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Appropriate Nitrogen Application for Alleviation of Soil Moisture-Driven Growth Inhibition of Okra (*Abelmoschus esculentus* L. (Moench))

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Abstract: Uneven rainfall, in the context of global warming, can cause soil moisture fluctuations (SMFs) that harm crop growth, and it is not yet known whether nitrogen (N) can mitigate the harm caused by a strong SMF. This paper uses okra as a test subject and sets three SMFs of 45–55% FC (W_1), 35–65% FC (W_2), and 25–75% FC (W_3) and three N applications of 0 kg hm^{-2} (N_0), 110 kg hm^{-2} (N_1), and 330 kg hm^{-2} (N_2) to investigate the effects of SMF and N application on the physiological and biochemical aspects of okra. The results demonstrated that okra exhibited the highest values in stem diameter, number of leaves, photosynthesis characteristics, antioxidant enzyme activity, and yield under the N_1 treatment. The average yield in the N_1 treatment was 149.8 g, significantly surpassing the average yields of the N_0 (129.8 g) and N_3 (84.0 g) treatments. Stomatal density, antioxidant enzyme activity, malondialdehyde content, and proline content in okra leaves were highest in the W_3 treatment, indicating that plants experienced stress in the W_3 treatment. However, the agronomic traits and yields of okra in the N_1 treatment were higher than those in the N_0 and N_1 treatments, indicating that the crop damage caused by W_3 could be mitigated by an appropriate amount of N application. The N_1W_1 treatment emerged as the most suitable combination for okra growth in this study, exhibiting the highest stem diameter, leaf count, photosynthetic characteristics, and yield (201.3 g). Notably, this yield was 67.8% higher than the lowest treatment (N2W3), signifying a significant improvement.

Keywords: okra; nitrogen; soil moisture fluctuation; physiological characteristics; yield

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1. Introduction

Okra (*Abelmoschus esculentus* L. Moench), also known as lady's finger, is an annual herb of the mallow family. It is said to be native to Africa, Northern Australia, and Southeast Asia and is produced and marketed all over the world [1,2], covering 2.5 million hectares and producing 10.5 million tons annually [3]. It has edible fruits, young leaves, blossoms, buds, and seeds [4], as well as medical properties that include the treatment of diabetes, reducing blood cholesterol levels, preventing and combating cancer, and boosting immunity [5]. With its numerous health benefits and increasing popularity as a functional food ingredient, okra shows promising potential for applications in the functional food and pharmaceutical industries [6]. However, its cultivation encounters a number of obstacles, the most significant of which is a lack of precipitation, which severely restricts its yield potential [7].

Changes in rainfall patterns are predominantly caused by global warming, with the Intergovernmental Panel on Climate Change (IPCC) noting that global average temperatures are 1.1 °C above pre-industrial records [8], leading to numerous extreme weather conditions such as floods and severe droughts [9,10], posing a substantial risk to agricultural production. Adjustments in the amount and type of extreme rainfall have a significant impact on habitats by altering their interannual water–carbon balance [11,12]. Additionally, these changes have the potential to threaten global water, food, and energy security [13,14]. However, precipitation can cause soil moisture fluctuation (SMF), a common and frequent natural process that soils encounter, leading to physical, chemical, and biological transformations from anaerobic to aerobic conditions [15]. Suralta [16] and Niones [17] have shown that SMF has a detrimental effect on the production of dry matter and harvesting.

Chaturvedi [18] subjected okra to drought stress (60% FC) at four development stages and discovered that drought stress substantially decreased the growth rate and proportion of okra biomass allocated to the nutritional and flowering stages. Barzegar [19] demonstrated that compared to 100% ETc (actual evapotranspiration rate) irrigation, both 33% and 66% ETc irrigation decreased fruit yield, relative water content, and water use efficiency while increasing antioxidant enzyme activity and proline content. Bahadur [20] determined the effects of 5-, 10-, and 15-day interval irrigation on the growth, physiology, and yield of okra. The impact of irrigating at a 10-day interval on gas exchange and okra yield was discovered to be positive. N administration under low tissue water potentially mitigates stress injury to the crop by maintaining metabolic activities [21,22]. By boosting the growth of roots, defending the photosynthetic system, activating the antioxidant defense, and enhancing osmoregulation, N supply increases crop drought resistance [23,24].

It is clear that soil moisture has a significant influence on the growth, development, and yield of okra, but at present, most of the relationships between okra and soil moisture are only studied in terms of irrigation techniques such as irrigation volume and irrigation frequency [7,18–20]. Furthermore, there have been few investigations to investigate the effects of SMF on okra growth, and it is unknown whether N can mitigate the damage caused by strong SMFs. Therefore, in this paper, we designed three SMFs and three N levels to investigate the effects of SMF on the morphological indexes, physiological response, and biological yield of okra under different N applications in a more systematic manner, which not only enriches the theory of the relationship between SMF and N in crops but also provides a scientific foundation for adapting to climate change.

2. Material and Methods

2.1. Soil Conditions of the Pot Experiments

The experimental soil was a cultivated river tide soil developed from river alluvium at the foundation of Hunan Agricultural University's Cultivation Garden. After being gathered from a depth of 0–20 cm, the soil was air-dried, pulverized, and mixed. Moreover, 500 g of the soil was taken and sieved through 2 mm and 0.149 mm sieves for the analysis of the soil's fundamental physical and chemical characteristics, while the rest of the soil was sieved through a 0.5 cm sieve and used for the potting soil experiment.

The culture pots were built of rubber buckets with a top diameter of 30 cm, a bottom diameter of 22 cm, and a height of 24 cm. Every one of them was filled with 12 kg of soil, which had a loamy clay texture. Table 1 displays the fundamental physical and chemical parameters of the soil, determined using standard methods: soil texture by the pipette method; field capacity and soil bulk density by the ring knife method; total nitrogen by the semi-micro Kjeldahl method, total phosphorus by the concentrated sulfuric acid–perchloric acid method; alkaline hydrolysis nitrogen by the alkaline diffusion method; available phosphorus by the molybdenum antimony colorimetric method; available potassium by flame photometric colorimetry; organic matter by the volumetric method with potassium dichromate; and pH value by the potentiometric method [25].

Table 1. Fundamental physical and chemical properties of soil.

Soil Texture(%)			Field Capacity (v/v%)	Soil Bulk Density (g cm^{-3})	Total Nitrogen (g kg^{-1})	Total Phosphorus (g kg^{-1})	Alkaline Hydrolysis Nitrogen (mg kg^{-1})	Available Phosphorus (mg kg^{-1})	Available Potassium (mg kg^{-1})	Organic Matter (g kg^{-1})	pH Value
Sand	Silt	Clay									
43.2	25.2	31.5	27.6	1.1	1.28	0.84	164.5	10.3	200.0	19.63	5.53

2.2. Experimental Design and Crop Management

The okra seedlings used in the study were purchased from Weifang, Shandong province, and the variety was "Wujiao". The plant experiments were in accordance with local and national regulations. We gained permission so that our studies complied with the relevant institutional, national, and international guidelines and legislation. The soil pot experiment was carried out from June to September 2021 at the Experimental Base for the Effective Use of Soil Fertilizer, Faculty of Resources and Environment, Hunan Agricultural University (28°11′ N, 113°4′ E; Figure 1A). To prevent rain from affecting the fluctuations in soil moisture, a rain-proof canopy was used as a shelter. The experiment was designed with two factors: SMF and N application. The SMC suitable for okra growth was used as the median value for up and down fluctuations, and three SMFs were established (Figure 1B): 45–55% FC (weak SMF: W_1), 35–65% FC (medium SMF: W_2), and 25–75% FC (strong SMF: W_3). These fluctuations were monitored daily by a rapid soil moisture measuring instrument (SU-LB, Beijing Meng Chuang Wei Ye Technology Co., Ltd., Beijing, China), and when the soil moisture content reached the lower limit of the fluctuation, the water was manually replenished to the upper limit (Table S1). The N application was designed at three levels, 0 kg hm^{-2} (N_0), 110 kg hm^{-2} (N_1), and 330 kg hm^{-2} (N_2), and was conducted in an interactive experiment with a total of nine treatments (Figure 1C). For a total of 36 pots, random blocks were arranged and replicated four times per treatment.

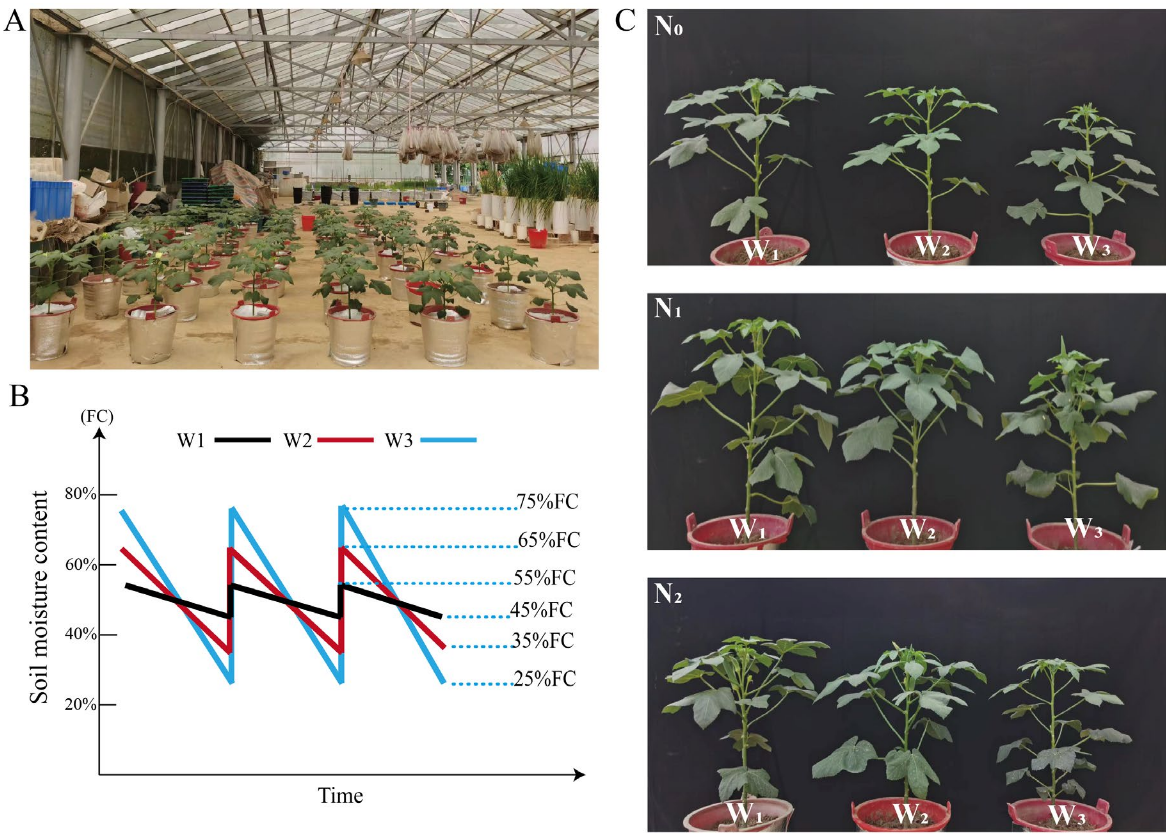

Figure 1. Experimental site (**A**), schematic diagram of SMF amplitude (**B**), and effect of SMF amplitude on the growth of okra under different N application (**C**).

As fertilizer sources for the experiment, ammonium sulfate (N 21%) was used for the N fertilizer, potassium phosphate (P_2O_5 52%, K_2O 35%) was used for the phosphorus fertilizer, and potassium sulfate (K_2O 52%) and potassium phosphate were used for the potassium fertilizer. The same amount of phosphorus and potassium were added to each treatment, all the phosphorus fertilizer was used as the base fertilizer, 60% of N and potassium were used as the base fertilizer, the base fertilizer was dissolved in water and evenly mixed into the soil before potting, the soil moisture was adjusted to 62.5% FC, and okra seedlings were transplanted a few days later. Fertilization during the seedling and flowering stages utilized 40% of the N and potassium, with each phase providing 20%.

Fertility management for okra: On 8 June 2021, selected okra seedlings (one leaf and one heart) with uniform growth were transplanted into pots, and the soil moisture was maintained at 60% FC. When okra had three leaves and one heart, the moisture treatment was applied.

2.3. Soil Moisture Content

For the definition of soil moisture content in this study and the method of calculating it, we refer to Brady and Weil [26].

2.3.1. Soil Volumetric Moisture Content

Soil volumetric water content is the proportion of soil water per unit volume, a dimensionless quantity, often expressed as θv.

$$\theta v = \frac{Vw}{Vs} \times 100\% \tag{1}$$

where Vw represents the volume occupied by water and Vs represents the volume occupied by soil.

2.3.2. Soil Mass Moisture Content

The mass water content, an important hydrological constant in soil, expresses the ratio of the mass of water in the soil to the mass of the corresponding solid-phase material, often expressed as θm.

$$\theta m(\%) = \frac{m1 - m2}{m2} \times 100 \tag{2}$$

$$\theta v = \theta m \times \rho \tag{3}$$

where $m1$ represents the wet soil mass; $m2$ represents the quality of dried soil; and ρ represents the soil capacity, and in this paper, the soil capacity after potting was 1.28 $g \cdot cm^{-3}$.

2.3.3. Field Water Holding Capacity

Field water holding capacity refers to the soil's water holding capacity when capillary-suspended water reaches its maximum, which is an essential indicator of the soil's ability to hold water and is used in moisture analysis and drought evaluation indexes.

2.3.4. Relative Soil Water Content

This refers to the soil moisture content as a percentage of the field water holding capacity.

$$RWC(\%) = \frac{\theta m}{\theta f} \times 100\% \tag{4}$$

where RWC represents the soil relative moisture and θf represents the field water holding capacity.

2.4. Agronomic Traits

The height of the plant was determined by extending a measuring tape from the bottom of the plant stem to the point where it had developed the most. At the first internode above the cotyledon node, the stem diameter was measured using vernier calipers. The maximum leaf length and maximum leaf width were determined using a measuring tape. The number of leaves was determined by visually numbering them.

2.5. Gas Exchange Measurements

At the seedling stage, three representative okra leaves were selected from each plant, and the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were measured using a LI-6400 portable photosynthetic system (LI-COR, Lincoln, NE, USA) on a sunny, cloudless day (21 July) between 9:00 and 11:00 AM.

2.6. Stomatal Morphology

On 23 July 2021, at 8:00 AM, three random samples of okra leaves (approximately 2 × 2 mm) from the central portion of the leaf were fixed with the fixative 2.5% glutaric aldehyde in 0.1 mol L^{-1} phosphate buffer (pH 7.0) and stored at 4 °C [27]. The tissues were then rinsed six times with phosphate buffer, fixed for three hours in 1.0% (v/v) phosphate, and rinsed thoroughly with the same phosphate buffer. The tissues were dehydrated with various alcohol concentration gradients and desiccated to the critical point and then mounted on an observation table and treated with gold spray using a high-pressure coating device. Individual stomata were observed and photographed using a scanning electron microscope (SEM-6380LV, Tokyo, Japan).

Using Image J version 1.51k (Wayne Rasband/NIH, Bethesda, MD, USA) [28], the stomatal characteristics were observed, and a square with a side length of 500 µm was drawn at a magnification of 100. The stomatal density (pcs mm^{-2}) within the square was then calculated by multiplying the number of stomata within the square by 4. At a magnification of 250, ten stomata were chosen for length measurements.

2.7. Antioxidant Enzymes, Malondialdehyde and Proline

At the seedling stage, the latest fully grown leaves of okra were harvested, wrapped in tinfoil, rapidly frozen using liquid nitrogen, and then stored in a −80 °C refrigerator for preservation, and each treatment was replicated three times. Then, they were sent to Shanghai ZCIBIO Technology Co., Ltd. (Shanghai, China) for the determination of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde, and proline, utilizing an enzyme-linked immunoassay kit. SOD activity was determined using the photochemical reduction method with azurotetrazole [29], POD activity was determined using the colorimetric method with guaiacol [29], CAT activity was determined using the hydrogen peroxide method [30], malondialdehyde content was determined using the thiobarbituric acid (TBA) colorimetric method [31], and proline content was determined using the acid ninhydrin method [32].

2.8. Fruit Yield

The harvested yield of individual plants was tallied, and the average was calculated.

2.9. Statistical Analysis

With SPSS 22.0 (SPSS, Chicago, IL, USA), ANOVA and correlations were carried out, and the least significant difference (LSD) technique was employed to compare means at $p < 0.05$, where p represents the p-value. A low p-value indicates strong evidence against the null hypothesis, whereas a high p-value implies poor evidence.

3. Results

3.1. Dynamics of Soil Volumetric Water Content under Different Irrigation Conditions

As shown in Figure 2, the soil volumetric water content of W_1 treatment fluctuated between 16.1% and 19.7%, which corresponded to 45–55% of the field water holding capacity; the soil volumetric water content of W_2 treatment fluctuated between 12.5% and

23.3%, which corresponded to 35–65% of the field water holding capacity; and the soil volumetric water content of W_3 treatment fluctuated between 9.0% and 26.9%, which was equivalent to 35–65% of the field water holding capacity. This suggests that moisture monitoring and manual irrigation as supplemental watering methods successfully met the requirement of SMF in the experimental design.

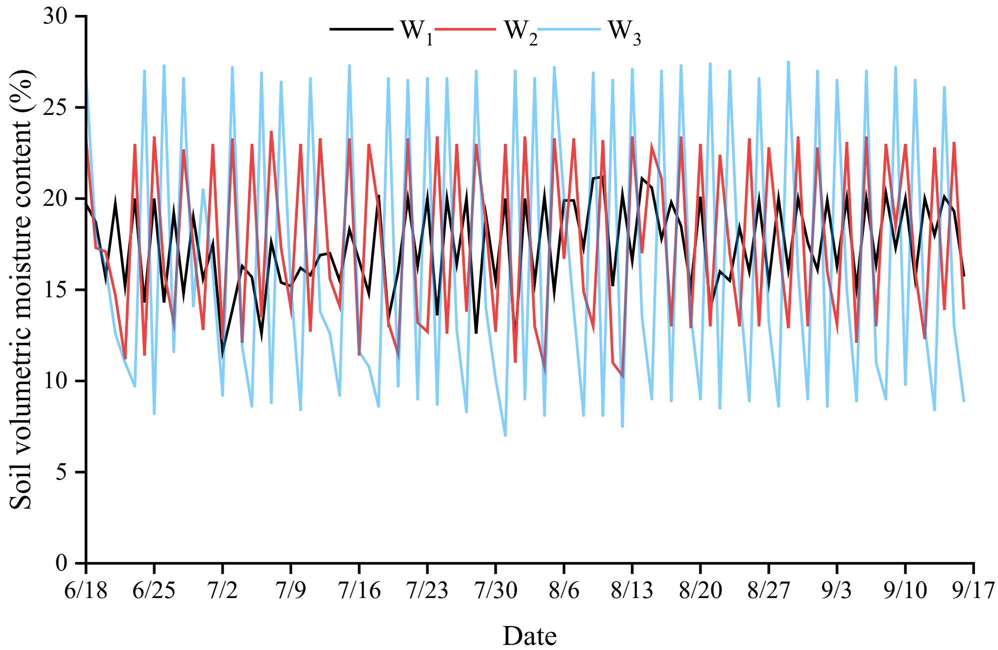

Figure 2. Dynamics of soil volumetric water content under various irrigation treatments.

3.2. Agronomic Traits

The SMF and N application significantly influenced the height, stem diameter, maximum leaf length, maximum leaf width, and number of leaves of okra, with the highest height, maximum leaf length, and maximum leaf width of 42.2 cm, 17.2 mm, and 24.7 mm for the N_2W_1 treatment, respectively, with significant increases of 22.5%, 28.05%, and 21.9% when compared to the lowest treatment of N_2W_3. The stem diameter and number of leaves were highest in the N_1W_1 treatment at 13.5 mm and 20 leaves, respectively, significantly higher by 31.9% and 28.5% compared to the lowest treatment N_2W_3 (Table 2).

Table 2. Effects of SMF and N application treatments on the agronomic traits of okra.

Treatment		Plant Height (cm)	Stem Diameter (mm)	Maximum Leaf Length (cm)	Maximum Leaf Width (cm)	Number of Leaves
N_0	W_1	38.7 ± 6.13 abc	12.1 ± 0.17 b	15.8 ± 0.47 ab	22.3 ± 0.94 abc	15.7 ± 0.94 bc
	W_2	34.3 ± 4.92 bc	11.9 ± 0.23 b	15.5 ± 1.47 ab	21.0 ± 1.63 bcd	15.7 ± 0.47 bc
	W_3	34.3 ± 2.05 bc	10.9 ± 0.45 c	14.3 ± 0.94 b	20.3 ± 0.94 cd	14.3 ± 0.47 c
N_1	W_1	40.0 ± 1.63 ab	13.5 ± 0.56 a	16.0 ± 0.82 ab	24.7 ± 0.47 a	20.0 ± 2.16 a
	W_2	36.0 ± 0.82 abc	13.0 ± 0.45 a	15.7 ± 0.47 ab	23.3 ± 0.47 ab	17.0 ± 0.82 abc
	W_3	34.7 ± 2.49 bc	11.5 ± 0.61 bc	15.3 ± 0.47 ab	21.5 ± 1.47 bcd	15.3 ± 2.62 bc
N_2	W_1	42.2 ± 0.62 a	12.0 ± 0.65 b	17.2 ± 1.03 a	24.7 ± 1.70 a	17.7 ± 1.25 abc
	W_2	36.0 ± 1.41 abc	12.1 ± 0.23 b	14.3 ± 0.24 b	20.3 ± 0.47 cd	16.7 ± 0.47 bc
	W_3	32.7 ± 1.70 c	9.2 ± 0.33 d	12.3 ± 0.24 c	19.3 ± 0.47 d	14.3 ± 0.47 c

Note: The data in the table are the mean ± standard deviation of the observed values. The different lowercase letters after the same number represent the significant difference between the treatments at 0.05 level.

With the increase in the N application, the plant height, maximum leaf length, and maximum leaf width of W_1 increased, while the stem diameter and leaf number initially increased and then decreased. The plant height of W_2 increased, while the stem diameter, maximum leaf length, maximum leaf width, and number of leaves initially increased and then decreased. The plant height, stem diameter, maximum leaf length, maximum leaf width, and number of leaves of W_3 initially increased and then decreased. The plant height, stem diameter, maximum leaf length, maximum leaf width, and leaf number of each N treatment decreased as the amplitude of SMF increased (Table 2).

3.3. Fruit Yield

As shown in Figure 3, the N_1W_1 treatment produced the most okra fruit with a yield of 201.3 g, which was 67.8% greater than the N_2W_3 treatment. With the increase in N application, the fruit yield of okra in W_1 and W_3 initially increased and then decreased, whereas the fruit yield of okra in W_2 decreased. The fruit yield of N_0 and N_1 decreased as the amplitude of SMF increased, while the fruit yield of N_2 increased and then decreased.

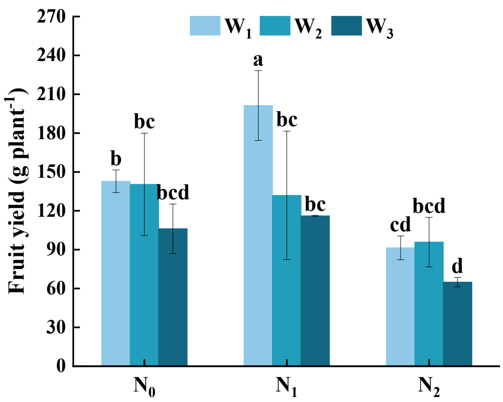

Figure 3. Effects of SMF and N application treatments on okra fruit yield. Different letters indicate differences up to a 5% significant level.

3.4. Photosynthesis

As shown in Figure 4, the N_1W_1 treatment had the highest Pn, Gs, Ci, and Tr concentrations in okra leaves at 26.8 mol m^{-2} s^{-1}, 0.45 mmol m^{-2} s^{-1}, and 279 mol mol^{-1}, respectively. The Pn, Ci, and Tr of W_1 increased and then decreased as the N application increased, while Gs did not change significantly. Pn decreased in W_2, Gs did not change significantly, Ci and Tr increased and then decreased, Pn decreased in W_3, Gs did not change significantly, and Ci and Tr increased. The Pn, Ci, and Tr of N_0 increased and then decreased, and Gs decreased as the amplitude of SMF increased, whereas the Pn, Gs, Ci, and Tr of N_1 and N_2 decreased.

3.5. Stomatal Morphology

As shown in Figure 5A–C, the amplitude of SMF significantly affected the stomatal density and stomate pore length of okra leaves. With the increase in SMF amplitude, the stomatal density of okra leaves increased. The stomatal density of the W_3 treatment was 339 pcs mm^{-2}, which was considerably higher by 24.2% than the W_1 treatment. As depicted in Figure 5B–D, the stomate pore length of okra leaves decreased with increasing SMF amplitudes, and the stomate pore length of the W_1 treatment was 24.9 μm, which was considerably increased by 33.8% when compared to the W_3 treatment.

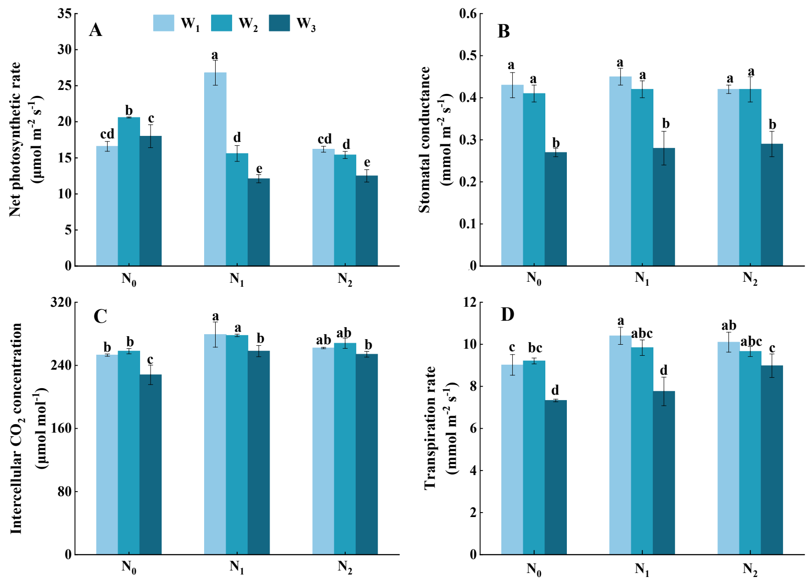

Figure 4. Effects of SMF and N application treatments on the photosynthetic rate (**A**), stomatal conductance (**B**), intercellular CO_2 concentration (**C**), and transpiration rate (**D**) of okra. Different letters indicate differences up to a 5% significant level.

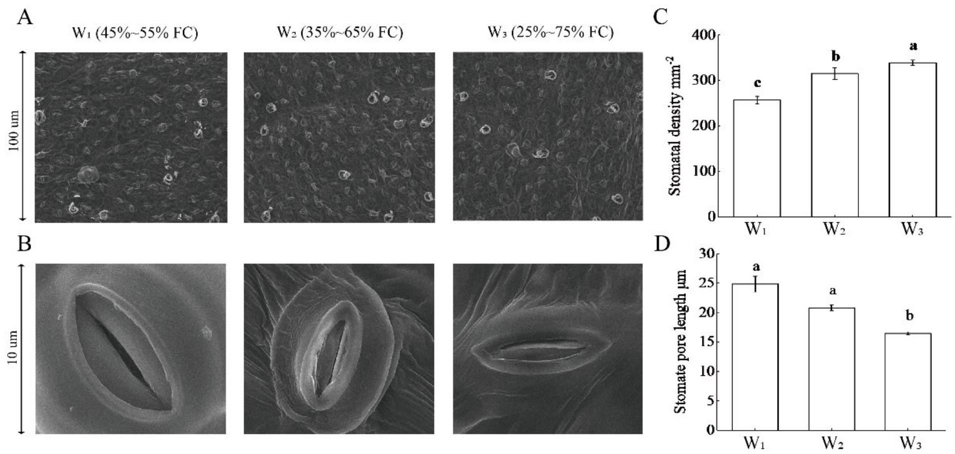

Figure 5. Effects of SMF on the stomatal density diagram (**A**), stoma size diagram (**B**), stomatal density statistics (**C**), and stomate pore length statistics (**D**) of okra leaves. Different letters indicate differences up to a 5% significant level.

3.6. Antioxidant Enzymes

As shown in Figure 6, the SMF and N application had significant impacts on the antioxidant enzyme activities of okra leaves. The SOD activity of okra leaves was highest in the N_1W_3 treatment at 1370 U g^{-1}, a significant increase of 36.9% compared to N_0W_3 in the lowest treatment (Figure 6A); POD activity was highest in the N_2W_3 treatment at 2128 U g^{-1}, a significant increase of 32.5% compared to N_0W_2 in the lowest treatment (Figure 6B); and CAT activity was highest in the N_1W_3 treatment at 541 U g^{-1}, a significant increase of 38.8% compared to N0W1 in the lowest treatment (Figure 6C). With the increase in N application, SOD and POD activities increased and CAT activities increased and then decreased in W_1, SOD, POD, and CAT activities increased and then decreased in W_2, and SOD, POD, and CAT activities increased in W_3. With the increase in SMF amplitude, the SOD and CAT activities of N_0 increased and then decreased, the POD activities increased, and the SOD, POD, and CAT activities of N_1 and N_2 increased (Figure 6).

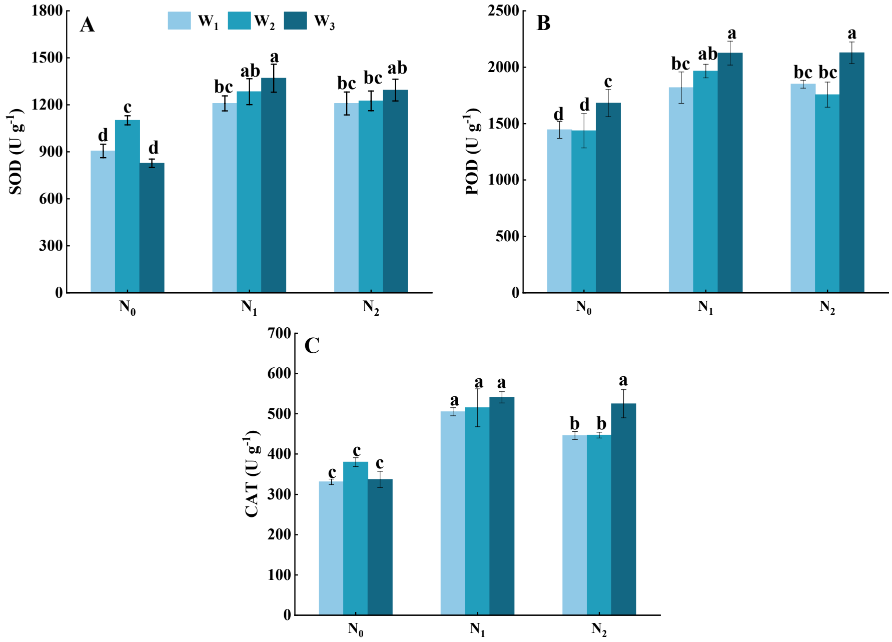

Figure 6. Effects of SMF and N application treatments on the activities of SOD (**A**), POD (**B**), and CAT (**C**) oin okra. Different letters indicate differences up to a 5% significant level.

3.7. Malondialdehyde and Proline

Malondialdehyde is a byproduct of cell membrane lipid peroxidation, and its concentration reflects the level of adversity-induced plant stress [33]. As shown in Figure 7A, the malondialdehyde content increased with the increasing SMF amplitude at the same level of the N application, with an average increase of 11.6% in the W_3 treatment compared to the W_1 treatment. Compared to the W_1 treatment, the malondialdehyde content of the W_3 treatment increased by 11.6% on average. As the N application increased between irrigation treatments, the malondialdehyde content decreased. In comparison to the N_2W_1 treatment, the N_0W_3 treatment produced malondialdehyde content that was significantly higher at 151 nmol g^{-1}. This represents a significant increase of 20.5%.

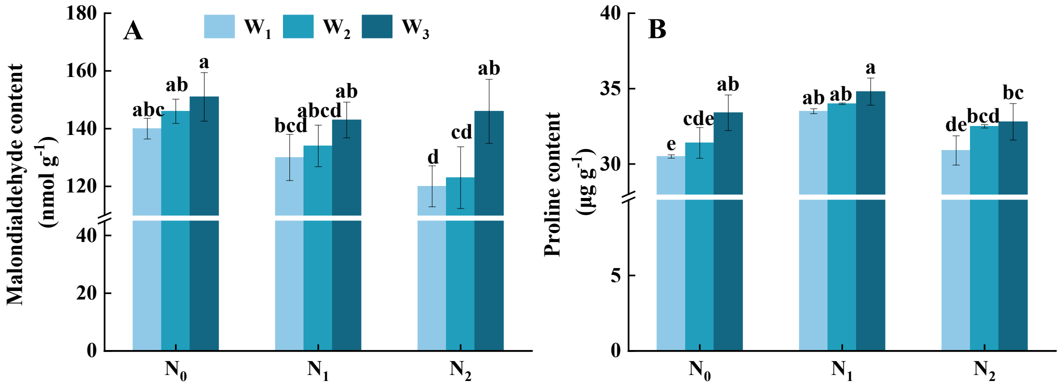

Figure 7. Effects of SMF and N application treatments on the malondialdehyde (**A**) and proline (**B**) of okra. Different letters indicate differences up to a 5% significant level.

Proline is an important osmoregulatory substance in the plant cytosol and a component of plant proteins. It is extensively distributed throughout the plant's body in its free form. Under conditions of duress, such as drought and salinity, plants accumulate enormous amounts of proline. As shown in Figure 7B, the proline content increased with increasing SMFs under the same N application treatments, and the proline content of the W_3 treatment amplitude increased by an average of 6.1% when compared to the W_1 treatment. The proline content increased and then decreased between irrigation treatments as the amount of N applied increased. The N_1W_3 treatment had the highest proline content concentration at 34.8 µg g^{-1}, a significant increase of 12.4% compared to the N_0W_1 treatment.

3.8. Correlation Analysis

The correlation analysis results (Figure 8) indicated that the agronomic parameters of okra were positively correlated with yield, with stem diameter and leaf number being significantly positively correlated with yield, while other indexes were not significantly positively correlated. The net photosynthetic rate, stomatal conductance, and interstitial CO_2 concentration were significantly positively correlated with yield, whereas the transpiration rate was not significantly positively correlated. The antioxidant enzyme activity and malondialdehyde content of okra leaves were negatively correlated with yield, and the correlation was not significant. Proline was positively correlated, but the correlation was not significant.

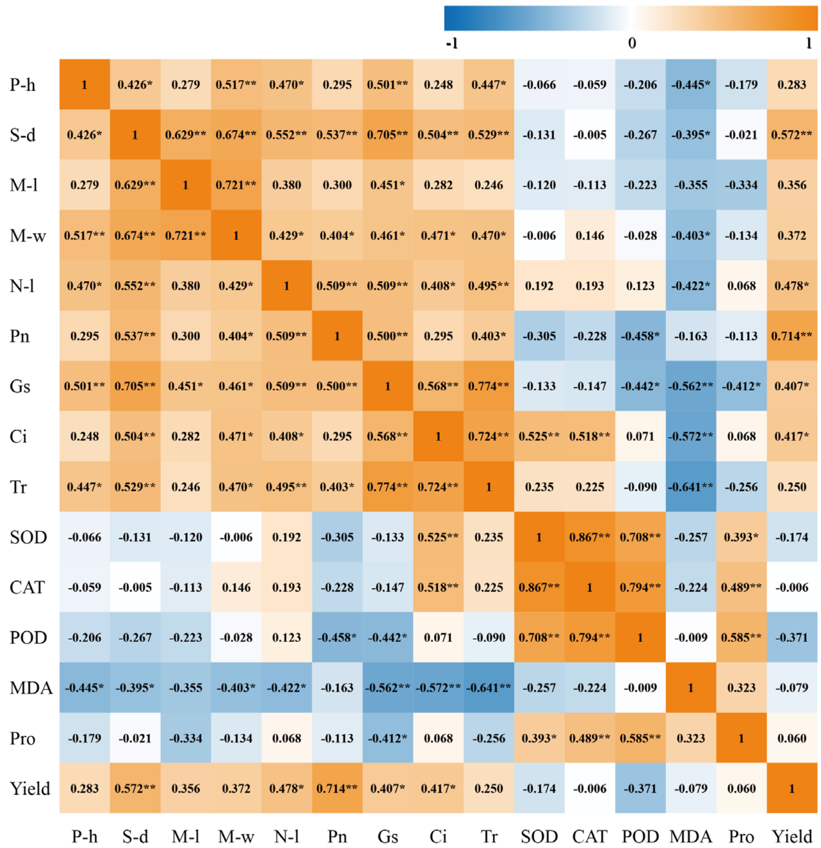

Figure 8. Correlation analysis between agronomic traits, physiological indicators, and yield. P-h, plant height; S-d; stem diameter; M-l, maximum leaf length; M-w, maximum leaf width; N-l, number of leaves; Pn, net photosynthetic rate; Gs, stomatal conductance; Ci, intercellular CO_2 concentration; Tr, transpiration rate; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; MDA, malondialdehyde; Pro, proline; Yield, fruit yield; * represents a significant correlation at the $p < 0.05$ level, and ** represents a significant correlation at the $p < 0.01$ level.

4. Discussion

The nutrient environment of the soil, which may be formed by proper fertilization and irrigation, has the potential to supply the plant with the necessary nutrients, and the height of the plant can serve as an indicator of its nutritional growth. In this study, the okra's plant height, stem diameter, maximum leaf length, maximum leaf width, and number of leaves decreased significantly with increasing SMFs, indicating that under the strong SMF (W_3, 25–75% FC), plant growth was inhibited, which was detrimental to the nutrient uptake of okra [34] and resulted in poor growth and a low fruit yield (Figure 3). Additionally, it is important to note that excessive nitrogen can also have detrimental effects on crops [35]. The average yield of the N_3 treatment was 84.0 g, which was significantly lower than the N_1 (149.8 g) and N_0 (129.8 g) treatments (Figure 3). These results emphasize the detrimental consequences of high nitrogen concentrations on okra growth. Conversely, the appropriate N application resulted in a considerable increase in okra plant height, stem diameter, maximum leaf length, maximum leaf width, and number of leaves, which is consistent with previous research findings [36]. The results of the correlation analysis showed (Figure 8) that the agronomic traits of okra were positively correlated with fruit yield, with the stem diameter and number of leaves reaching significant levels with fruit yield, further demonstrating that appropriate N applications are beneficial to the growth and yield of okra. In the presence of drought, nutrient deficits can exacerbate the inhibiting effects of drought stress, making them even more pronounced [37]. In this study, okra grew better under the strong SMF with the appropriate N application because the appropriate N application could reduce the inhibitory effect on plant growth and alleviate the adverse effects caused by the strong SMF [23,38].

In this study, the number of fallen leaves varied greatly during the late stage of okra growth (Table S2), and the degree of senescence was not the same across the treatments [39], which would affect the results of the measured data, so this study only investigated and explored the physiological characteristics of okra during the seedling stage. One of the most important ways to achieve high crop yields is to regulate photosynthetic characteristics and the accumulation of photosynthetic products through proper water and N management [40,41]. The Pn, Gs, Ci, and Tr of okra leaves were highest in the N_1W_1 treatment (Figure 4), which means that using 110 kg hm^{-2} N application and weak SMF is a reasonable and suitable water and N management method for okra, which is beneficial to improve the photosynthetic capacity of okra leaves. The Pn, Gs, Ci, and Tr were significantly lower in the W_3 treatment than in the W_1 treatment, and the increased SMF amplitude reduced the photosynthetic rate of okra leaves, which was detrimental to the growth of okra. When plant growth was inhibited, the transpiration rate was reduced mainly through stomatal closure (Figure 5B) to reduce leaf water loss [42]. The Pn, Ci, and Tr all exhibited an "S" trend with increasing N applications, whereas Gs was not significantly different, indicating that the appropriate N application can enhance photosynthesis in okra leaves. And, the Pn of N_3 was significantly lower than that of N_0 and N_1, which implied that photosynthesis of okra leaves was inhibited under high nitrogen conditions (Figure 4). Excess nitrogen may lead to an imbalance in the photosynthesis process, affecting the photosynthetic capacity of leaves. Photosynthesis is essential for normal plant growth, and its function has a direct effect on plant yield, with more than 90 percent of plant yield originating from photosynthetic products [43]. The correlation analysis results (Figure 8) indicated that okra fruit yield was substantially and positively correlated with the Pn, Gs, and Ci, which coincidentally also proved to be the case. In this study, N application increased okra fruit yield, while excessive fertilizer application resulted in a lower yield, as has been confirmed by Taheri [44] and Agegnehu [45]. Furthermore, the stomatal density of okra leaves increased under the W_3 treatment (Figure 5C) and stomate pore length decreased (Figure 5D), which is consistent with previous studies reporting an increase in stomatal density and a decrease in stomatal size due to drought [46,47]

Plants produce active oxygen during aerobic metabolism. When the amount of active oxygen in a biofilm reaches a specific threshold, it begins to peroxidize the lipids of the biofilm and causes damage to the plants. Antioxidant enzymes in plants have the ability to partially repair this damage [48,49]. Studies have shown that SOD and POD activities are significantly affected by water and nutrients [50–52]. In this study, the appropriate application of N significantly increased the activity of antioxidant enzymes in okra leaves (Figure 6), which was beneficial in delaying leaf senescence [53]. Additionally, under the W_3 treatment, the antioxidant enzyme system acts to enhance antioxidant enzyme activity and scavenge excess reactive oxygen species [54,55] which is a reflection of the stress on okra growth. The highest SOD and CAT activities were also observed in okra under the W_3 treatment with appropriate N application. This may be due to the fact that the appropriate N application under the strong SMF has the effect of increasing antioxidant enzyme activity in leaves, which ultimately results in the increased scavenging of reactive oxygen species and the alleviation of the oxidative damage to which it is subjected [56,57].

In adverse conditions, proline has the potential to contribute to osmoregulation as well as the elimination of free radicals [58]. Under stress, plants produce proline to lower their water potential and enhance protection against further damage [59]. According to the findings of this study, the proline content of the W_1 treatment was much lower than that of the W_3 treatment, which suggests that the soil wetness in the W_3 treatment subjected okra to duress [60]. The accumulation of reactive oxygen species in plants as a response to stress results in the oxidation of polyunsaturated fatty acids found in membrane lipids, which in turn results in the production of malondialdehyde. This in turn results in the polymerization of enzyme proteins into chains, which increases the permeability of the membrane and causes damage to the membrane system [61]. As a consequence of this, ion channels, membrane proteins, and associated enzymes are all impacted and damaged, and the amount of malondialdehyde in the sample represents the level of structural membrane damage as well as the degree of membrane lipid peroxidation [55]. In this study, the malondialdehyde content of the W_3 treatment was significantly higher than that of the W_1 treatment, which indicates that the strong SMF caused some oxidative damage to okra [62]. However, the antioxidant enzyme activities were increased under the W_3 treatment (Figure 6), which indicates that SOD, POD, and CAT activities played a role in the process of oxidative damage to the plants and, to some extent, resisted the damage caused by the strong SMF.

One of the important environmental issues facing the world today is global climate change and uneven rainfall distribution [8–10]. In the context of this climate change, the water requirements and adaptability of plant growth have become particularly important. Plants rely on water to absorb nutrients and carry out photosynthesis, so fluctuations in water availability have a significant impact on plant growth and development [63]. This study reveals that okra plants grow best under weak SMFs (Figure 9). Such SMFs enhance the efficiency of carbon dioxide and water vapor exchange in okra leaves by adjusting the size and density of their stomata. This indicates that plants achieve more efficient water utilization by adjusting stomatal characteristics during the process of adapting to water fluctuations. Additionally, the study also found that under this SMC, the antioxidant enzyme activity, malondialdehyde content, and proline content in okra leaves were insufficient (Figure 9). Antioxidant enzymes are an important defense mechanism for plants in the face of environmental stress, helping plants withstand oxidative stress and maintain cellular homeostasis. However, under weak SMFs, okra did not appear to experience significant stress, so the antioxidant system did not come into play, resulting in an increase in okra fruit yield. The results of this study reveal certain adaptive mechanisms of plants in response to water fluctuations.

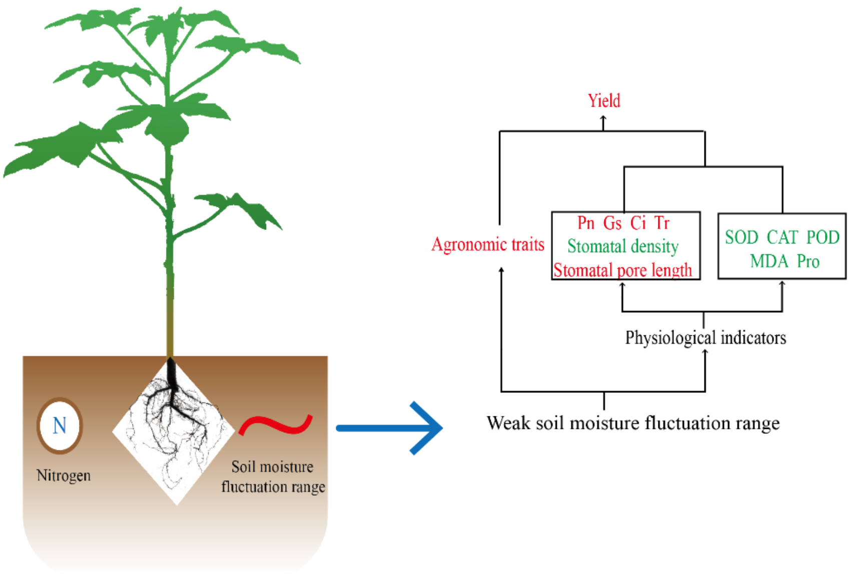

Figure 9. Mechanisms of physiological changes in okra under SMF. Note: the green font represents indicators with reduced levels under weak SMF, and the red font represents indicators with increased levels under weak SMF.

5. Conclusions

The effects of SMF and N application on the agronomic characteristics, photosynthetic characteristics, antioxidant enzyme activity, malondialdehyde content, proline content, and yield of okra were significant. The weak SMF promoted okra growth by boosting photosynthesis and protecting the plant from oxidative damage, thereby increasing yield. Under the strong SMF, okra growth was inhibited; however, the administration of N could mitigate its negative effects and reduce its growth-inhibiting effect.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10050425/s1, Table S1: Soil volumetric water content and irrigation in different treatments; Table S2: Effects of SMF and N application treatments on the number of fallen leaves of okra.

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