI	SPAD	Pn	Tr	Gs	Y(II)	Y(NPQ)	Y(NO)
Year(Y)	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	0.006 **	<0.001 ***
Seedling emergence time(T)	<0.001 ***	-	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Cropping pattern (P)	<0.001 ***	<0.001 ***	0.135	0.206	<0.001 ***	0.037*	0.639	<0.001 ***	0.043*	<0.001 ***
Nitrogen level (N)	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Maize planting density (M)	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Y × T	<0.001 ***	-	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Y × P	<0.001 ***	0.068	0.146	<0.001 ***	<0.001 ***	0.524	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
T × P	<0.001 ***	-	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Y × M	0.008 **	<0.001 ***	0.03*	0.197	0.004 **	0.562	0.181	<0.001 ***	<0.001 ***	0.224
T × M	<0.001 ***	-	<0.001 ***	<0.001 ***	0.007 **	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
P × M	<0.001 ***	0.001**	0.026*	0.013 *	0.562	<0.001 ***	<0.001 ***	<0.001 ***	0.003**	<0.001 ***
Y × N	0.942881	0.004 **	0.301	0.752	0.756	0.335	0.04 *	<0.001 ***	<0.001 ***	<0.001 ***
T × N	<0.001 ***	-	<0.001 ***	0.015 *	0.037 *	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
P × N	0.005 **	<0.001 ***	0.824	0.138	0.825	0.457	0.002**	0.089.	<0.001 ***	0.02*
M × N	0.132603	0.577	0.302	0.732	0.071.	0.025 *	0.387	<0.001 ***	<0.001 ***	<0.001 ***
Y × T × P	<0.001 ***	-	0.236	0.003 **	<0.001 ***	<0.001 ***	<0.001 ***	0.553	<0.001 ***	<0.001 ***
Y × T × M	<0.001 ***	-	0.988	0.036 *	0.003 **	0.059.	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Y × P × M	<0.001 ***	0.321	0.024 *	<0.001 ***	0.122	0.122	0.518	<0.001 ***	<0.001 ***	0.021 *
T × P × M	<0.001 ***	-	<0.001 ***	<0.001 ***	0.004 **	0.03 *	<0.001 ***	0.009**	0.003 **	0.004 **
Y × T × N	0.004 **	-	0.006 **	0.006 **	0.041 *	<0.001 ***	0.024 *	<0.001 ***	<0.001 ***	0.692
Y × P × N	0.893512	0.003 **	0.429	<0.001 ***	0.152	<0.001 ***	<0.001 ***	0.04 *	<0.001 ***	0.431
T × P × N	<0.001 ***	-	<0.001 ***	<0.001 ***	0.065.	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	0.017 *
Y × M × N	<0.001 ***	0.001 **	0.028 *	0.335	0.152	0.051.	0.003 **	<0.001 ***	<0.001 ***	<0.001 ***
T × M × N	0.026 *	-	0.99	0.53	<0.001 ***	<0.001 ***	0.297	<0.001 ***	<0.001 ***	<0.001 ***
P × M × N	0.021 *	0.042*	0.541	0.048 *	0.281	0.095.	0.059.	0.448	<0.001 ***	<0.001 ***
Y × T × P × M	<0.001 ***	-	0.786	0.061.	0.186	0.034 *	<0.001 ***	<0.001 ***	0.002 **	<0.001 ***
Y × T × P × N	0.903	-	0.753	0.018 *	0.064.	<0.001 ***	<0.001 ***	0.004 **	<0.001 ***	0.199
Y × T × M × N	0.362	-	0.661	<0.001 ***	0.013 *	0.88	0.005 **	<0.001 ***	<0.001 ***	0.089.
Y × P × M × N	0.083	0.337	0.111	0.101	0.247	<0.001 ***	<0.001 ***	0.03 *	<0.001 ***	<0.001 ***
T × P × M × N	0.112	-	0.064.	<0.001 ***	<0.001 ***	0.034 *	<0.001 ***	0.001 **	<0.001 ***	0.356
Y × T × P × M × N	0.864	-	0.195	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***

The values are probability values. * Significant difference at the 0.05 probability level; ** significant difference at the 0.01 probability level; *** significant difference at the 0.001 probability level.

3.2. Dense Planting Regulated Dry-Matter Accumulation and Boosted Grain Yield of Intercropped Maize with Nitrogen Reduction

3.2.1. Aboveground Dry-Matter Accumulation (DMA)

Figure 1 shows that prior to 90 DAE, the dry-matter accumulation of the intercropped maize was decreased by 21.6–24.8% ($p < 0.05$) compared to the sole maize. However, intercropping showed a higher dry-matter accumulation of the maize by 24.8–26.1% ($p < 0.05$)

compared to sole cropping from 90 DAE to 145 DAE. The results showed that intercropped maize had a recovery growth after the wheat harvest. The dry-matter accumulation under nitrogen reduction (N1) was lowered by 3.5–5.1% starting at 75 DAE in both years compared with the traditional nitrogen level (N2). The dry-matter accumulation also increased with the maize density during the growth period. In the intercropping system, the maize's dry-matter accumulation declined slightly with N1 before 75 DAE and was reduced significantly ($p < 0.05$) by 4.2–4.6% compared with N2 after 90 DAE in 2020 and 2021, respectively. The dry-matter accumulation of the intercropped maize was enhanced by an increase in the planting density; the average value under M2 and M3 was 12.3–22.6% and 29.6–43.3% ($p < 0.05$) higher, respectively, than that under M1. After the wheat harvest, dense planting in intercropping could boost the dry-matter accumulation of the maize even if the nitrogen was reduced: IN1M1, IN1M2, and IN1M3 were increased by 21.7–26.9%, 37.2–40.4%, and 50.0–58.3%, respectively, compared with the traditional treatment (SN2M1).

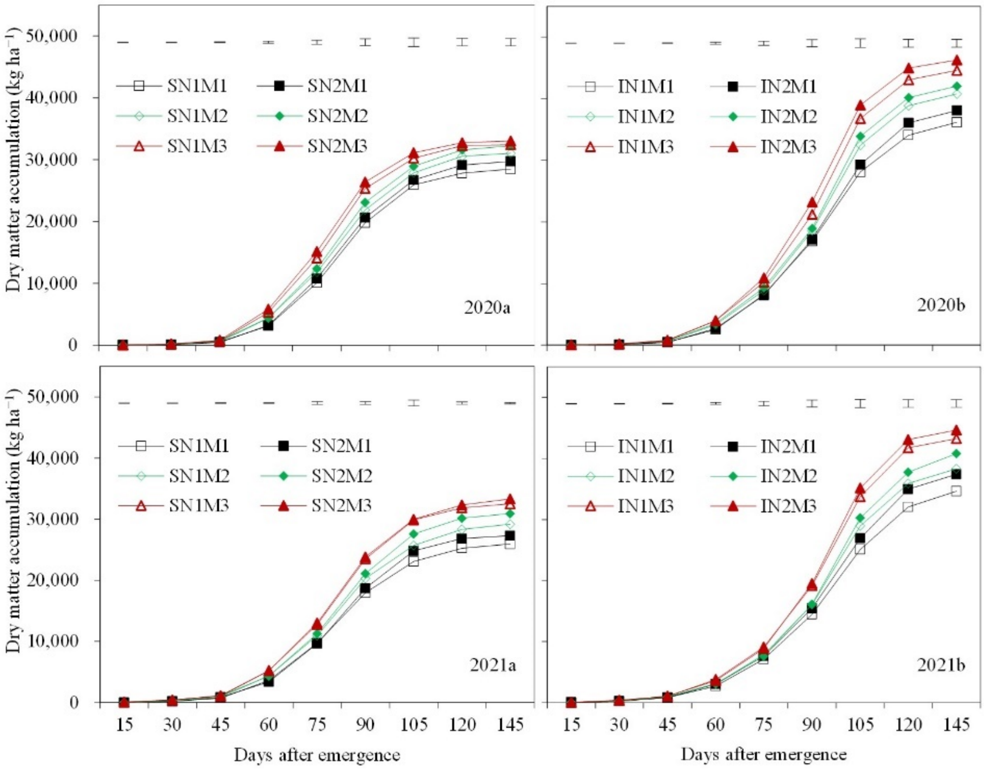

Figure 1. Dry-matter accumulation of maize under sole cropping system in 2020a and 2021a and intercropping system in 2020b and 2021b. The dry-matter accumulation was sampled at 15, 30, 45, 60, 75, 90, 105, 120, and 145 DAE in the maize-growing season. The vertical bars above the curve are the standard errors. The length of vertical bars represents the magnitude of the least significant difference (LSD) at $p = 0.05$ among treatments within a measurement time ($n = 3$). Treatment abbreviations are described in Table 1.

3.2.2. Grain Yield (GY)

Figure 2 shows that intercropping significantly improved the maize's grain yield by 34.1–35.6% ($p < 0.01$) compared to sole cropping. The grain yield under N1 was 5.0–8.4% lower than that under N2. Among the three maize planting densities, M2 had a 6.7–13.4% greater grain yield than M1, but no significant differences were found between M2 and M3. For the intercropped maize, N1 lowered the maize's grain yield by 6.0–11.7% ($p < 0.01$) compared with N2; M2 and M3 raised the grain yield by 6.7–14.1% and 4.6–12.6%, respectively, compared with M1, respectively. When integrating the three factors of cropping pattern, nitrogen level, and planting density, IN2M2 produced the highest grain yield among all treatments. Compared with SN2M1, IN2M2 improved the grain yield by 49.4–52.7%. Under the condition of nitrogen reduction, dense planting in intercropping could still boost the grain yield: IN1M2 the enhanced grain yield by 31.3–45.9% more than SN2M1. Overall, dense planting in the intercropping system could compensate for the loss in grain yield of the maize caused by nitrogen reduction.

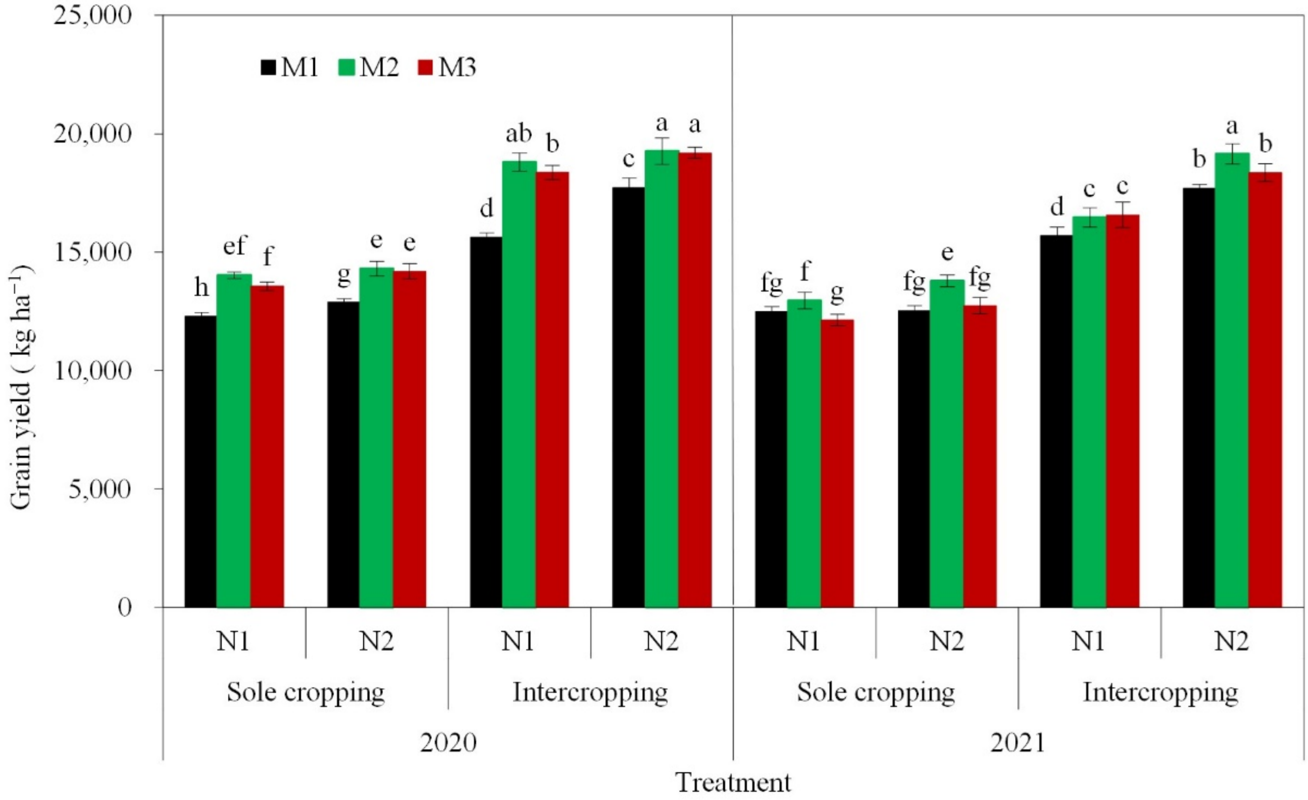

Figure 2. Grain yield of maize under sole cropping and intercropping systems in 2020–2021. See Table 1 for the treatment codes. Data are presented as the mean ± standard error (n = 3). Within a year for a given figure pane, different lowercase letters indicate treatment means that were significantly different at $p < 0.05$ according to Duncan's multiple-range test.

3.3. Dense Planting Affected Nitrogen Status of Intercropped Maize with Nitrogen Reduction

Figure 3 demonstrated that the NNI was higher than 1.0 from 45 to 90 DAE and was less than 1.0 after 105 DAE. At 45 DAE, the NNI of the intercropped maize was decreased by 11.2% (13.0% compared to sole maize), but from 105 to 145 DAE, the NNI of the intercropped maize increased by 8.2–9.7% and 7.7–12.7% compared to the sole maize in 2020 and 2021, respectively. Nitrogen reduction led to a 4.4% and 3.6% drop in the average NNI in 2020 and 2021, respectively. A high planting density caused a 3.7% and 5.5% decline in the average NNI in 2020 and 2021, respectively. After integrating these factors, SN2M1 and SN1M3 reached the maximum and minimum of the averaged NNI. Compared to the traditional treatment (SN2M1), IN1M2 lowered the NNI before 90 DAE but improved it after 105 DAE, which corresponded to the maize's grain-filling stage.

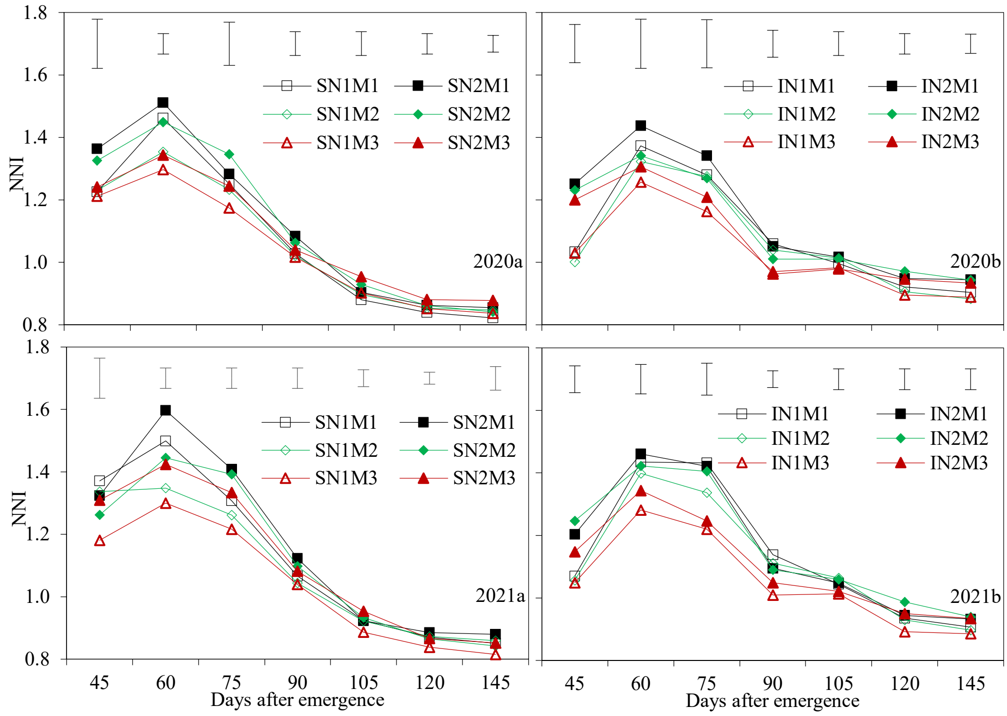

Figure 3. Nitrogen nutrition index (NNI) of maize under sole cropping system in 2020a and 2021a and intercropping system in 2020b and 2021b. NII was determined at 45, 60, 75, 90, 105, 120, and 145 DAE in the maize-growing season. The vertical bars above the curve are standard errors. The length of vertical bars represents the magnitude of the least significant difference (LSD) at p = 0.05 among treatments within a measurement time (n = 3). Treatment abbreviations are described in Table 1.

3.4. Dense Planting Optimized Chlorophyll Content and Photosynthetic Gas-Exchange Characteristics of Intercropped Maize with Nitrogen Reduction

3.4.1. Chlorophyll Relative Content (SPAD)

The chlorophyll relative content (SPAD) of the maize increased before 90 DAE and then decreased until the maize harvest (Figure 4). Intercropping firstly declined the SPAD of the maize at 60 and 75 DAE but then improved it at 105d and 120 DAE compared with sole maize. The SPAD under N1 decreased by 3.2–8.1% and 3.8–7.6% compared with N2 in 2020 and 2021, respectively, except at 45 and 90 DAE in 2020. Dense planting had a negative influence on the SPAD; M2 and M1 had no significant difference in the SPAD before 105 DAE, but M2 decreased the SPAD by 3.0–3.8% and 3.2–9.6% compared to M1 at 105 and 120 DAE, respectively; and M3 lowered the SPAD by 3.6–7.2% and 3.3–13.4%, respectively, compared to M1 after 75 DAE. N1 application significantly lowered the SPAD value of the intercropped maize compared to N2 at the late-filling stage of the maize. Dense planting had less of an effect on the SPAD in the intercropped maize; significant decreases in the SPAD caused by dense planting were only observed at 105 and 120 DAE in 2020 and 105 DAE in 2021. integrating the three factors of nitrogen reduction, dense planting, and intercropping (IN1M2) showed a disadvantage in the SPAD value in the ear-leaf for the maize at 60 and 75 DAE compared to the traditional treatment (SN2M1), but caught up with it at 90 DAE.

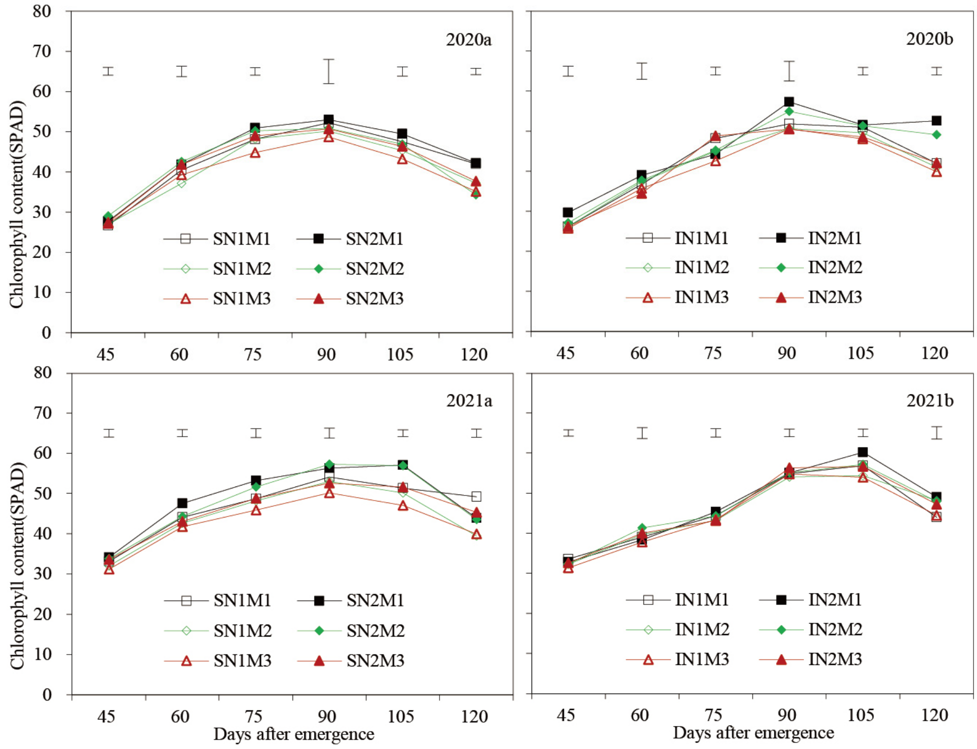

Figure 4. Chlorophyll content (SPAD) of ear-leaf for maize under sole cropping system in 2020a and 2021a and intercropping system in 2020b and 2021b. The SPAD value was sampled at 45, 60, 75, 90, 105, and 120 DAE in the maize-growing season. The vertical bars above the curve are standard errors. The length of vertical bars represents the magnitude of the least significant difference (LSD) at $p = 0.05$ among treatments within a measurement time ($n = 3$). Treatment abbreviations are described in Table 1.

3.4.2. Net Photosynthetic Rate (Pn)

The net photosynthetic rate (Pn) reached a maximum at 60–75 DAE and then gradually decreased (Figure 5). Intercropping significantly reduced the Pn value by 6.8% before 75 DAE in 2020 but improved the Pn value by 10.3–30.9% after 90 DAE during the grain-filling stage of the maize in 2020 and 2021 compared with sole cropping. Pn under N1 was lower than N2 during the entire growth period and was particularly significant at 105 and 120 DAE. The Pn of M2 and M3 was lower than that of M1; the Pn at 105 and 120 DAE for M3 were significantly reduced by 11.4–24.5% compared to that of M1. In the intercropping system, nitrogen reduction led to a sharp decrease in the Pn at 105 and 120 DAE, the Pn of M2 was similar to that of M1, and M3 led to a substantial decline in the Pn during 75–120 DAE in 2020 and at 105 DAE in 2021. Comprehensively, when using the three factors of cropping pattern, nitrogen-application level, and planting density, the intercropping with nitrogen reduction and a medium density (IN1M2) did not significantly affect the Pn of the maize's green leaves compared to sole cropping with traditional nitrogen and density (SN2M1) at the vegetative growth stage of maize (from 45 to 75 DAE) in 2020–2021, and IN1M2 decreased the Pn by 15.3% in 2020 but increased the Pn by 12.9% in green leaves at the reproductive growth stage of the maize (from 90 to 120 DAE). Above all, nitrogen reduction in the intercropped maize had a stronger negative effect on the Pn than that in sole maize; this negative effect of nitrogen reduction on the Pn of green leaves in the maize was weakened by moderately increasing the planting density.

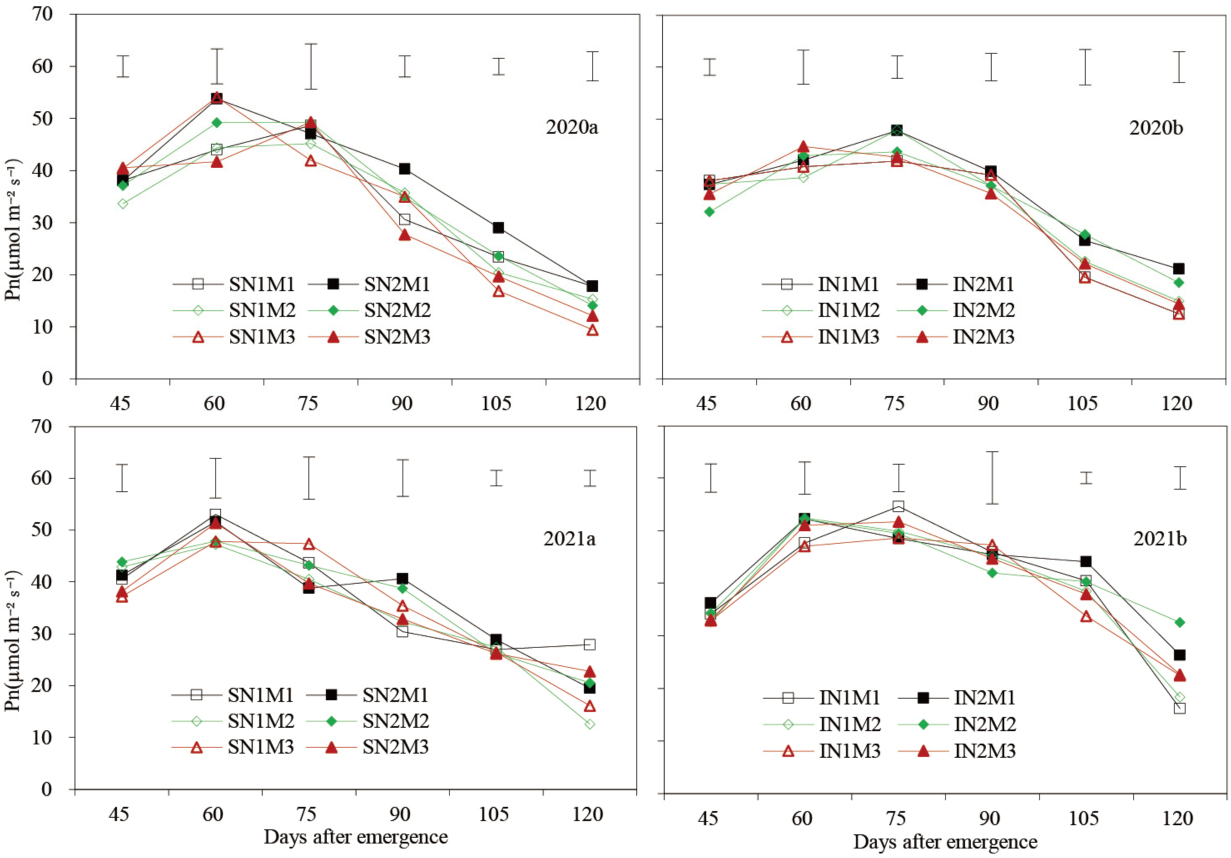

Figure 5. Net photosynthetic rate of ear-leaf for maize under sole cropping system in 2020a and 2021a and intercropping system in 2020b and 2021b. The Pn value was sampled at 45, 60, 75, 90, 105, and 120 DAE in the maize-growing season. The vertical bars above the curve are standard errors. The length of vertical bars represents the magnitude of the least significant difference (LSD) at $p = 0.05$ among treatments within a measurement time ($n = 3$). Treatment abbreviations are described in Table 1.

3.4.3. Transpiration Rate (Tr)

The transpiration rate (Tr) showed an increasing trend until 60 DAE then decreased as the growth period advanced (Figure 6). A lower Tr ($p < 0.05$) was observed at the early growth stage (45 and 60 DAE in 2020; 45–75 DAE in 2021) in intercropping compared with sole cropping, then it was higher in intercropping than in sole cropping at the late growth stage (75–120 DAE in 2020; 90–120 DAE in 2021). N1 decreased the Tr compared to N2 during the entire growth period in both years. Increasing the density reduced the Tr of the maize leaves; the average Tr across the growth period under M2 was reduced by 6.8% and 6.2% compared to M1, respectively; while that of M3 was 14.1% and 12.5% lower than M1, respectively, in both years. When synthesizing the three factors used in this study, IN1M2 significantly decreased the Tr by 17.0% compared to SN2M1 in the maize's green leaves at the vegetative growth stage of the maize in 2020, but IN1M2 increased the Tr by 8.4–46.4% in the maize green leaves at the reproductive growth stage of the maize in 2020–2021.

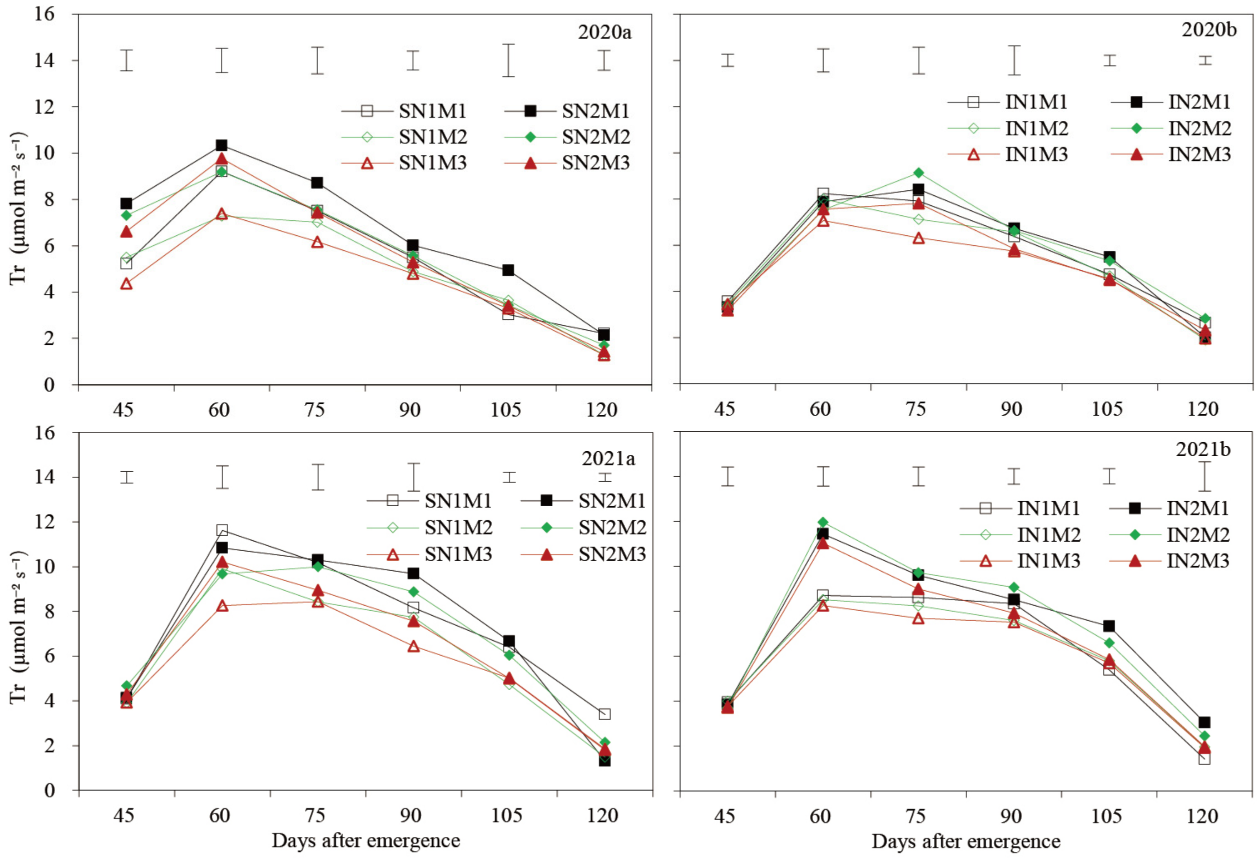

Figure 6. Transpiration rate (Tr) of ear-leaf for maize under sole cropping system in 2020a and 2021a and intercropping system in 2020b and 2021b. The Tr value was sampled at 45, 60, 75, 90, 105, and 120 DAE in the maize-growing season. The vertical bars above the curve are standard errors. The length of vertical bars represents the magnitude of the least significant difference (LSD) at $p = 0.05$ among treatments within a measurement time ($n = 3$). Treatment abbreviations are described in Table 1.

3.4.4. Stomatal Conductance (Gs)

The stomatal conductance (Gs) had a similar trend to the Tr of green leaves in the maize (Figure 7). Intercropping firstly lowered the Tr ($p < 0.05$) at the early growth stage (45 and 60 DAE in 2020; 45–75 DAE in 2021) compared with sole cropping, but then improved it at the late growth stage (75–120 DAE in 2020; 105–120 DAE in 2021). Nitrogen reduction (N1) resulted in a decreased Gs ($p < 0.05$) during the entire growth stage in both years except at 105 DAE in 2020 and 45 and 105 DAE in 2021. The average Gs value across the entire growth period of the maize was the highest for M1 and the lowest for M3 among three planting densities. IN1M2 decreased the Gs by 21.8–23.5% in maize green leaves at the vegetative growth stage of the maize in 2020–2021 but increased the Gs by 12.1% compared to SN2M1 at the reproductive growth stage in 2021. These results indicated that intercropping integrated with low nitrogen and a medium planting density could maintain a high Gs; hence, the IN1M2 treatment can be employed to enhance the efficient use of soil water under arid conditions.

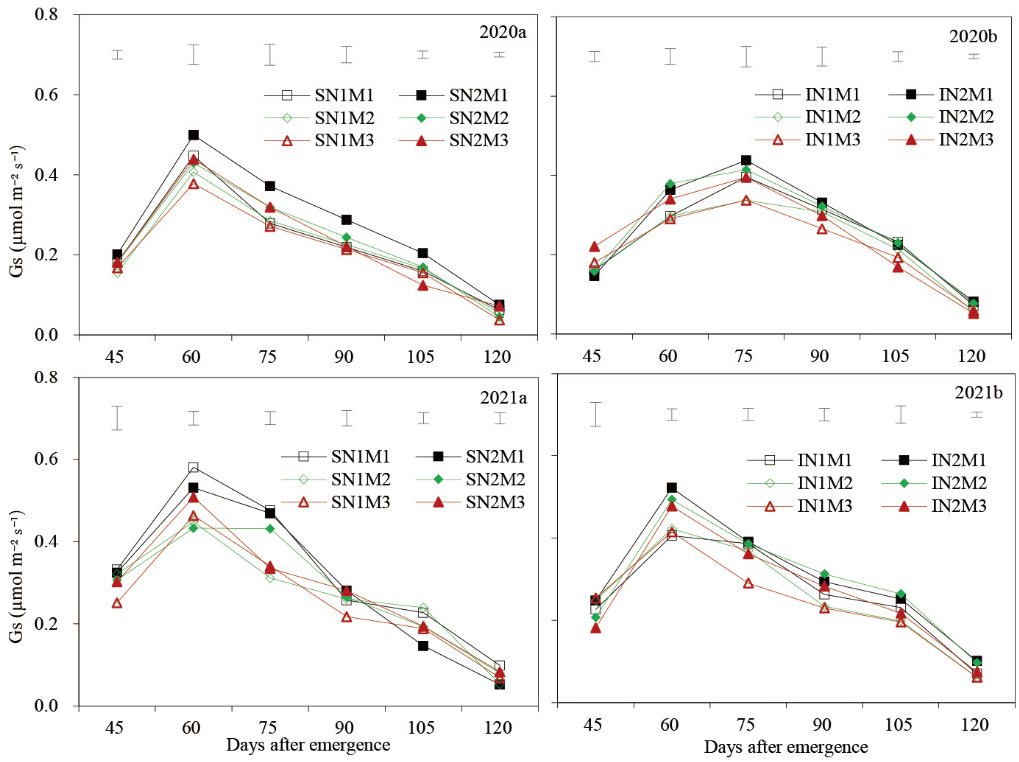

Figure 7. Stomatal conductance (Gs) of ear-leaf for maize under sole cropping system in 2020a and 2021a and intercropping system in 2020b and 2021b. The Gs value was sampled at 45, 60, 75, 90, 105, and 120 DAE in the maize-growing season. The vertical bars above the curve are standard errors. The length of vertical bars represents the magnitude of the least significant difference (LSD) at $p = 0.05$ among treatments within a measurement time ($n = 3$). Treatment abbreviations are described in Table 1.

3.5. Effects of Dense Planting and Nitrogen Reduction on Chlorophyll a Fluorescence of Intercropped Maize

3.5.1. The Effective Quantum Yield of PSII in Light: Y(II)

Table 4 demonstrates the chlorophyll a fluorescence parameter at 75, 90, and 105 DAE in 2020–2021. The effective quantum yield of PSII in light Y(II) decreased with the growth period of maize. It was significantly affected by the cropping pattern, nitrogen level, maize planting density; the interactions among these three factors varied between the two years. The Y(II) of the intercropped maize was significantly higher by 9.2–10.2% than that of sole maize in three measurements taken during 2020–2021. N1 lowered the Y(II) by 1.7–5.9% compared to N2. Y(II) also declined with an increase in the maize planting density in all the measurements taken in the two years. Compared to that under M1, the Y(II) under M2 was significantly reduced by 5.4–6.6% in all the measurements taken in 2020–2021; while the Y(II) under M3 was significantly reduced by 11.3–11.5% in the two years. When considering the integrated cropping pattern, nitrogen level, and maize planting density, the intercropping with nitrogen reduction and a medium density (IN1M2) did not significantly decrease the Y(II) compared with sole cropping with traditional nitrogen and density (SN2M1). Altogether, these results showed that planting at a rational density combined with a 25% reduction in nitrogen in the wheat–maize intercropping did not have an adverse impact on the photochemical efficiency of the PSII of the maize in an arid irrigation region.

Table 4. Chlorophyll a fluorescence parameters of ear-leaf for sole and intercropped maize ear leaves at 75, 90, and 105 DAE in 2020–2021 in an arid irrigated region.

Year	Cropping Pattern [a]	Nitrogen Level	Planting Density	Y(II) [b]			Y(NPQ)			Y(NO)		
				75 DAE	90 DAE	105 DAE	75 DAE	90 DAE	105 DAE	75 DAE	90 DAE	105 DAE
2020	Sole cropping	N1	M1	0.637cc	0.505d	0.488b	0.09cd	0.121e	0.161f	0.273de	0.373a	0.351bc
			M2	0.579ef	0.485e	0.452d	0.089cde	0.139de	0.181e	0.332b	0.376a	0.367b
			M3	0.52g	0.443f	0.354f	0.096c	0.173ab	0.246a	0.383a	0.383a	0.4a
		N2	M1	0.64c	0.558abc	0.492b	0.117b	0.137de	0.186e	0.243f	0.305d	0.322de
			M2	0.585de	0.514d	0.443d	0.134a	0.156bcd	0.221bc	0.281de	0.33c	0.336cd
			M3	0.563f	0.506d	0.461cd	0.127ab	0.167abc	0.211cd	0.31c	0.327c	0.328de
	Intercropping	N1	M1	0.64c	0.568ab	0.531a	0.075f	0.142d	0.158f	0.285d	0.289ef	0.311e
			M2	0.651c	0.549c	0.491b	0.081def	0.152cd	0.196de	0.268e	0.299de	0.313e
			M3	0.598d	0.504d	0.408e	0.078ef	0.147d	0.238ab	0.324b	0.349b	0.354b
		N2	M1	0.694a	0.573a	0.524a	0.069f	0.15cd	0.207cd	0.237f	0.277f	0.269f
			M2	0.679ab	0.573a	0.48bc	0.079def	0.15cd	0.237ab	0.242f	0.277f	0.282f
			M3	0.669b	0.555bc	0.483bc	0.094c	0.184a	0.234ab	0.237f	0.261g	0.283f
2021	Sole cropping	N1	M1	0.561cd	0.502de	0.472b	0.114cd	0.151bc	0.163e	0.326de	0.347de	0.365c
			M2	0.532ef	0.463f	0.422ef	0.112d	0.153b	0.148f	0.356bc	0.384c	0.430b
			M3	0.501gh	0.422g	0.419ef	0.13b	0.125f	0.121g	0.369b	0.453a	0.46a
		N2	M1	0.549de	0.543b	0.497a	0.129b	0.144bcde	0.158e	0.322def	0.312gh	0.345d
			M2	0.513fg	0.531bc	0.416ef	0.145a	0.140e	0.211b	0.343cd	0.329efg	0.373c
			M3	0.488h	0.446f	0.365g	0.122bc	0.127f	0.199c	0.390a	0.427b	0.435b
	Intercropping	N1	M1	0.584b	0.526bc	0.492a	0.115cd	0.169a	0.22a	0.301g	0.305h	0.288f
			M2	0.557d	0.530b	0.473b	0.116cd	0.148bcde	0.186d	0.327de	0.322fg	0.341d
			M3	0.567bcd	0.512cd	0.426e	0.114cd	0.128f	0.209b	0.319efg	0.359d	0.365c
		N2	M1	0.609a	0.581a	0.492a	0.087f	0.141de	0.19d	0.304fg	0.278i	0.318e
			M2	0.580bc	0.523bc	0.445d	0.095e	0.150bcd	0.208b	0.325de	0.327fg	0.347d
			M3	0.563cd	0.519cd	0.459c	0.129b	0.142cde	0.193cd	0.311efg	0.339ef	0.348d

[a] Treatment abbreviations are described in Table 1. [b] Y(II), the effective quantum yield of PSII; Y(NPQ), the quantum yield of regulated non-photochemical energy dissipation; Y(NO), the quantum yield of non-regulated non-photochemical energy dissipation. Data are presented as mean ± standard error (n = 3). Within a column for a given year, means followed by different lowercase letters were significantly different at $p < 0.05$ according to Duncan's multiple-range test. Analysis of variance is presented in Table 2.

3.5.2. The Quantum Yield of Regulated Non-Photochemical Energy Dissipation of PSII: Y(NPQ)

The quantum yield of regulated non-photochemical energy dissipation (Y(NPQ)) increased with the growth period of the maize. The cropping pattern, nitrogen level, and maize planting density had a significant effect on the Y(NPQ) at 75 and 105 DAE, and the interaction of two or three factors varied between the two years (Table 2). The Y(NPQ) of intercropped maize was 12.7–27.1% lower than that of the sole maize at 75 DAE, but that of intercropped maize was 5.3–20.6% greater than for sole maize at 105 DAE in both years. Nitrogen reduction resulted in a decreased Y(NPQ) in 2020; it was decreased by 7.4–17.9% at three determining stages but was only decreased by 9.7% at 105 DAE in 2021. The Y(NPQ) was increased with an increase in the planting density: the Y(NPQ) under M2 and M3 was increased by 8.4–17.4% and 12.6–30.5%, respectively, compared to M1 in 2020, while M3 increased the Y(NPQ) by 11.2% at 75 DAE and decreased it by 13.7% at 90 DAE in 2021 compared to M1, but no significant differences were found between M2 and M3 in the two years. IN1M2 decreased the Y(NPQ) by 10.1–31.1% at 75 DAE but increased the Y(NPQ) by 2.8–10.9% and 5.4–17.7% at 90 and 105 DAE, respectively, compared to SN2M1. These results indicated that intercropping integrated with low nitrogen and a medium planting density could maintain a high Y(NPQ); hence, the IN1M2 treatment can be used to enhance the photoprotection of PSII of the leaves of maize cultivated under arid conditions.

3.5.3. The Quantum Yield of Non-Regulated Non-Photochemical Energy Dissipation: Y(NO)

The quantum yield of non-regulatory non-photochemical energy dissipation (Y(NO)) increased over the growth period of the maize. It was significantly affected by the cropping pattern, nitrogen level, and maize planting density, but the interaction of two or three factors was not significant (Table 2). The Y(NO) of the intercropped maize was lower than that of the sole maize by 13.9–14.3% at three determining stages in 2020–2021. A 25% reduction in nitrogen increased the Y(NO) by 12.6–16.3% in 2020 compared to the traditional nitrogen level, but was not significant in 2021. The Y(NO) was augmented with an increase in the maize planting density in all of the measurements in the two years. The Y(NO) under a medium density (M2) was significantly raised by 7.8–13.3% compared to the traditional density (M1) in 2021, but there was no significant difference in 2020; that of a high density (M3) was significantly raised by 11.4–20.0% compared to the traditional density (M1) in the two years. When synthesizing the three factors used in this study, there were no significant differences between the IN1M2 and SN2M1 treatments. These results showed that planting at a rational density combined with a 25% reduction in nitrogen in the wheat–maize intercropping did not have a significant effect on the photodamage to PSII in the leaves of maize cultivated under arid conditions.

3.6. Relationships between Grain Yield and Photosynthetic Physiological Parameters of Maize

3.6.1. Grey Relation Analysis (GRA) between Photosynthetic Characteristics and Yield

A grey relation analysis (GRA) was applied to assess the degree of importance of each photosynthetic parameter to the grain yield of the maize (Table 5). The GRA values ranged from 0.523 to 0.678. The main factors that affected the grain yield of the maize were the photosynthetic physiological indexes; the effect degree decreased in the order of Pn, Tr, SPAD, and chlorophyll a fluorescence parameters; and the effect degree also decreased in the order of Y(II), Y(NPQ), and Y(NO). The results showed that the Pn and Tr of the individual maize played the most significant roles in group productivity under the condition of dense planting.

Table 5. The incidence matrix among grain yield and photosynthetic characteristics as well as rankings for maize in arid irrigated regions.

Degree of Association	SPAD	Pn	Gs	Tr	Y(II)	Y(NPQ)	Y(NO)
Grain yield	0.619	0.678	0.592	0.627	0.601	0.563	0.523
Comprehensive ranking	3	1	5	2	4	6	7

SPAD, chlorophyll content; Pn, net photosynthetic rate; Gs, stomatal conductance; Tr, transpiration rate; Y(II), the effective quantum yield of PSII; Y(NPQ), the quantum yield of regulated non-photochemical energy dissipation; Y(NO), the quantum yield of non-regulated non-photochemical energy dissipation.

3.6.2. Correlation Analysis between Photosynthetic Characteristics

The correlation analysis demonstrated that the SPAD showed a positive correlation with Pn, Gs, Tr, and Y(II); and a negative correlation with Y(NO) (Table 6). The Pn had a positive correlation with the Y(II) and a negative correlation with the Y(NO). The Gs and Tr were positively correlated. The Y(II) was positively correlated with the Y(NPQ) but negatively correlated with the Y(NO). The results showed that the function and content of chlorophyll was the key factor in raising leaf photosynthesis in this study because under the condition of dense planting, light absorption was the essential process in the photosynthesis, which was conducted in the chlorophyll. In this study, dense planting in an intercropping system alleviated leaf shading and benefited the formation of chlorophyll, thus improving the maize's photosynthesis and yield.

Table 6. Correlation coefficients between photosynthetic characteristics of ear-leaf for maize in an oasis region of northwestern China.

Parameter	SPAD	Pn	Gs	Tr	Y(II)	Y(NPQ)	Y(NO)
SPAD	1	0.744 **	0.847 **	0.868 **	0.614 *	−0.303	−0.735 **
Pn		1	0.447	0.426	0.689 *	−0.452	−0.703 *
Gs			1	0.936 **	0.427	−0.219	−0.498
Tr				1	0.326	−0.109	−0.446
Y(II)					1	−0.847 **	−0.770 **
Y(NPQ)						1	0.313
Y(NO)							1

SPAD, relative chlorophyll content; Pn, net photosynthetic rate; Gs, stomatal conductance; Tr, transpiration rate; Y(II), the effective quantum yield of PSII; Y(NPQ), the quantum yield of regulated non-photochemical regulated energy dissipation; Y(NO), the quantum yield of non-regulated non-photochemical non-regulated energy dissipation. The values are Pearson's correlation coefficients. * Significant difference at the 0.05 probability level; ** significant difference at the 0.01 probability level.

4. Discussion

4.1. Effects of Dense Planting and Nitrogen Reduction on Dry-Matter Accumulation and Grain Yield of Intercropped Maize

Dry-matter accumulation is the basis for forming a grain yield [32]. Dense planting and nitrogen application are both important agronomic practices that improve the dry-matter accumulation and yield [33,34]. This study explored the response of the dry-matter accumulation and grain yield of maize to dense planting under nitrogen reduction in an intercropping system. Intercropping significantly improved the dry-matter accumulation at maturity, whereas it decreased the dry-matter accumulation of the maize during the co-growth period of the maize and wheat, which was consistent with previous studies [9,10]. This phenomenon was attributed to interspecific competition and complementarity [9,35]. Reduced nitrogen causes a reduction in the dry-matter accumulation, especially in intercropping systems [36–38]. In this study, a 25% nitrogen reduction caused a decrease in the dry-matter accumulation of the maize both during the co-growth period of the wheat and maize and after the wheat harvest. Previous studies have shown that the total dry-matter production increased linearly with an increased plant density or followed a logarithmic relationship [33,34]. Our study demonstrated that dense planting in an intercropping system could compensate for the reduction in the dry-matter accumulation caused by a nitrogen reduction. Under the nitrogen-reduction condition, a medium density of intercropped maize (IN1M2) increased the dry-matter accumulation at maturity by 37.2–40.4% compared with sole maize under traditional nitrogen and density (SN2M1); the increase for a high density was 50.0–58.3% (IN1M3). Dry-matter accumulation of the maize occurred through photosynthetic production, which depended heavily on the leaf canopy characteristics, while dense planting increased maize's leaf area and light interception to improve the maize's productivity [39,40]. Intercropping has the potential to accommodate larger groups and obtain a higher biomass yield and grain yield; examples include pigeon pea–sorghum intercropping and cactus–sorghum intercropping [41–43]. The spatial and temporal arrangement of two crops with different growth habits on neighboring strips enhanced the efficient use of resources so that intercropping could support a larger population, thus increasing the dry-matter accumulation and grain yield [44,45]. This was the basis of the idea that nitrogen reduction and dense planting in intercropping systems could coordinate population and individual development to achieve a better dry-matter accumulation.

Intercropping could boost grain yield, particularly in maize-based intercropping systems [46,47]. Previous studies showed that intercropping of legumes and cereals enabled major increases in land productivity with less fertilizer nitrogen use [48]. However, nitrogen reduction decreased the grain yield in cereal–cereal intercropping [37,38]. In this study, nitrogen reduction resulted in varying degrees of yield reduction; the grain yield for different densities followed an order of medium density > high density > traditional density, especially in the intercropping system. Similarly, dense planting improved the grain yield of sorghum intercropped with pigeon pea and cactus [41–43]. Therefore, in an appropriate range of densities, the grain yield was increased with an increase in density, which was mainly caused by maximizing the radiation interception during the early growth period of the crops [49]. However, an overcrowded plant population intensified intraspecific competition, deteriorated the canopy environment, and caused a drop in the grain yield per plant due to the impaired individual photosynthetic capacities [40,50,51]. This explains why a high planting density did not improve the grain yield in this study [52]. In our study, in the condition of reduced nitrogen, the grain yield of the intercropped maize under a medium planting density exhibited the strongest compensation effect on the grain yield. This was because intercropping boosted the light interception and nitrogen use efficiency, alleviated the degradation of the canopy environment, and increased the light captured by the below canopy [37,53].

4.2. The Photosynthetic Characteristics of Intercropped Maize with Dense Planting and Nitrogen Reduction

The improved leaf photosynthesis could increase the yield under the condition that other factors were kept constant [54]. Our research indicated that nitrogen reduction and a high density led to a yield loss due to restricted photosynthesis characteristics including SPAD, Pn, Gs, and Tr, which was consistent with the results of previous studies [55–57]; while intercropping significantly alleviated this negative effect of nitrogen reduction and dense planting after the wheat harvest. This was mainly because a significant portion of the nitrogen in the leaves was linked to chloroplasts and the actions of the photosynthetic enzymes, and nitrogen concentration in the leaves and photosynthesis were correlated linearly [55,58]. Nitrogen deficiency was characterized by reduced size of chloroplasts, eminent osmiophilic globules, and large grana stacks [59]. In addition to nitrogen reduction, dense planting also inhibited plants' photosynthesis and the synthesis of chlorophyll by shaping the light distribution and intensifying the shading of the crop canopy, which directly caused a decline in the leaf chlorophyll content and net photosynthetic rate [26,50]. This adverse light condition was also harmful to the chloroplast morphology and the ultrastructure of leaves [26,60] and led to the damaged membrane systems and mitochondria, which were primarily responsible for the decrease in the photosynthetic capacity [61,62].

In our study, intercropping firstly decreased the SPAD value and gas-exchange parameters of the intercropped maize compared with the sole maize during the co-growth period of the wheat and maize. This weak performance of the intercropped maize was mainly due to the wheat's more developed root system and stronger ability to compete for water and nutrients [44,63]. Nevertheless, intercropping alleviated the negative effect of nitrogen reduction and dense planting on the maize's photosynthetic characteristics after the wheat harvest due to the stronger light intensity and higher resource availability provided by intercropping [35]. It is known that leaves grown in high irradiance show an increased leaf nitrogen content and photosynthetic capacity compared with shadowed leaves [14,64]. Intercropping increased the maize canopy transmittance and improved the light environment of the middle and lower canopy, which was beneficial to the photosynthetic characteristics [16]. Thus, we speculated that intercropping could enhance the adaptability of maize to reduced nitrogen levels and dense planting, and this view was also reflected in the results of this study.

4.3. The Regulation Effects of Dense Planting and Nitrogen Reduction on Chlorophyll a Fluorescence of Intercropped Maize

Chlorophyll fluorescence is a non-invasive measurement of photosystem II activity that reflects a plant's response to environmental change. It allows the separation of the components that comprise all of the energy dissipation of chlorophyll in the thylakoid membrane [30]. The Y(II) gives the proportion of absorbed light that is used in PSII photochemistry [30]. Some studies have shown that a nitrogen deficiency decreased the quantum yield of PSII electron transport and the maximal efficiency of PSII photochemistry, suggesting that nitrogen deficiency induced some damage to PSII [65,66]. The low-light environment at a high planting density caused a decline in the electron transport rate and the formation of assimilatory power; this low-light stress could injure the photosynthetic center [26]. Our results indicated that intercropping increased the Y(II) but nitrogen reduction and dense planting decreased it, which was consistent with previous studies [26,67]. Meanwhile, the Y(II) of the intercropped maize under nitrogen reduction and a medium density (IN1M2) was similar to that of the traditional nitrogen supply and planting density of the sole maize (SN2M1). This result suggested that a 25% nitrogen reduction and a medium density in intercropping did not influence the proportion of absorbed light of the maize leaves.

Fluorescence quenching consists of non-photochemical quenching (Y(NPQ)) and non-regulated quenching (Y(NO)) [30]. The Y(NPQ) reveals the photoprotective process that removes excess excitation energy within chlorophyll-containing complexes and prevents the likelihood of the formation of damaging free radicals [68]. In this study, nitrogen reduction decreased the Y(NPQ), which was consistent with a previous study [69]. The Y(NPQ) of the intercropped maize under nitrogen reduction and a medium density (IN1M2) was lower than that of the traditional nitrogen supply and planting density of sole maize (SN2M1) during the co-growth period of the wheat and maize, but they showed no significant differences after the wheat harvest (90 and 105 DAE). This phenomenon was in line with a previous study that indicated that the photosynthesis of intercropped maize was inhibited during the co-growth period but then showed growth recovery after the wheat harvest [70].

The Y(NO) is an indicator of photosynthetic system damage [68]. Our results showed that intercropping decreased the Y(NO) but that nitrogen reduction and dense planting increased it. The Y(NO) of the intercropped maize under nitrogen reduction and a medium density (IN1M2) was similar to that of the traditional nitrogen supply and planting density of sole maize (SN2M1). This may have been related to the shading environment in the sole cropping and dense planting conditions, which was harmful to the photosynthetic performance and the mesophyll cell ultrastructure [71]. A lack of nitrogen caused chloroplast degradation and a decrease in the activity of the photosynthetic enzyme [55,58], so it decreased the Y(NPQ) and increased the Y(NO) of leaves in the crops. On one hand, intercropping improved the light environment to avoid low-light stress on PSII [26]; on the other hand, it enhanced nitrogen absorption by the roots [44]. In this way, intercropping weakened the changes in Y(NPQ) and Y(NO) of the maize leaves with nitrogen reduction and a high density, which was also favorable to the growth and photosynthetic abilities of the leaves and contributed to the high yield of the maize.

4.4. Effects of Dense Planting and Nitrogen Reduction on the Intercropped Maize's Nitrogen Status

A nitrogen-status diagnosis is essential for precise nitrogen management. The cropping pattern, planting density, water conditions, and other factors could influence the nitrogen status of plants [72–74]. The nitrogen nutrition index(NNI) is a classical and practical tool used to study the associated with nitrogen uptake at the canopy level [29,30]. In our study, the NNI was higher than 1.0 before 90 DAE and lower than 1.0 after 105 DAE, which reflected the nitrogen deficiency in the late growth period and indicated a lack of nitrogen in the maize crops. Intercropping decreased the NNI of the maize before 90 DAE but boosted it after 105 DAE. This was due to the strong competition for nutrition by the wheat during the co-growth period and the absorption of residual nitrogen from the wheat strip after the wheat harvest [44]. Nitrogen reduction and a high planting density lowered the NNI, particularly in the intercropping system, due to an imbalance between the nitrogen supply and the absorption, which was in accordance with previous studies [38,72]. Therefore, integrated nitrogen reduction and dense planting in an intercropping system (IN1M2) could relieve the nitrogen deficiency during late growth period compared with the traditional treatment (SN2M1). An improved nitrogen status is tightly connected to the canopy's photosynthetic physiology [18], so IN1M2 had an advantage in gas exchange and light energy distribution compared with the traditional treatment (SN2M1).

5. Conclusions

Nitrogen reduction significantly decreased the dry-matter accumulation/grain yield, relative chlorophyll content (SPAD), net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) of the maize. Dense planting compensated for the decrease in grain yield caused by nitrogen reduction but also impeded the photosynthesis of the individual maize. However, intercropping alleviated the negative effects of nitrogen reduction and dense planting on the photosynthesis and improved the grain yield. Combining a 25% nitrogen reduction (270 kg ha^{-1}) and a medium planting density (10,400 plant ha^{-1}) in intercropping (IN1M2) boosted the grain yield of the maize by 31.3–45.9% compared with that of the sole maize under traditional nitrogen and density (SN2M1). This yield advantage was derived from the greater population size and stable individual photosynthesis capacity. The SPAD, Pn, and Tr of the ear-leaves of the maize under IN1M2 were similar or superior to those of SN2M1 at the reproductive growth stage. Moreover, IN1M2 increased the quantum yield of regulated non-photochemical energy dissipation (Y(NPQ)), did not significantly decrease the effective quantum yield of PSII in light (Y(II)), and did not significantly increase the quantum yield of non-regulated non-photochemical energy dissipation (Y(NO)) of the maize compared to SN2M1. In addition, IN1M2 improved the nitrogen status of the maize at the grain-filling stage. Consequently, IN1M2 resulted in a significant increase in the grain yield and a stable photosynthetic performance of the maize, so we can suggest it as a practical agronomic technique for maize production in arid irrigated regions.

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