

Article

Effects of Nitrogen Fertilizer and Planting Density on Growth, Nutrient Characteristics, and Chlorophyll Fluorescence in Silage Maize

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Abstract: The optimal combination of the nitrogen fertilizer application and planting density with reference to the silage maize yield and quality remains unclear. We hypothesized that increasing both would increase yields following the law of diminishing returns. Yayu26, a silage maize cultivar, was used in a split-plot experiment to investigate the effects of nitrogen fertilizer and planting density on growth, nutrient characteristics, and chlorophyll fluorescence. The main plots were assigned to three planting densities: 60,000 (A1), 75,000 (A2), and 90,000 (A3) plants hm^{-2} , and the subplots were assigned to four nitrogen fertilizer rates: 0 (B1), 120 (B2), 240 (B3), and 360 (B4) kg hm^{-2} . The results showed that increasing the nitrogen application rate and planting density both enhanced silage maize yield. Nitrogen accumulation and agronomic use efficiency peaked at a planting density of 75,000 hm^{-2} . Structural equation modeling showed that the nitrogen application rate and planting density affected nitrogen accumulation and nutrient properties by influencing chlorophyll fluorescence parameters and nitrogen agronomic efficiency, ultimately resulting in a positive effect on the yield. The A3 $\times$ B2 treatments exhibited higher nitrogen accumulation, potentially compensating for any deficiencies in the dry-matter yield. Therefore, the A3 $\times$ B2 treatment was evaluated as the optimal treatment to achieve sustainable and economically feasible silage maize production.

Keywords: silage maize; nitrogen fertilizer; planting density; yield; forage quality



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1. Introduction

Silage maize (*Zea mays* L.) is a protein- and energy-rich forage crop that is commonly used in animal feed. As living standards continuously improve, the demand for high-quality protein increases. Increased production of silage maize would be well suited to meet this need. In addition, the roughage it supplies could be used to further develop the animal husbandry industry [1,2]. Although it is cultivated on a relatively small scale in China, its production has surged since 2015, prompted by Chinese policies promoting animal feed production. Supported by favorable policies and financial incentives, the planting acreage reached 2.33 million hm^2 by 2022.

The forage yield and quality of silage maize are influenced by several factors, including environmental conditions, cultural practices, and seed genetics [3]. Cultivation-specific factors, such as cultivation density and nitrogen fertilizer application, play important roles in determining the silage maize yield and quality [4]. Previous studies have shown that the individual silage maize plant biomass decreases with increasing planting density [5]. However, an increase in nitrogen fertilizer doses can significantly increase the yield while

decreasing the crude protein content [3] (although the effect on the crude protein content is not consistent across the literature). Optimizing the combination of the planting density and nitrogen application rate can enhance fertilizer absorption and utilization by silage maize, thereby promoting yield and quality.

Nitrogen is an essential element for plant growth, with silage maize exhibiting extreme sensitivity to nitrogen fertilizer stimulation, contributing approximately 45% to the unit yield of maize [6]. Other studies have shown that nitrogen application leads to significant enhancements in the plant growth, crude protein content, soil–plant analysis development (SPAD) value, and fresh and dry biomass of silage maize [7]. Although nitrogen can also enhance photosynthetic efficiency [8], its use efficiency remains relatively low. Despite increased nitrogen fertilizer application in maize fields in recent years, the nitrogen fertilizer use rate has dropped to 40–60% [9]. Excessive nitrogen application not only adversely affects the silage maize yield and quality but also leads to significant residual soil nitrogen pollution [10]. Therefore, exploring a rational strategy of nitrogen fertilizer application can boost the silage maize yield and quality while effectively utilizing nitrogen fertilizer to mitigate resource waste and soil pollution.

Planting density is another factor that significantly influences the yield of crops. Studies have shown that lower planting densities enhance the individual growth performance of plants [5]. Increasing the planting density up to a certain threshold can enhance the land utilization rate. However, it also triggers competition among silage maize populations, reducing the leaf photosynthetic efficiency. On the whole, as planting densities are increased from low levels, yields improve until the optimum density is reached, beyond which yields begin to decline [11]. The decrease in the feed quality because of high-density planting primarily stems from canopy closure, which affects substance absorption and transport. Consequently, it hampers the accumulation and growth of digestible components in maize straw and grains. The optimal planting density for silage maize is influenced by several factors, including temperature differences, soil properties, fertility levels, variety selection, and moisture content.

To better understand the combined effect of nitrogen fertilizer application rates and planting densities on silage maize, a split-plot experiment was conducted at the Huangjiapu experimental station near Taoyuan County, Changde City, Hunan Province, China. The objectives of this study were to (i) clarify the growth, nutritional quality, and chlorophyll fluorescence parameters of silage maize with different nitrogen fertilizer and planting density treatments and (ii) assess the correlations among the yield and growth, nutritional quality, and chlorophyll fluorescence parameters of silage maize.

We hypothesized that (i) increases in nitrogen fertilizer and planting density would have a cumulative effect on the silage maize yield; (ii) nitrogen fertilizer and planting density would increase the silage maize yield following the law of diminishing returns; and (iii) the growth, nutritional quality, and chlorophyll fluorescence parameters would jointly affect the yield of the silage maize. Testing these hypotheses is useful for understanding the growth regulation mechanism of silage maize, as well as analyzing the pathways through which nitrogen fertilizer and planting density regulate its yield.

2. Materials and Methods

2.1. Experimental Materials

Yayu26—a common silage maize cultivar, known for its high field yield and silage quality, that is cultivated locally—was grown in field trials at the Huangjiapu experimental station (29°101′ N, 111°309′ E) in Taoyuan County, Changde City, Hunan Province, China, from June to September 2019. The experimental sites featured a subtropical monsoon climate. The meteorological data during the growing seasons of the silage maize in 2019 are presented in Figure S1. The soil type at the site was laterite, with a pH of 6.1, and the 0–15 cm soil layer contained 32.8 g/kg of soil organic carbon, 172 mg/kg of available N, 11.8 mg/kg of available P, 117 mg/kg of available K, and 1.81 g/kg of total N. Silage maize seeds were obtained from Sichuan Southwest Kelian Seed Industry Co., Ltd., Chengdu City,

China. Nitrogen fertilizer (urea containing 46% N) was procured from Sichuan Lutianhua Co., Ltd., situated in Naxi District, Luzhou City, Sichuan Province, China.

2.2. Experimental Design

The experiments were conducted using a split-plot design with three replicates, totaling 36 subplots. The main plots were designated for three planting density rates: 60,000 plants hm^{-2} (A1), 75,000 plants hm^{-2} (A2), and 90,000 plants hm^{-2} (A3). Subplots were assigned four nitrogen fertilizer rates: 0 kg hm^{-2} (B1), 120 kg hm^{-2} (B2), 240 kg hm^{-2} (B3), and 360 kg hm^{-2} (B4). Each subplot measured 12 m^2 (6 m long $\times$ 2 m wide), with six rows spaced 33 cm apart. A 0.2–0.3 m wide buffer plot, dug to a depth of 30 cm, was arranged between neighboring nitrogen-fertilizer-rate plots to prevent nitrogen transport and root extension. Border rows were excluded from all the samples.

Phosphorus (calcium superphosphate) and potassium (potassium sulfate) were applied as basal fertilizers to each plot at rates of 12 kg hm^{-2} of P_2O_5 and 50 kg hm^{-2} of K_2O , respectively. Nitrogen fertilizer—at rates of 0 kg hm^{-2} (B1), 120 kg hm^{-2} (B2), 240 kg hm^{-2} (B3), and 360 kg hm^{-2} (B4)—was combined with 12 kg hm^{-2} of P_2O_5 and 50 kg hm^{-2} of K_2O and then evenly applied as basal fertilizer to the prepared land during seed sowing. Sowing was conducted on 4 June 2019. Throughout the growth season, a prophylactic regimen of fungicides, insecticides, and herbicides was employed to manage diseases, pests, and weed infestation. No significant occurrences of disease, pests, or weeds were noted, and no effects on growth were observed during the season. Harvesting took place on 15 September 2019.

2.3. Field Sampling and Laboratory Analysis

2.3.1. Measurement of the Agronomic Traits and Whole-Plant Yield

During the jointing, heading, and dough stages of silage maize, 10 representative plants were selected from each plot for measurements of plant height, stem diameter, leaf length, and leaf width using Vernier calipers or tape measures. The plant height refers to the distance from the stem (with the leaf, sheath, and spike) base to the top of the spikes. The stem diameter (at 1/2 the height of the plant) was measured with digital Vernier calipers. The leaf length and leaf width were determined by measuring the largest leaf of each representative plant with tape measures. The yield (dry matter) was determined by harvesting the upper plants within a 4 m^2 (2 m $\times$ 2 m) area centered in each experimental plot and drying them to a constant weight at 60 °C.

2.3.2. Measurement of the Leaf Chlorophyll Fluorescence Characteristics

Additionally, while assessing agronomic traits, the third functional or fully expanded leaves from the tops of 10 representative plants were surveyed for Φ_i2 (the actual photosynthetic rate), $\Phi_i\text{NO}$ (the light energy interception coefficient), and $\Phi_i\text{NPQ}$ (the non-photochemical quenching coefficient) using a MultispeQ (Photosynq Inc., East Lansing, MI, USA). Furthermore, the relative chlorophyll (RC) content was measured using a Minolta SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan). The measurement was conducted five times for each leaf, with the mean of these measurements considered as being representative for the given leaf.

2.3.3. Measurement of the Nutrient Characteristics

After assessing agronomic and photosynthetic traits during the wax-ripening period, 10 representative plants were harvested from each plot. Plant samples underwent oven-drying at 105 °C and were fully sieved through 1.00 and 0.425 mm nylon mesh sieves for quality characteristic measurements. For the plant samples, the hectoliter-weight ash content was determined using muffle furnace burning and the differential weight method. The crude fiber content was measured by employing the paradigm method (A220 semi-automatic fiber analyzer, ANKOM, Macedon, NY, USA). The ether extract (crude fat) content was determined via the Soxhlet extractor method. Furthermore, the crude protein

and total nitrogen contents were assessed using the Kjeldahl method (Kjeltec 8400, FOSS, Hilleroed, Denmark).

2.4. Data Analysis

2.4.1. Nitrogen Accumulation Amount (NAA), Nitrogen Agronomic Efficiency (NAE), and Nitrogen Use Efficiency (NEE) Analyses

NAA, NAE, and NEE were calculated using the following equations to assess the effects of the nitrogen application rate and planting density on the nitrogen uptake and utilization of the silage maize:

$$\text{NAA (kg hm}^{-2}\text{)} = \text{Plant nitrogen contraction} \times \text{Dry-matter yield} \quad (1)$$

$$\text{NAE (kg kg}^{-1}\text{)} = (\text{Dry-matter yield of the nitrogen treatment} - \text{Dry-matter yield without nitrogen application}) / \text{nitrogen application amount} \quad (2)$$

$$\text{NEE (\%)} = [(\text{Nitrogen accumulation amount of the nitrogen treatment} - \text{Nitrogen accumulation amount without nitrogen application}) / \text{nitrogen application amount}] \times 100\% \quad (3)$$

2.4.2. Statistical Analysis

Analysis of variance (ANOVA) was conducted using IBM SPSS Statistics, version 22.0 (International Business Machines Corporation, Armonk, NY, USA), to compare the differences between the variables. Each plot was considered as a basic experimental unit. All the data are presented as means $\pm$ standard deviations, as calculated from three replicated plots for each sampling. The mean values of the significant differences were separated using Duncan's multiple range test at a significance level of $p < 0.05$. Origin 2022 (OriginLab, Northampton, MA, USA) was used to draw bar graphs. In addition, a two-way ANOVA with an F-test was performed to assess the significance of the effects of the nitrogen fertilizer, planting densities, and their interactions on silage maize growth characteristics. A three-way ANOVA with an F-test was performed to assess the significance of the effects of the nitrogen fertilizer, planting densities, growth period, and their interactions on silage maize chlorophyll fluorescence parameters. Pearson's correlation analysis was mainly performed in R (version 4.1.3; <https://www.r-project.org> (accessed on 18 June 2024)). The relationships among the treatments, plant growth, nutrient properties, and chlorophyll fluorescence parameters were determined using the corrplot package (version 0.92), and the results were visualized using the ggplot2 package (version 3.5.1). Path model analysis and bootstrap simulations were performed using the IBM SPSS Amos 17.0 software.

3. Results

3.1. Yield and Nitrogen Utilization

The dry-matter yield is the most important indicator for evaluating the interaction between the fertilizer application and planting density, while NAA, NAE, and NEE reflect the utilization of the nitrogen fertilizer by the silage maize. The dry-matter yield increased with increasing planting density, but the positive effects for increasing the nitrogen application rate on the yield varied across the planting densities (Figure 1A). In the 60,000-plant·hm⁻² (A1) plots, the dry-matter yields of the nitrogen application treatments (B2, B3, and B4) did not significantly differ from that of the no-nitrogen-application treatment (B1). However, at 75,000 plants·hm⁻² (A2), the dry-matter yield increased from 17.41 to 20.92 t·hm⁻², and at 90,000 plants·hm⁻² (A3), it rose from 18.62 to 22.05 t·hm⁻² as the nitrogen fertilizer rate was increased from 0 to 360 kg hm⁻², respectively. The maximum dry-matter-yield values were observed with nitrogen fertilization at 360 kg hm⁻² (B4) and a planting density of 90,000 plants·hm⁻² (A3).

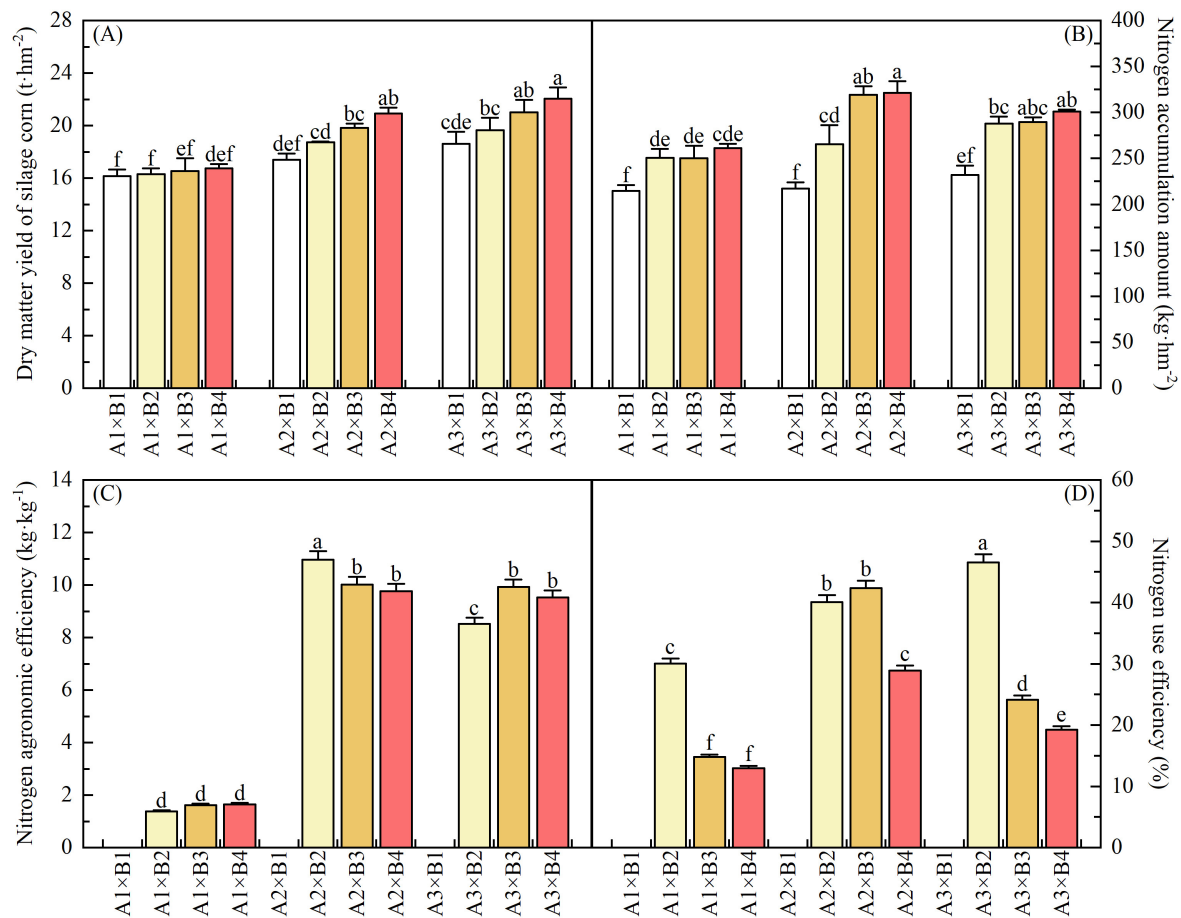


Figure 1. Dry-matter yields and nitrogen utilization efficiencies of silage maize at different nitrogen application rates and planting densities. (A) Dry-matter yield of silage corn; (B) nitrogen accumulation amount; (C) nitrogen agronomic efficiency; (D) nitrogen use efficiency. Different lowercase letters above the bars represent significant differences between treatments ($p < 0.05$).

Compared to B1, the nitrogen accumulation significantly increased with increasing nitrogen application treatment rates, showing increments, at different planting densities, of 17–22% (60,000 plants·hm⁻²), 22–48% (75,000 plants·hm⁻²), and 24–30% (90,000 plants·hm⁻²) (Figure 1B). There was no significant difference in nitrogen accumulation between nitrogen application treatments at 60,000 (A1) and 90,000 (A3) plants·hm⁻². However, the N accumulation increased with the increase in the nitrogen application rate at 75,000 plants·hm⁻² (A2), with the A2 × B4 treatment exhibiting the highest values.

NAE ranged from 1.38 to 1.65 kg kg⁻¹ in the 60,000-plant·hm⁻² (A1) plots, from 9.76 to 10.97 kg kg⁻¹ in the 75,000-plant·hm⁻² (A2) plots, and from 8.51 to 9.93 kg kg⁻¹ in the 90,000-plant·hm⁻² (A3) plots (Figure 1C). The highest NAE value was observed in the A2 × B1 treatment. There was no significant difference in NAEs between the B2 and B3 nitrogen application rates at the A2 and A3 planting densities.

NUE decreased with increasing nitrogen application rate within the same planting density treatment (Figure 1D). Furthermore, NUE significantly increased from 30 to 47% in the B2 treatment as the planting density rose from 60,000 (A1) to 90,000 (A3) plants·hm⁻². Similarly, NUE significantly increased from 15 to 42% in the B3 treatment and from 13 to 29% in the B4 treatment as the planting density was increased from 60,000 (A1) to 75,000 (A2) plants·hm⁻². However, NUE decreased from 42 to 24% in the B3 treatment and from 29 to 19% in the B4 treatment as the planting density was increased from 75,000 (A2) to 90,000 (A3) plants·hm⁻², respectively.

3.2. Growth Characteristics

The silage maize yield is a comprehensive index of growth characteristics. Testing the growth characteristics of silage maize can better illustrate the effects of the fertilizer application and planting density on the yield. The interaction between the planting density and nitrogen fertilizer use significantly influenced the silage maize growth, with the planting density exerting a significant influence on agronomic traits (Table 1). Among these, the mean values of the stem diameter, leaf length, leaf width, and flag leaf areas decreased with increasing planting density. The reductions were 8, 10, and 19%, as the planting density was increased from 60,000 (A1) to 75,000 (A2) and to 90,000 (A3) plants·hm^{−2}, respectively. The maximum values of the stem diameter, leaf length, leaf width, and flag leaf area were observed in treatments A1 × B4, A1 × B4, A1 × B3, and A1 × B3, respectively. In addition, the mean values of the number of leaves ear^{−1} increased by 9% as the planting density was increased from 60,000 (A1) to 90,000 (A3) plants·hm^{−2}. Furthermore, the maximum values of the plant height, ear length, ear diameter, and ear height were observed in treatments A2 × B3, A2 × B1, A1 × B2, and A2 × B4, respectively.

Table 1. Growth characteristics of silage maize at different nitrogen application rates and planting densities.

Treatment	Plant Height (cm)	Stem Diameter (mm)	Leaf Length (cm)	Leaf Width (cm)	Flag Leaf Areas (cm ²)	Ear Length (cm)	Ear Diameter (mm)	No. of Leaves Ear ^{−1}	Ear Height (cm)
A1 × B1	284 ab	17.6 a	63.8 abcd	8.07 ab	386 ab	25.6 ab	43.4 a	7.40 ab	130 ab
A1 × B2	273 ab	18.5 a	61.6 abcd	7.64bc	354 abc	26.9 ab	48.0 a	7.30 ab	133 a
A1 × B3	270 ab	18.4 a	65.2 abc	8.62 a	421 a	23.6 ab	47.2 a	7.02 ab	130 ab
A1 × B4	260 ab	19.2 a	68.2 a	8.16 ab	417 a	25.2 ab	44.8 a	6.63 b	112 b
A2 × B1	277 ab	17.5 a	55.9 cd	7.55 bc	317 bc	29.0 a	47.0 a	7.73 a	124 ab
A2 × B2	284 ab	17.4 a	65.9 ab	7.82 abc	386 ab	23.4 ab	47.0 a	7.18 ab	130 ab
A2 × B3	292 a	18.6 a	62.7 abcd	8.08 ab	380 ab	24.5 ab	46.2 a	7.25 ab	135 a
A2 × B4	290 a	16.7 a	63.9 abcd	7.81 abc	374 abc	22.9 ab	46.6 a	7.33 ab	137 a
A3 × B1	252 b	16.7 a	64.3 abcd	7.56 bc	365 abc	23.8 ab	47.3 a	7.82 a	132 a
A3 × B2	285 a	17.1 a	55.1 d	7.17 c	296 c	27.3 ab	44.8 a	7.65 a	123 ab
A3 × B3	279 ab	17.0 a	56.6 bcd	7.32 bc	312 bc	25.9 ab	47.6 a	7.57 a	120 ab
A3 × B4	280 ab	17.2 a	55.7 cd	7.30 bc	304 bc	21.2 b	47.9 a	7.74 a	135 a
A1	272 A	18.4 A	64.7 A	8.12 A	395 A	25.3 A	45.9 A	7.09 B	127 A
A2	286 A	17.5 AB	62.1 AB	7.82 A	365 A	24.9 A	46.7 A	7.37 AB	132 A
A3	274 A	17.0 B	57.9 B	7.34 B	320 B	24.6 A	46.9 A	7.69 A	128 A
B1	271 A	17.3 A	61.3 A	7.73 A	357 A	26.2 A	45.9 A	7.65 A	129 A
B2	281 A	17.7 A	60.9 A	7.54 A	346 A	25.8 A	46.6 A	7.37 A	129 A
B3	281 A	18.0 A	61.5 A	8.01 A	372 A	24.6 A	47.0 A	7.28 A	129 A
B4	277 A	17.7 A	62.6 A	7.76 A	366 A	23.1 A	46.4 A	7.23 A	129 A
Statistics									
A × B	ns	ns	*	ns	*	ns	ns	ns	*

Different lowercase letters represent significant differences between treatments ($p < 0.05$). Different capital letters represent significant differences between different nitrogen application rates or planting densities ($p < 0.05$). *, $p < 0.05$; ns, not significant. A × B represents the interaction between the planting density and nitrogen. A1, A2, and A3 represent 60,000, 75,000, and 90,000 plants ha^{−1}, respectively, while B1, B2, B3, and B4 represent 0, 120, 240, and 360 kg ha^{−1} nitrogen, respectively.

3.3. Nutritional Characteristics

The feed value of the silage maize can be evaluated using the ash content, crude fiber content, ether extract content, and crude protein content. The ash content of the silage maize increased with increasing nitrogen application rate, ranging from 4.24 to 5.12% in the nitrogen application (B2, B3, and B4) treatments and from 3.90 to 4.14% in the nitrogen-free (B1) treatments (Figure 2A). Among these, the ash content in the A2 × B4 treatments was the highest, at 5.12%, significantly exceeding that in the A2 × B1 treatments. However, the ash content of the silage maize at different planting densities did not show any significant differences at the same nitrogen application rates.

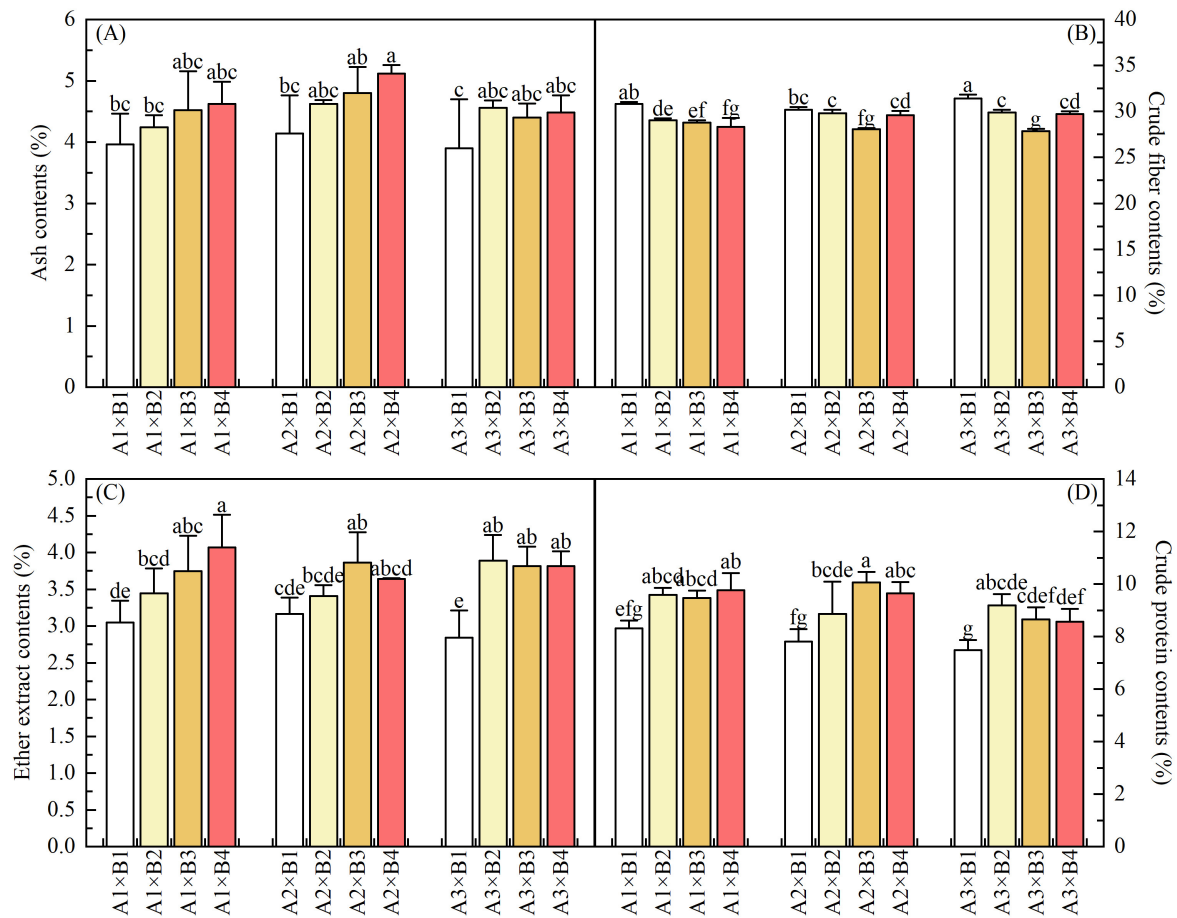


Figure 2. Nutrient properties of silage maize at different nitrogen application rates and planting densities. (A) Ash contents; (B) crude fiber contents; (C) ether extract contents; (D) crude protein contents. Different lowercase letters above the bars represent significant differences between treatments ($p < 0.05$).

Similar to the ash content, the ether extract and protein contents of the silage maize increased with increasing nitrogen application rate (Figure 2C,D). Additionally, the proportion of the ether extract in the silage maize at different planting densities did not differ significantly at the same nitrogen application rate. The ether extract content ranged from 3.41 to 4.07% in the nitrogen application treatments (B2, B3, and B4) and from 2.84 to 3.16% in the nitrogen-free treatments (B1). The ether extract proportion was significantly higher, by 22.9 and 33.3%, in the A1 × B3 and A1 × B4 treatments, respectively, than in the A1 × B1 treatment. In addition, it was 22.3%, significantly, higher in the A2 × B3 treatment than in the A2 × B1 treatment. Furthermore, at 90,000 plants·hm⁻² (A3), the ether extract proportion was 34.4–37.0% higher in nitrogen application (B2, B3, and B4) treatments than in the A3 × B1 treatment (Figure 2C). Compared to B1, the protein content in the nitrogen application (B2, B3, and B4) treatments significantly increased by 13.8–17.6% at 60,000 plants·hm⁻² (A1), 13.4–28.8% at 75,000 plants·hm⁻² (A2), and 14.4–22.7% at 90,000 plants·hm⁻² (A3) (Figure 2D). The proportion of the protein decreased with increasing planting density at a nitrogen fertilization of 360 kg hm⁻² (A4). Finally, the maximum values of the ether extract and protein proportions were observed in treatments A1 × B4 and A2 × B3, respectively.

The crude fiber content exhibited a negative response to nitrogen fertilizer rates, with the crude fiber proportion in nitrogen application (B2, B3, and B4) treatments significantly decreased by 5.76–8.16% at 60,000 plants·hm⁻² (A1) and 4.76–11.23% at 90,000 plants·hm⁻² (A3) compared to B1 (Figure 2B). Additionally, the crude fiber proportion was 7.07%,

significantly lower in the A2 × B3 treatment than in the A2 × B1 treatment. The maximum values of the crude fiber were observed in the A3 × B1 treatments.

3.4. Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence reflects the primary reaction processes of photosynthesis. Phi2 indicates the actual photosynthetic capacity of the plant. PhiNPQ reflects the ability of the plant to dissipate excess light energy to heat, which reflects the photoprotective capacity of the plant. PhiNO is an important indicator of light damage. These parameters are important references for understanding the efficiency of plant photosynthesis. The chlorophyll fluorescence parameters of the silage maize were significantly influenced by the nitrogen application rate and growth period rather than the planting density, although the interactions of the planting density, nitrogen application rate, and growth period also affected the growth characteristics of the silage maize (Table 2). Three-way ANOVA results showed that the growth period, nitrogen application rate, and their interactions significantly affected Phi2 and PhiNPQ (Table 2). The Phi2 value of the silage maize decreased with increasing growth period, with values ranging between 0.67 and 0.74 (jointing stage), 0.61 and 0.73 (heading stage), and 0.61 and 0.67 (dough stage) (Figure 3A). Higher Phi2 values were observed for the A2 treatment at the jointing stage and the A3 treatment at the heading stage, with the maximum values observed for A2 × B3 (0.74) at the jointing stage. Unlike Phi2, the PhiNPQ value of the silage maize increased with increasing growth period, with values ranging between 0.015 and 0.077 (jointing stage), 0.043 and 0.131 (heading stage), and 0.078 and 0.154 (dough stage) (Figure 3C). The highest PhiNPQ value was observed in the A3 treatment at the jointing and dough stages, with the maximum values observed in the A3 × B2 treatment at both stages.

Table 2. Chlorophyll fluorescence parameters of silage maize at different nitrogen application rates and planting densities.

	Phi2	PhiNO	PhiNPQ	Relative Chlorophyll Content
Planting density	ns	ns	ns	ns
Nitrogen fertilizer application rate	*	ns	**	**
Growth period	***	ns	***	ns
Planting density × Nitrogen fertilizer application rate	ns	***	ns	ns
Planting density × Growth period	**	**	*	ns
Nitrogen fertilizer application rate × Growth period	ns	*	ns	*
Planting density × Nitrogen fertilizer application rate × Growth period	ns	ns	ns	ns

Planting density × Nitrogen fertilizer application rate, Planting density × Growth period, Nitrogen fertilizer application rate × Growth period, and Planting density × Nitrogen fertilizer application rate × Growth period represent the interactions. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant.

Three-way ANOVA revealed that PhiNO was primarily influenced by the interaction between the planting density and nitrogen application rate (Table 2). Among these, PhiNO in A1 increased with increasing nitrogen application rate during the jointing stage, while in A1 and A2, it increased at the dough stage. Conversely, in A2 and A3 during the heading stage and A3 during the dough stage, PhiNO decreased with increasing nitrogen application rate (Figure 3B). Higher PhiNO values were observed in treatments A1 × B4, A2 × B1, and A3 × B1 at the jointing, heading, and dough stages, respectively.

Three-way ANOVA showed that the RC content was primarily influenced by the nitrogen application rate (Table 2). In the A3 treatment, the RC content increased from 56.42–60.58 to 64.73–69.48 as the nitrogen fertilizer rate was increased from 0 to 360 kg hm^{−2}, respectively (Figure 3D). In the A2 treatment, the RC content initially increased and then decreased with increasing nitrogen application rate, with the highest

value observed in the A2 × B3 treatment at all three growth stages. The RC content in the A1 treatment also increased with increasing nitrogen application rate at the two stages, indicating that increasing the nitrogen application rate was beneficial for enhancing the relative chlorophyll content of the silage maize.

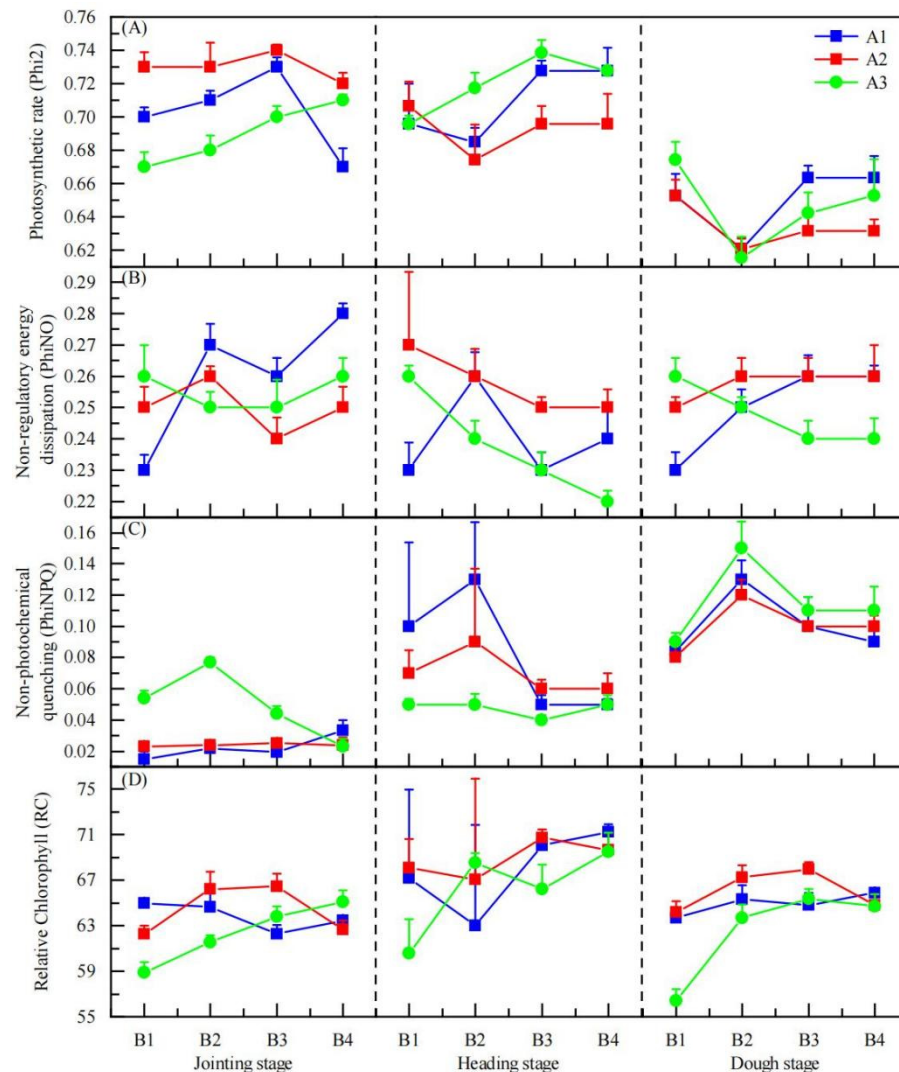


Figure 3. Chlorophyll fluorescence parameters of silage maize with three growth periods at different nitrogen application rates and planting densities. (A) Photosynthetic rate; (B) non-regulatory energy dissipation; (C) non-photochemical quenching; (D) relative chlorophyll content.

3.5. Chlorophyll Fluorescence Parameter Correlations with Silage Maize Characteristics

Pearson's correlation analysis showed significant positive correlations between the dry-matter yields and N utilization characteristics (NAA, NAE, and NRE) of the silage maize and the nitrogen application rate and planting density (Figure 4). Similarly, the nitrogen application rate exhibited significant positive correlations with the ash, ether extract, and protein contents of the silage maize ($p < 0.001$) but showed a negative correlation with the crude fiber content ($p < 0.001$). Conversely, the growth characteristics (stem diameter, leaf length, leaf width, and flag leaf area) and protein content were significantly negatively correlated with the planting density ($p < 0.05$). However, the PhiNPQ value of the silage maize exhibited a positive and significant correlation with the planting density ($p < 0.001$). The RC content showed a positive correlation with the nitrogen application rate at the heading and dough stages. Additionally, it was significantly correlated with the N utilization and nutrient characteristics of the silage maize ($p < 0.05$).

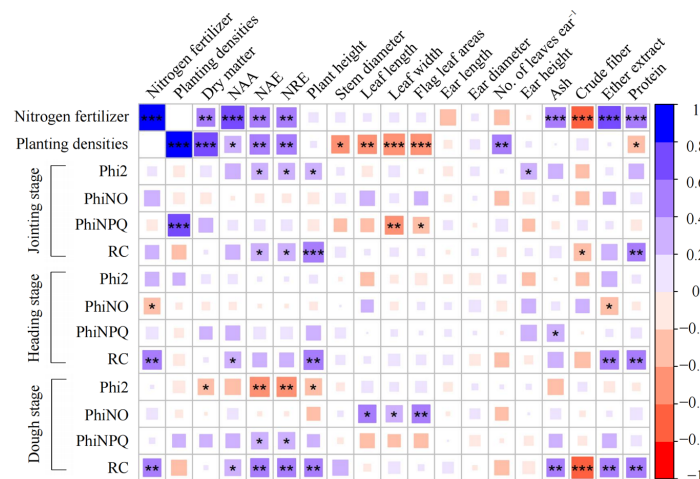


Figure 4. Heat map of correlation coefficients between chlorophyll fluorescence parameters and treatments, plant growth, and nutrient properties. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; blank cell, not significant.

3.6. Silage Maize Yield Structural Equation

The structural equation showed that the nitrogen application rate and planting density influenced the yield of the silage maize via the NAA and the ash, crude fiber, and protein contents (Figure 5). Among these, the NAA and protein content played major roles in determining the dry-matter content of the silage maize. Furthermore, the nitrogen application rate not only directly affected the NAA and the ash, crude fiber, and protein contents but also indirectly affected the NAA and the crude fiber and protein contents through the relative chlorophyll content at the dough stage. The planting density directly influenced the protein content while also indirectly influencing the NAA and crude fiber content. Overall, the nitrogen application rate and planting density of the silage maize affected the N accumulation and nutrient properties by influencing the chlorophyll fluorescence parameters and NAE, ultimately resulting in a positive effect on the yield.

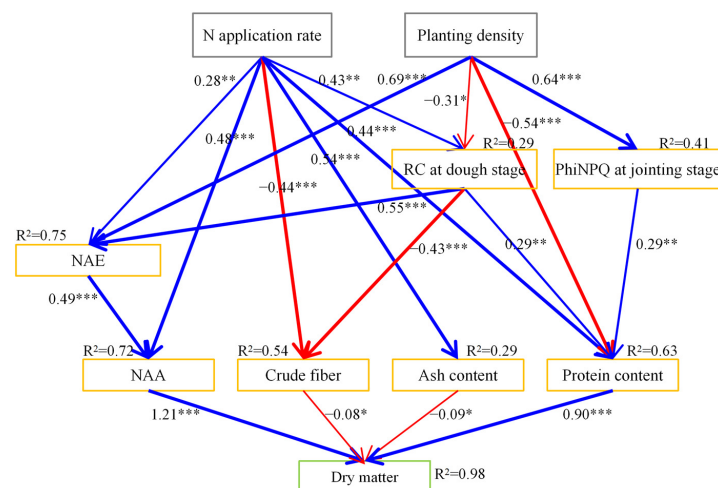


Figure 5. Structural equation model of multivariate effects on dry matter of silage maize by nitrogen application rate and planting density. Arrows indicate the hypothesized direction of causation. Blue arrows indicate positive relationships, while red arrows indicate negative relationships. The widths of the arrows represent the strength of the relationship. The numbers next to the arrows are the standardized path coefficients. Asterisks represent significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The proportion of the explained variance (R^2) appears alongside each response variable in the model. The goodness-of-fit statistics for the model were CMIN/DF = 1.173, CFI = 0.987, GFI = 0.870, NFI = 0.923, and RMSEA = 0.070.

4. Discussion

4.1. Effects of Nitrogen Application Rate and Planting Density on Silage Maize Yield

Research on nitrogen application to enhance silage maize yields has been extensively conducted [3]. Studies indicate that increasing the nitrogen fertilizer application rate significantly boosts the silage maize yield at planting densities of 60,000 and 90,000 plants·hm⁻² [4]. In this study, the silage maize yield rose with increasing nitrogen application rates at planting densities of 75,000 and 90,000 plants·hm⁻². However, nitrogen application failed to significantly increase the silage maize yield at a 60,000-plant·hm⁻² planting density. Liu et al. also reported that increasing the nitrogen fertilizer application rate at a planting density of 60,000 plants·hm⁻² did not significantly change the yield of silage corn [12]. Given the high levels of the soil organic carbon, total nitrogen, and available nitrogen in the experimental field in this study, nitrogen may not limit the growth of silage maize at a planting density of 60,000 plants·hm⁻². Hence, the maize plants at this density did not effectively utilize the applied nitrogen fertilizer [13]. The NAE results further support this finding, indicating a significantly lower NAE at the 60,000-plant·hm⁻² planting density compared to 75,000 and 90,000 plants·hm⁻² (Figure 1). A previous study has also shown that optimal planting densities were different at different nitrogen application levels [14]. Excessive nitrogen fertilizer application has no positive effect on the silage maize yield [15]. This is because when the available nitrogen content in the soil is high, excessive nitrogen fertilizer application does not significantly enhance the nitrogen accumulation and utilization of the silage maize. This observation may align with our finding that increasing the nitrogen fertilizer application rate from 240 to 360 kg hm⁻² did not significantly increase the yield, nitrogen accumulation, and nitrogen agronomic efficiency of the silage maize. Given the negative effects associated with excessive nitrogen fertilizer application, such as soil acidification, water pollution, excessive reactive nitrogen loss, and greenhouse gas emissions, which contribute significantly to global environmental pollution [16,17], applying 240 kg hm⁻² of nitrogen fertilizer may represent the optimal treatment to balance silage maize yields and environmental protection (Figure 1).

Increasing the planting density is a key cultivation measure for enhancing the yield [18,19]. However, as the planting density increases, so does competition for resources among individual plants. Excessive densities can result in canopy closure, increasing the competition between the lower and middle cluster layers and reducing the nutrient accumulation and material growth [20]. Furthermore, high-density planting reduces soil nutrient effectiveness by limiting space for root growth in the soil [21]. The correlation between the maize kernel yield and planting density exhibits a parabolic curvilinear pattern [5]. Silage maize necessitates harvesting grains and straw, thus necessitating a balance between the two to achieve the highest dry-matter yield. Our findings indicate that as the planting density was increased from 60,000 to 75,000 plants·hm⁻², the silage maize yield exhibited a consistent increase, albeit without a significant difference between 75,000 and 90,000 plants·hm⁻² (Figure 1). Qian et al. [22] similarly observed a significant increase in the silage maize yield when the planting density was increased from 65,000 to 80,000 plants·hm⁻², with no significant difference between 80,000 and 95,000 plants·hm⁻². This finding suggests that high planting densities can hamper silage maize growth, compensating for yields per hectare with a higher number of plants exhibiting lower yields per plant [23].

4.2. Effects of Nitrogen Application Rate and Planting Density on Silage Maize Nutrient Characteristics

Silage maize nutrient characteristics are classified into positive and negative quality parameters based on their feed values [24]. Among these, feed qualities with high crude protein and ether extract contents signify positive quality parameters, while the crude fiber content, negatively correlated with feed digestibility, serves as a negative quality parameter [25]. The positive and negative quality parameters of silage maize exhibit distinct responses to nitrogen fertilizer increments. Nitrogen is an important element that affects plant photosynthesis, and its deficiency adversely affects the silage nutritional

composition [26]. However, the effect of the nitrogen application rate on the protein content of silage maize is still unclear. Some studies show that increments in nitrogen fertilizer can significantly decrease the fiber content while increasing the protein accumulation [27]. But other studies indicate that increased nitrogen fertilizer levels lead to an increase in the silage maize yield while decreasing the crude protein content [28]. Our study findings demonstrate that nitrogen fertilizer application significantly increases the silage maize protein content, reduces the crude fiber content, and improves the quality of the silage maize (Figure 2). This is because the nitrogen application increased the NAA of the silage maize and promoted protein synthesis. Furthermore, the correlation between the NAA and crude protein content in the silage maize also supported this viewpoint (Figure S2). Nitrogen fertilization generally has a positive effect on the nutritional worth of silage maize. Correlation analysis further reinforces this assertion, revealing a positive association between the nitrogen application rate and crude protein and fat contents, alongside a negative correlation with the crude fiber content (Figure 4). However, varying planting densities elicited diverse responses in the silage maize protein content to nitrogen fertilizer application rates, indicating the co-regulation of the silage maize quality by nitrogen fertilizer application rates and planting densities.

The planting density stands out as the most important factor influencing the nutritional characteristics of silage maize. Studies have shown that high planting densities correlate with an increase in the crude fiber content and decreased crude protein contents in grain and straw. This results in decreased digestible components in whole-plant maize and a decrease in the feed value [29,30]. The findings of this study revealed that the silage maize protein content initially increased and then decreased with increasing planting density, peaking at 75,000 plants hm^{-2} . Given the decline in the crude fiber content, the silage maize quality also peaked at a planting density of 75,000 plants hm^{-2} . This is because as the planting density of the silage maize increases, the space per plant diminishes, and the competition for water, air, and soil nutrients increases. Consequently, the nutrient accumulation per plant increases, affecting the protein content and, consequently, the overall quality of the silage maize in particular [31]. Correlation analysis revealed a significant negative relationship between the silage maize planting density and the protein content (Figure 4). Furthermore, the negative effects of the silage maize planting density on the nutritional properties were because of the dilution effect of the dry-matter accumulation [32]. Zhao et al. [3] also observed a decline in the nutritional value of silage maize with increasing yield, attributing this effect to the negative influence of the planting density on the nutritional value.

4.3. Effects of Nitrogen Application Rate and Planting Density on Silage Maize Chlorophyll Fluorescence Parameters

Photosynthesis is the process by which plants use solar energy to fix carbon dioxide. Photochemical alterations in PSII, triggered by nitrogen deficiency, can downregulate photosynthetic electron transfer, thereby reducing carbon dioxide fixation in plants [33]. However, the jointing stage holds significance in the growth and development of silage maize [34]. The findings of this study revealed that Phi2 levels in the silage maize were higher during the jointing stage and increased with increasing nitrogen application rates between the B1 and B3 treatments (Figure 3A). However, Phi2 in the A1 and A2 treatments exhibited a declining trend when nitrogen fertilizer application rates exceeded 240 kg hm^{-2} . This is attributed to excessive nitrogen application, causing plant leaf enlargement, imbalanced canopy structure, and decreased photosynthetic rates [35]. Additionally, excessive nitrogen application resulted in premature plant leaf senescence, with Phi2 levels in silage maize at various nitrogen treatment rates being lower than those without nitrogen treatment at the dough stage (Figure 3A). Although the photosynthetic level of the silage maize in the A3 treatment was lower than those in the A1 and A2 treatments during the jointing stage, it consistently exhibited an upward trend with increasing nitrogen fertilizer application rate (Figure 3A). This trend is supported by the chlorophyll content and non-photochemical quenching coefficient of the silage maize at the regreening stage. Nitrogen applica-

tion significantly increased the RC content and decreased PhiNPQ in the A3 treatment (Figure 3B,C). This result might be attributed to the reduced nitrogen availability per maize plant at higher planting densities. Yan et al. [36] found that high-density planting results in decreased plant leaf areas, canopy transmissions, chlorophyll contents, and photosynthetic rates, which could explain the main reason why the photosynthetic rate of the silage maize treated in A3 was lower than those in the A1 and A2 treatments (Figure 3A). Additionally, the leaf width and area of the silage maize in the A3 treatment were significantly lower than those in the A1 and A2 treatments, confirming this observation (Table 1). Increasing the planting density is an essential strategy to maximize the total light interception by the plant canopy and optimize the light energy to increase the crop yield [37]. In the A2 $\times$ B3 treatments (75,000 plants hm^{-2} and 240 kg hm^{-2}), the chlorophyll content and photosynthetic rate of the silage maize reached their peak levels at the regreening stage, while the photochemical and non-photochemical quenching coefficients were low. Combined with the yield and nutritional characteristics of the silage maize, the A2 $\times$ B3 treatments emerged as the most favorable in this study.

Although the effects of nitrogen fertilizer application and planting density on the growth of silage maize are complex, in this study, we employed field experiments to analyze the nitrogen utilization, yield, nutritional quality, and chlorophyll fluorescence characteristics of silage maize. We also evaluated the optimal nitrogen fertilizer and planting density treatments, considering both economic and ecological benefits. These research findings offer a solid theoretical foundation for the cultivation of silage maize in subtropical climate regions. However, importantly, the variable climatic conditions (precipitation and light) of silage corn varieties and subtropical climate zones may limit the broader applicability of these findings. The effectiveness of management practices across different climate zones may still require validation and calibration.

5. Conclusions

Increases in the nitrogen fertilizer application rate and planting density positively affected the silage maize yield, adhering to the principle of diminishing returns, wherein the yield increment decreased with excessively high rates of nitrogen fertilizer application and planting density. Although the nutritional attributes of the silage maize positively responded to nitrogen fertilizer application, they negatively responded to planting density. The interplay between the nitrogen fertilizer application rate and planting density influenced the chlorophyll fluorescence characteristics and agronomic nitrogen use efficiency of the silage maize, consequently affecting its nutritional characteristics and yield. The objectives of silage maize production are not only to increase the crop yield but also to emphasize environmental protection and resource conservation. At a planting density of 75,000 plants hm^{-2} , the protein content of the silage maize peaked with 240 kg hm^{-2} of nitrogen fertilizer. Although this treatment did not yield the highest silage maize output, the high nitrogen accumulation effectively compensated for the lack of yield. Thus, this may be the best treatment.

Considering the broad applicability of the results of this study, it is necessary to conduct long-term studies in the future to evaluate the sustained impacts of different nitrogen application rates and planting densities on silage maize, including the effects on the soil health and microbial activity over several growing seasons. At the same time, incorporating climate conditions (precipitation and light) into the influencing factors is expected to provide broader support for the management practices of silage corn in different climate regions. The environmental impacts of different nitrogen application rates should also be assessed, particularly focusing on nitrate leaching, greenhouse gas emissions, and nitrogen efficiency. Studies that consider these factors could help to develop more sustainable silage maize production practices.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy14071352/s1>: Figure S1: Meteorological data during the growing seasons of silage maize; Figure S2: Heat map of correlation coefficients. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; blank cell, not significant.

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