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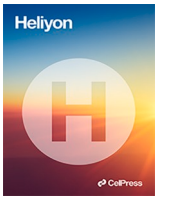

Research article

Evaluating crop nitrogen status in maize leaves: A predictive modelling approach using chlorophyll fluorescence parameters

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ABSTRACT

The consumption of chemical fertilizers has increased eight-fold since the 19th century, outstripping crop yields increases and, emphasizing the need for precise nitrogen (N) assessment in crops to optimize fertilization and mitigate environmental impacts. This study developed a model using chlorophyll fluorescence technology to accurately evaluate the N status in maize leaves while addressing the limitations of current labor-intensive and environmentally sensitive methods. Based on a long-term experiment initiated in 2011, maize hybrid Fumin 985 was sampled in 2021 and 2022 under two crop-straw management strategies (SM: no tillage with surface straw mulch, SP: plough tillage with straw incorporation) and six N application rates. Partial least squares regression (PLSR) models were formulated using chlorophyll fluorescence parameters (ChlF) to assess leaf N content (N leaf). The results indicated that a N application rate of 270 kg ha^{-1} sufficed to meet crop N requirements. Leaf characteristics such as N leaf, total pigment content (TP), and leaf dry weight (DW leaf) changed significantly with increasing N application rates, influencing rapid chlorophyll fluorescence (OJIP) dynamics. Principal component analysis (PCA) reduced ChlF from 35 to 21, and four models were developed, among which, the model using ChlF and TP was more accurate than the model using DW alone. Key ChlF parameters for PLSR model performance included ABS/RC, φ(Eo), ETo/CSm, and δ(Ro)/(1–δ(Ro)). Although non-destructive N leaf detection using chlorophyll fluorescence technology proved feasible, additional leaf characteristics, such as TP, are necessary to improve model accuracy. Considering local field conditions is essential for the application of this technology at a larger scale. Precise evaluation of N status using chlorophyll fluorescence is beneficial for a more efficient N management and sustainable agriculture.

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1. Introduction

From the 1890s to the early 21st century, average maize yields increased by 25 %, with approximately 12 % of the increase attributed to the application of nitrogen (N) fertilizers to improve N uptake by plants [1–3]. However, from 1961 to 2013, fertilizer consumption increased approximately eight-fold, far exceeding the proportional increase in crop yield over the same period [4,5]. In China, small-scale farmers use an average of 305 kg ha^{-1} of N fertilizer, significantly exceeding the global average of 74 kg ha^{-1} [6]. Excessive application of N fertilizer does not lead to an endless increase in crop yield but can instead lead to considerable off-site losses of N into the environment [7,8]. Therefore, it is crucial to appropriately reduce the N input to crops to develop sustainable agriculture.

Nitrogen is an essential nutrient for the development of photosynthetic organs in crops and plays a vital role in supporting normal growth and development [9]. Therefore, it is important to determine the criteria to allow appropriate reductions in N input in agricultural fields, as a N deficiency can lead to the degradation of chlorophyll and soluble proteins, which would result in reduced activity of important enzymes involved in photosynthesis, such as phosphoenolpyruvate carboxylase, and consequently, a decrease in photosynthetic rate [10], which serves as a key indicator of photosynthetic activity, and is influenced by factors such as leaf structure and biochemical composition. In particular, specific leaf weight and leaf N content (N leaf) are important factors that determine plant photosynthetic capacity [11,12]. However, the correlation between the photosynthetic rate per unit of leaf area and specific leaf weight is not clear, and the photosynthetic rate may reportedly decrease with increasing specific leaf weight [13]. Studies on N leaf effects on net photosynthesis have found that net crop-photosynthetic rate initially increases and then stabilizes with increasing N leaf [14,15]. In maize, N uptake rate peaks between the 10th and 14th-leaf stages, and by the start of reproductive growth, plants have absorbed approximately 70 % of the total N acquired during physiological maturation [16]. Therefore, accurately assessing the N status of crops and providing timely supplemental fertilization during specific growth stages is crucial for ensuring optimal crop growth and maximum yield.

Chlorophyll fluorescence measurements are commonly used to assess the physiological status of crops under various stress conditions [17] and, to monitor and quantify photosynthetic performance [18]. As chlorophyll molecules can absorb light through three distinct processes, they can drive photosynthesis process, dissipate light energy as heat, and fluoresce with light re-emitted by chlorophyll. Any change in any of these processes can affect the yield of others; thus, chlorophyll fluorescence is considered an accurate indicator of photosynthetic performance [19]. Analyzing the trajectory of movement of different phases (i.e. chlorophyll fluorescence induction kinetics curve, OJIP curve) provides valuable information on the photosynthetic system status and performance [20]. The chlorophyll fluorescence technique has revealed that high temperatures induce photoinhibition specifically in photosystem II (PSII), affecting electron transfer between the donor and acceptor sides of PSII, whereas photosystem I (PSI) remains unaffected [21]. Using chlorophyll fluorescence, an accurate evaluation of N effects on the photosynthetic system should allow the design of appropriate N management measures to optimize electron transfer between the donor and acceptor sides, thereby improving PSII performance and increasing electron transfer efficiency [22]. Thus, chlorophyll fluorescence has emerged as a powerful tool for comprehensive analysis of the photosynthetic systems.

Current methods used in domestic and international studies to assess crop N status can be broadly classified into three categories: The first one category involves soil-sampling and destructive plant tests that are both labor-intensive and time-consuming [23,24]. The second category comprises remote and proximal sensing methods that require the use of satellites, vehicles, or large-scale instruments for data collection [25,26]. Lastly, the third one involves estimations obtained by using soil and plant analyzer development (SPAD) or chlorophyll indices whose accuracy is often compromised by variations in genotype and environmental conditions [27,28]. Further research is required to explore the use of chlorophyll or chlorophyll indices as diagnostic criteria for leaf N content under varying growth conditions [29].

The importance of N in enhancing crop yields is well established. However, these methodological limitations described above have created a knowledge gap regarding the precise, simple, and non-destructive evaluation of N in crops, which is crucial for optimizing fertilization practices and minimizing the resulting environmental impacts. This study addresses this gap by investigating the potential of chlorophyll fluorescence technology, a non-destructive approach, that focuses primarily on estimating the N and potassium content in cereal crops such as wheat [29–31], to assess the N status in maize leaves. Specifically, the hypotheses tested are as follows: (i) A N deficiency in maize leaves significantly affects leaf photosynthesis, thereby altering the shape of the OJIP transient curve under dark-adapted conditions. (ii) Specific chlorophyll fluorescence parameters can serve as accurate indicators of N leaf and can be integrated into predictive models to guide precise N management. (iii) Adding leaf characteristics to the model can improve accuracy. To ensure the comparability and manipulation of N uptake rates under different conditions, we established treatments consisting of different N application rates in combination with different crop straw managements practices. Our study aimed to contribute to the advancement of agricultural science by providing a robust model for accurately assessing maize leaf N content. In general, the advancement and refinement of chlorophyll fluorescence-based techniques offer a promising avenue for effectively evaluating and managing the N in plants.

2. Materials and methods

2.1. Experimental design

The study was conducted at the Halahai Comprehensive Experimental Station of the Jilin Agricultural Science Institute in Nong'an County, Jilin Province (44°40′N, 125°07′E), China. This study was based on a long-term experiment using a single annual maize monoculture system that started in October 2011. Field sampling and measurements were performed during the 2021 and 2022

cropping seasons. The maize hybrid used for the experiments was Fumin985 (FM985), and the area of each experimental plot was 149.5 m^2, with a length of 23 m. The planting density was 65,000 plants ha^{-1}, with a row spacing of 65 cm.

This study tested two factors of variation. The first factor was crop straw management, with no tillage with surface straw mulch (SM) and plough tillage with straw incorporation (SP). Tillage was performed to a depth of approximately 15 cm, as shown in Supplementary Fig. S1. The second factor was N application rate (NAR), with six levels: 0 kg N ha^{-1} (N0), 90 kg N ha^{-1} (N90), 150 kg N ha^{-1} (N150), 210 kg N ha^{-1} (N210), 270 kg N ha^{-1} (N270), and 330 kg N ha^{-1} (N330). Since 2011, fertilizer application rates, fertilizer types, and crop straw management have remained the same. Nitrogen (CH_4N_2O) was applied as a base fertilizer (40 %) and was top-dressed (60 %). Phosphorus (P_2O_5) and potassium (K_2O) fertilizers were applied at 105 kg ha^{-1} at planting. To avoid potential confounding effects of herbicides, the same herbicide component was sprayed at the three-leaf stage every cropping year.

2.2. Sampling and measurements

2.2.1. Yield and yield components

To determine maize yield, 10.4 m^2 of each plot was harvested at the physiological maturity stage, with three replicate plots per treatment. In each replicate plot, 1000-kernel weight and number of kernels per ear were determined, based on the average size of 20 ears of uniform size. Kernels were oven-dried at 80 °C to a constant weight, and kernel yield was standardized to 14 % moisture.

2.2.2. Leaf area index, dry weight, and nitrogen accumulation

To determine leaf area index (LAI), six plants were randomly selected from each treatment at the tasseling (VT) stage. The length and width of each green leaf were measured, and the leaf area was calculated as follows: Leaf area (cm^2) = leaf length (cm) × leaf width (cm) × 0.75. Total leaf area per plant was calculated as the sum total of the leaf area of all the leaves on the plant. LAI was calculated as follows [32]:

$$LAI = S \times P / 10{,}000 \quad (1)$$

where S and P are leaf area per plant (m^2) and planting density (plant ha^{-1}), respectively.

To determine leaf weight, six plants per plot were randomly sampled at the VT stage. All leaves were oven-dried at 85 °C to a constant weight. After measuring all leaf weights, two plants were pooled as one sample, and three independent replicates were used for each analysis. Dried plant samples were ground into powder and digested with H_2SO_4–H_2O_2 [33]. Nitrogen concentration was determined using a continuous flow analyzer (AA3, Seal, Norderstedt, Germany). Leaf N and relative N content were calculated as follows [1]:

$$R = M \times G \quad (2)$$

where R is the N accumulation the leaves (g $plant^{-1}$); G is the leaf N concentration (%); and M is the leaf dry weight (g $plant^{-1}$).

2.2.3. Total pigment content

To determine pigment content, fresh leaf samples were collected from different positions at the VT stage. Approximately 2 g of fresh leaves (FW) was placed in a 15-ml solution containing a mixture of acetone and ethanol (v:v = 1:1). Pigments were extracted in the dark at room temperature (25 °C) for 24 h. Absorbance of the extracts was measured at 663 nm (A663), 645 nm (A645), and 470 nm (A470) using a spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan) [20]. Pigment contents were determined using the following formulas:

$$\text{Chlorophyll } a \text{ (Chl a, mg kg}^{-1}\text{ FW)} = 12.21 \times (\text{A663} - 2.81) \times \text{A645} \quad (3)$$

$$\text{Chlorophyll } b \text{ (Chl b, mg kg}^{-1}\text{ FW)} = 20.13 \times (\text{A645} - 5.03) \times \text{A663} \quad (4)$$

$$\text{Carotenoids (mg kg}^{-1}\text{ FW)} = (1000 \times \text{A470} - 3.27\ \text{Chl a} - 104\ \text{Chl b}) / 229 \quad (5)$$

$$\text{Total pigment content (TP, mg kg}^{-1}\text{ FW)} = \text{Chl a} + \text{Chl b} + \text{carotenoids} \quad (6)$$

2.2.4. Chlorophyll fluorescence

At the VT stage in the 2022 cropping season, a clip was attached to the tassel leaves after a 30-min dark-adaptation period. The chlorophyll fluorescence transient (OJIP transient) was measured using a Handy-PEA (Hansatech, King's Lynn, Norfolk, UK). The OJIP curve was obtained by inducing the original chlorophyll fluorescence transients with a 2-s treatment with red light at 3000 μmol m^{-2} s^{-1}. The original OJIP between the O and P phases was standardized as follows [20]:

$$Vt = (Ft - FO) / (FM - FO) \quad (7)$$

where F_O represents the fluorescence intensities at 20 μs and F_M is the maximum fluorescence intensity under saturating light conditions.

To detect information in the OJIP curve under different treatments, the relative variable fluorescence values between the O and P phases were calculated under the following N rate treatments: N0, N90, N150, N210, and N270 [20]:

$$\Delta \text{ Fluorescence} = (Vt_{(N)} - Vt_{(N270)}) \quad (8)$$

where $V_{t\ (N270)}$ is the standardized chlorophyll fluorescence transient under N270 and $V_{t\ (N)}$ is the standardized chlorophyll fluorescence transient under another N application rate.

2.3. Partial least squares regression prediction models

Chlorophyll fluorescence parameters were measured during the VT stage in 2022. A total of 35 chlorophyll fluorescence parameters (ChlF) were exported directly from the handy-PEA instrument (Table 1). Principal component analysis (PCA) was conducted for these 35 parameters. The weakest explanatory variables in the PCA, with component scores of less than 0.8, were excluded. This resulted in a reduction in the ChlF from 35 to 21. To further explore the potential of the parameters in predicting leaf N status, partial least squares regression (PLSR) was performed using four different partial least squares prediction models.

Model 1 included TP, DW, and 21 ChlF. The strength of this model lies in the integration of multiple leaf characteristics, which enhance the predictive power of the model. However, it may be more complex and may require more data for calibration. Model 2 included DW of leaves and 21 ChlF, focusing on the direct relationship between leaf weight and fluorescence parameters. This model is simpler and less data intensive than Model 1 but lacks the completeness provided by TP. In turn, Model 3 included TP and the 21 ChlF, emphasizing the role of pigments in photosynthesis and their relationship with fluorescence. Although this model offers a balance between complexity and predictive capability, it is sensitive to variations in pigment composition. Lastly, Model 4 contained only 21 ChlF, providing a streamlined approach requiring minimal additional leaf characteristics. Although it is the least complex, the model might not capture all the nuances of N status related to leaf characteristics. Finally, the performance of the PLSR models was analyzed by comparing the predicted with the actual values of leaf N content.

Table 1
Abbreviations and definitions of 35 chlorophyll fluorescence parameters. Bold font indicates the 21 parameters selected after principal component analysis.

Abbreviation	Definition
Fo/Fm	Primary photochemical reaction (electron transfer to Q_A) efficiency
Fv/Fm	PSII photochemical maximum quantum yield
N	Number of electrons required for complete reduction of all electron acceptors except Q_A
Fv/Fo	Ratio of photochemical reaction and nonphotochemical quenching rates in reaction center excitation energy utilization
Vj	Normalized variable fluorescence at J points (2 ms)
Vi	Normalized variable fluorescence at I point (30 ms)
dVG/dto	Rate of change in chlorophyll fluorescence parameter VG with time
dV/dto	Rate of change in chlorophyll fluorescence parameter V with time
Sm/t (Fm)	Energy required to energize all RCs to shut down completely
ABS/RC	Energy flux absorbed per unit PS II active reaction center (t = 0)
DIo/RC	Energy flux per unit PS II active reaction center heat dissipation (t = 0)
TRo/RC	Excitation energy flux captured per active reaction center (t = 0)
ETo/RC	Electron flux transferred per active reaction center (t = 0)
REo/RC	Specific electron flux per unit of PS II active reaction center reducing PS I terminal electron acceptor (t = 0)
phi Po (φPo)	Maximum quantum yield of the primary photochemical reaction (t = 0)
psi Eo (ψEo)	Efficiency of a single exciton captured by an active reaction center to drive electron transfer except Q_A (t = 0)
phi Eo (φEo)	Quantum efficiency of electron transfer from Q_A to the electron acceptor of the electron transfer chain except Q_A (t = 0)
delta Ro (δRo)	Efficiency of a single electron from the interphotosystem electron transport chain to the terminal electron acceptor on the PSI receptor side
phi Ro (φRo)	Quantum efficiency of PSI receptor-side terminal electron acceptor reduction
ABS/CSo	Energy absorbed per unit leaf cross section (t = 0)
DIo/CSo	Energy heat dissipation per unit leaf cross section (t = 0)
TRo/CSo	Energy flux captured by PSII-active reaction centers per unit leaf cross section (t = 0)
ETo/CSo	Energy flux per unit leaf cross section for electron transfer (t = 0)
REo/CSo	Specific electron flux per unit leaf cross-sectional area of reduced PS I terminal electron acceptor (t = 0)
ABS/CSm	Energy absorbed per unit leaf cross section ($t = t_{FM}$)
DIo/CSm	Energy heat dissipation per unit leaf cross section ($t = t_{FM}$)
TRo/CSm	Energy flux captured by PSII-active reaction centers per unit leaf cross section ($t = t_{FM}$)
ETo/CSm	Energy flux per unit leaf cross section for electron transfer ($t = t_{FM}$)
REo/CSm	Specific electron flux per unit leaf cross-sectional area of reduced PS I terminal electron acceptor ($t = t_{FM}$)
gamma (RC)/((1–gamma (RC))	PSII performance index based on absorbed light energy
phi (Po)/((1–phi (Po))	
psi (Eo)/(1–psi (Eo))	
PI abs	
dRo/(1–dRo)	
PI total	

2.4. Statistical analyses

The treatment effects were analyzed using SPSS (version 17.0; IBM, Armonk, NY, USA). Single-factor ANOVA was used to analyze the effects of crop–straw management and N application level on different indicators of maize. Multifactorial ANOVA was used to analyze the interaction effects between two or three factors (year, crop–straw management, and N application level) on different indicators. The significance of differences among treatments was determined using Fisher's LSD test, at $P < 0.05$ level of significance. PLSR analysis was performed using R Studio (R version 4.3).

3. Results

3.1. Relationship between nitrogen application rate, yield, and leaf N content

The analyses of the relationship between N application rates, crop yield, and N leaf revealed that yield and N leaf increased with increasing N application rate and then stabilized. The critical N rate, beyond which no significant further increase in yield or N leaf was observed was lower for plants in SP than for those in SM plots (Fig. 1A–J). Therefore, we concluded that 270 kg N ha^{-1} was sufficient to meet the nutritional needs of corn plants throughout the growth period. Subsequently, only treatments N0, N90, N150, N210, and N270 were subjected to statistical analysis.

3.2. Yield components

The highest correlation coefficient was found for the relationship between 1000-kernel weight and yield, followed by that between yield and kernels per ear (Fig. 2A–C). Compared with N0, 1000-kernel weight, kernels per ear, and harvest ear number were significantly higher under in N270. With its higher 1000-kernel weight and greater number of kernels per ear, SP demonstrated a slight advantage over SM in terms of yield components (Supplementary Table S1).

3.3. Leaf characteristics

Nitrogen fertilization had a significant effect on N leaf, DW leaf, LAI, and TP. The interaction between N and S significantly affected N leaf and DW leaf (Table 2). Similarly, crop straw management significantly affected DW leaf. N leaf, DW leaf, LAI, and TP initially

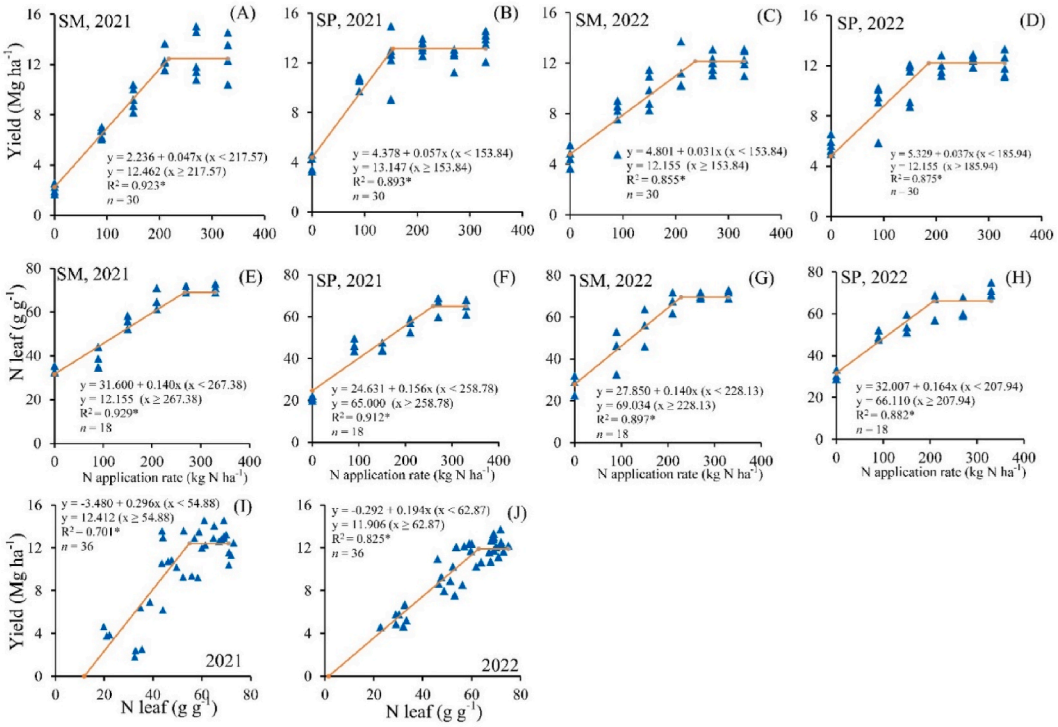

Fig. 1. Linear plus plateau curves of the relations between maize yield (A–D), leaf nitrogen content (N leaf) (E–H) and N application rate (0, 90, 150, 210, 270, 330 kg N ha^{-1}), and between maize yield (I: 2021, J: 2022) and N leaf. R^2: correlation coefficient. *$P < 0.05$. *n*: number of replicates. SM: no tillage with surface straw mulch. SP: plough tillage with straw incorporation. 2021: 2021 year. 2022: 2022 year.

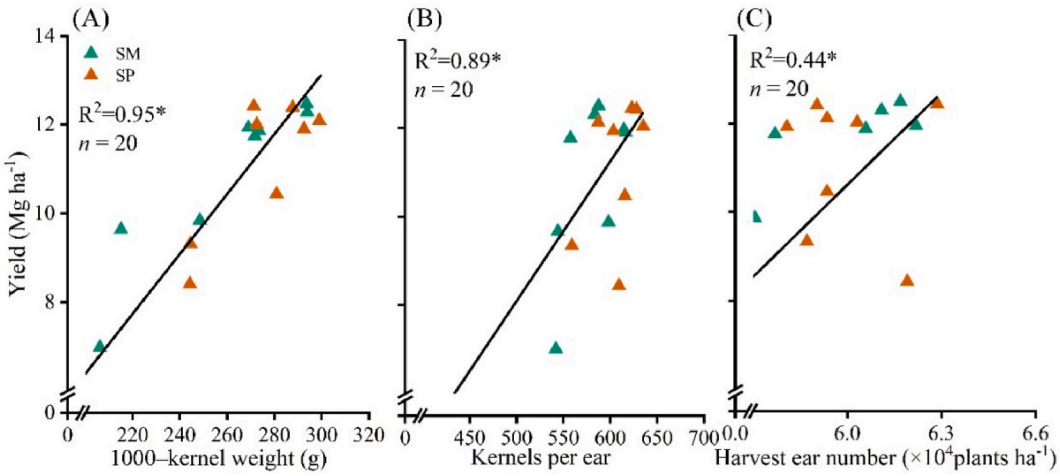

Fig. 2. Linear regressions of the relations between yield and yield components (A) 1000-kernel weight, (B) kernels per ear, and (C) harvest ear number. R^2: correlation coefficient. *$P < 0.05$. SM: no tillage with surface straw mulch; SP: plough tillage with straw incorporation.

Table 2
Effects of different crop–straw management practice and N application treatment on leaf characteristics of maize plants in two cropping years.

Year	Crop–straw management	N application rate (kg ha^{-1})	N leaf (kg ha^{-1})	DW leaf (g plant^{-1})	LAI	TP (mg kg^{-1} FW)
2021	SM	0	21.01c	37.45c	2.86b	8.51d
		90	46.46b	57.48b	4.88a	15.51c
		150	45.14b	69.27a	5.03a	22.88b
		210	56.14a	75.27a	5.17a	26.11a
		270	58.67a	76.67a	5.13a	25.65a
	SP	0	39.19d	58.90b	2.78c	9.28c
		90	33.59cd	64.52b	4.07b	15.03b
		150	42.18b	68.24b	4.8 ab	24.71a
		210	65.77b	68.69b	5.33a	24.65a
		270	70.33a	73.33a	5.4a	26.86a
2022	SM	0	30.88c	32.76b	3.00b	11.44c
		90	49.54b	40.51a	5.15a	17.52b
		150	54.85a	46.18a	5.6a	20.03 ab
		210	64.33a	47.9a	5.62a	21.71a
		270	62.33a	51.00a	5.52a	24.11a
	SP	0	27.94d	37.63c	2.92b	11.10c
		90	44.06c	39.23bc	5.50a	18.47b
		150	55.39b	49.24 ab	5.34a	22.18a
		210	67.13a	46.74a	5.67a	22.27a
		270	70.33a	50.33a	5.43a	23.26a
	ANOVA					
	Y		113.406***	258.579***	20.997***	0.297 ns
	S		3.465*	7.046*	1.176 ns	1.250 ns
	N		175.313***	44.425***	120.816***	195.191***
	Y × S		9.559**	2.398 ns	1.409 ns	0.557 ns
	Y × N		18.211***	3.969*	1.977 ns	13.222***
	S × N		8.838***	8.332***	0.612 ns	1.363 ns
	Y × S × N		9.556***	4.479**	2.066 ns	0.597 ns

increased and subsequently plateaued with increasing N application rate (Table 2). The DW leaf and N leaf plants growing in SP plots were significantly higher than those in of plants growing in SM plots.

N significantly affected Chl a, Chl b, and carotenoids, whereas S significantly affected only carotenoids (Fig. 3A–C). As N application rate increased, Chl a, Chl b, and carotenoids initially increased and subsequently plateaued (Fig. 3D, E, G, and H). Furthermore, carotenoid content was greater under the SP treatment than that under the SM treatment (Fig. 3F and I).

3.4. Correlation analysis

Leaf characteristics and yield were significantly and positively correlated (Fig. 4), with the strongest correlation being between yield and TP, followed by those between yield, LAI, N leaf, whereas the weakest one between was detected between yield and DW leaf.

(A)

ANOVA	Chl a
Y	5.093*
S	0.603ns
N	149.236***
Y×S	0.042ns
Y×N	7.425***
S×N	0.701ns
Y×S×N	1.146ns

(B)

ANOVA	Chl b
Y	0.741ns
S	0.081ns
N	96.442***
Y×S	1.713ns
Y×N	6.194**
S×N	0.822ns
Y×S×N	0.663ns

(C)

ANOVA	Carotenoid
Y	2.740ns
S	6.452*
N	61.715***
Y×S	0.328ns
Y×N	10.605***
S×N	1.072ns
Y×S×N	0.822ns

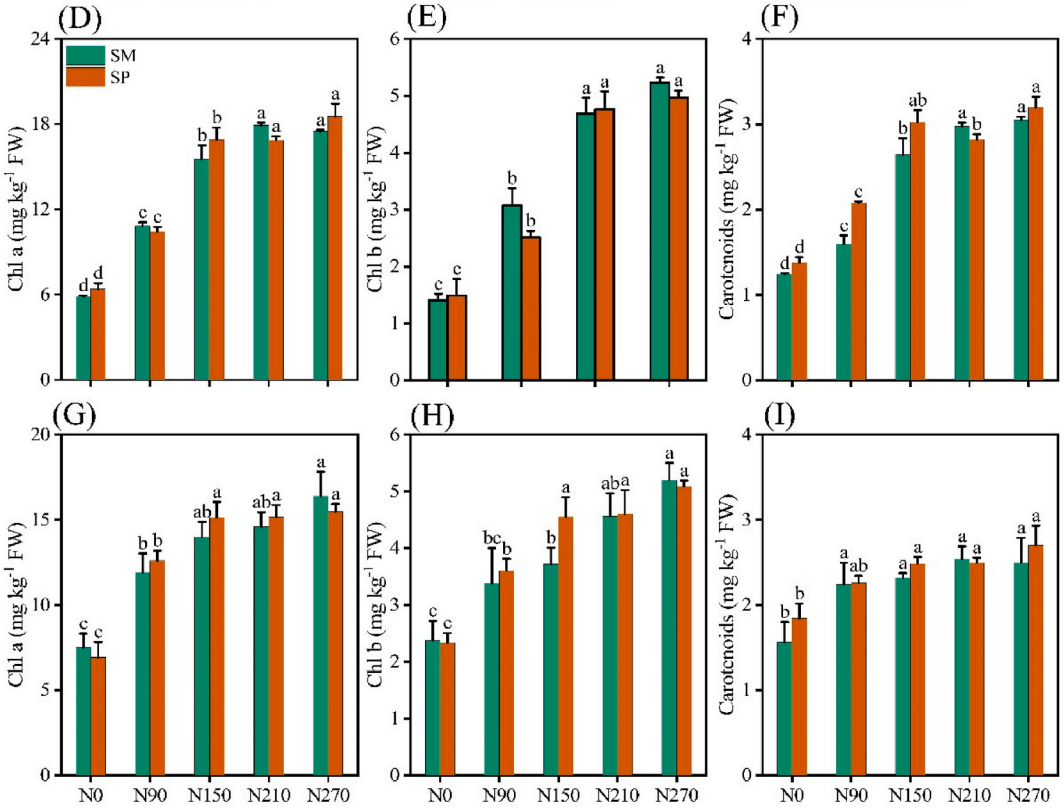

Fig. 3. Chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and carotenoid contents under different crops straw management (SM: no tillage with surface straw mulch; SP: plough tillage with straw incorporation) and N application rate (0, 90, 150, 210, 270 kg N ha^{-1}). FW: fresh weight. (A, B, C) ANOVA results; (D, E, F) 2021; (G, H, I) 2022. Different lowercase letters indicate significant differences among N application rate under the same straw return management ($P < 0.05$). *$P < 0.05$; ** $0.001 < P < 0.01$; ***$P < 0.001$; ns: $P > 0.05$. Y: year; S: crop straw management; N: N application rate. Data shown are means ± SE, $n = 3$.

Furthermore, NAR was significantly and positively correlated with leaf characteristics. Notably, among leaf characteristics, DW leaf did not significantly correlated with N leaf (Fig. 4).

3.5. OJIP curve and relative variable fluorescence

The OJIP curve showed distinct peaks at steps K, J, and I below N270, whereas step P remained unchanged. Furthermore, the largest peaks were at N0, whereas the smallest were observed for N210. Specifically, Δ fluorescence increased in the K–I phase in SM at N210, whereas it decreased in SP (Fig. 5A–D).

3.6. Construction of partial least squares regression models

3.6.1. Principal component analysis

Principal component analysis (PCA) was performed on the 35 parameters of the OJIP test for the different treatments (Fig. 6). PC1 provides a comprehensive understanding of the PSII performance and characteristics, whereas PC2 primarily reflects the status of the PSI acceptor. The weakest explanatory variables in the PCA (i.e. those with component scores less than 0.8) were excluded from further analysis, thus reducing the fluorescence parameters from 35 to 21 (Table 1).

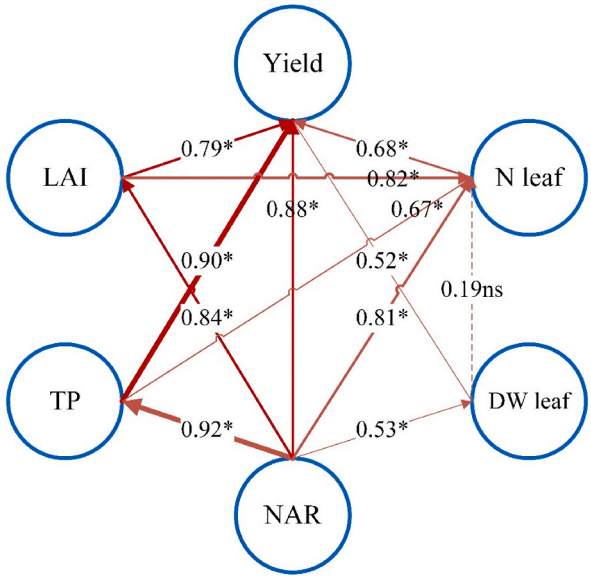

Fig. 4. Correlation analysis between leaf characteristics and maize yield and nitrogen (N) application rate at tasseling (VT) stage ($n = 48$). Leaf characteristics: LAI: leaf area index; TP: total pigment content; DW leaf: dry weight of leaf; N leaf: N content of ear leaf. NAR: nitrogen application rate. *$P < 0.05$; ns: $P > 0.05$. The values in the graph represent the correlation coefficient (r), the arrow thickness represents the magnitude of the correlation.

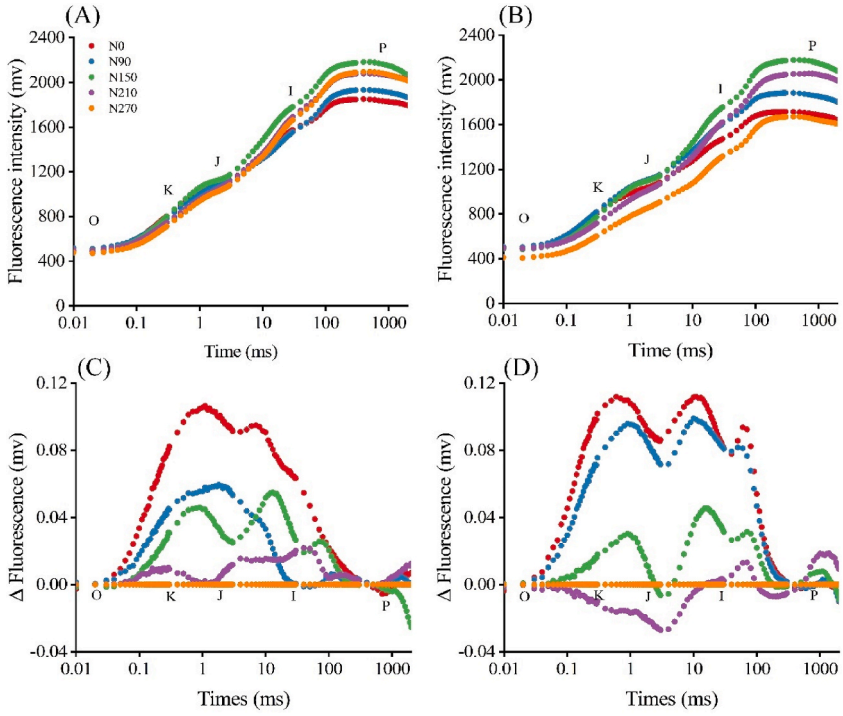

Fig. 5. Chlorophyll fluorescence kinetics for different crop straw management and N application rate (0, 90, 150, 210, 270 kg N ha^{-1}) at the tasseling (VT) stage in maize in 2022. (A, C) no tillage with surface straw mulch; (B, D) plough tillage with straw incorporation. O: minimum fluorescence intensity; K: fluorescence intensity of 0.3 ms occurring in a light environment; J: fluorescence intensity of 2 ms occurring in a light environment; I: fluorescence intensity of 30 ms occurring in a light environment; P: maximum fluorescence intensity of PSII after stopping accepting light quantum.

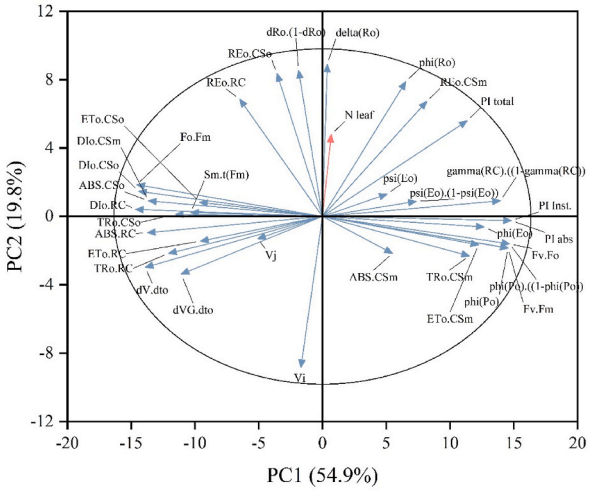

Fig. 6. Principal component analysis of chlorophyll fluorescence parameters. Refer to Table 1 for definitions of parameters. N leaf: leaf N content.

3.6.2. Partial least squares regression models

The results of the validation and corresponding components of the four regression models are shown in Table 3. Model 1 had the highest R^2 value (Fig. 7A–D), whereas Model 2 had the lowest root mean square error of cross validation (RMSECV). In turn, Model 4 exhibited the highest RMSECV values. In particular, the inclusion of TP and ChlF in Models 2 and 3 proved to be more effective in predicting N leaf than DW leaf. DW leaf did not positively influence the performance of Models 1 and 3. Among ChlF, ABS/RC, φ(Eo), ETo/CSm, and δ(Ro)/(1–δ(Ro)) consistently emerged as crucial factors in all models contributing significantly to the predictive power of all PLS models (Table 3).

4. Discussion

Nitrogen is an essential element for plant growth and productivity and is a crucial component of chloroplast structure [9]. Because of the resulting reduction in the number of developed florets and kernels [32], insufficient N can decrease kernels per ear, thereby significantly affecting yield (Supplementary Table S1). Our research revealed that N application rates significantly influenced leaf N content and TP (Table 2, Fig. 3), which in turn affected photosynthetic performance. The increase in TP with N application underscores the critical role of N in pigment synthesis, particularly chlorophyll synthesis, which is essential for capturing light energy and driving photosynthesis. Insufficient N can substantially obstruct the electron transport chain, as evidenced by the shifts in the OJIP fluorescence transient curve. Rapid chlorophyll fluorescence induction kinetics has greatly advanced the study of electron transfer between the donor and acceptor sides of Photosystem II (PSII) in plants [20,34,35]. The distinct peaks at steps K and I of the OJIP curve under low-N conditions suggest disruptions in the electron transport chain [34,36], potentially due to damage to the oxygen-evolving center (OEC) or limitations in electron acceptance at QA [37,38]. The mitigation of these peaks by N application indicates the restoration of photosystem II (PSII) function, highlighting the importance of N in maintaining the integrity and efficiency of the photosynthetic electron transport chain. Our study introduces the application of chlorophyll fluorescence technology to evaluate the N status of maize leaves, thus addressing the pressing need for precise, simple, and non-destructive agricultural assessment methods.

Current methods for assessing crop N status, such as soil sampling and SPAD-based measurements, are labor-intensive and susceptible to environmental variations [23–28]. These limitations have created a knowledge gap regarding the precise, simple, and nondestructive evaluation of N nutrition in crops. The partial least squares regression (PLSR) models developed in this study demonstrate the potential of chlorophyll fluorescence parameters (ChlF) to predict leaf N status (the coefficient of determination: $R^2 > 0.5$). The inclusion of TP and ChlF further improved the predictive precision of the models ($R^2 = 0.96$, RMSECV = 1.26), suggesting a synergistic relationship between pigment content and photosynthetic efficiency [39]. However, it has been suggested that the net photosynthetic rate per unit of leaf area is not related to the leaf dry mass per unit area [13]. The exclusion of dry weight (DW) from the models, based on its nonsignificant correlation with N content (Fig. 4), simplified the assessment process without compromising predictive power (Fig. 7). Although Model 4, with only ChlF, had the lowest R^2 and the highest RMSECV values, the relationship between the predicted and measured N leaf was relatively close across the range of measured values. Therefore, it is reasonable to recommend using only chlorophyll fluorescence to evaluate the leaf N content. Further, the following two additional points deserve consideration. First, using only ChlF to predict the N leaf should be acceptable, considering that relatively low precision is needed to estimate an appropriate N application rate (±60–90 kg N ha^{-1}). Second, because the addition of TP tightens the relationship between predicted and measured leaf N contents by almost one-to-one, it might be a useful recommendation to include TP in a predictive model, because N leaf is readily determined by a simple extraction procedure and a spectrophotometer.

Chlorophyll fluorescence technology supported by PLSR models is a feasible and non-destructive method for assessing the status of N nutrition in maize plants. This practical tool can effectively help farmers and agronomists to efficiently manage N application rate,

Table 3
Values of the root mean square error of cross validation (RMSECV) and the relevant variables for the four partial least squares predictive models formulated in this study.

PLS model	RMSECV	Relevant variables
Model 1	1.27	TP, DW leaf, ABS/RC, φ(Eo), ETo/CSm, δ(Ro)/(1–δ(Ro))
Model 2	1.00	DW leaf, ABS/RC, φ(Eo), Eto/CSm, δ(Ro)/(1–δ(Ro))
Model 3	1.26	TP, ABS/RC, φ(Eo), Eto/CSm, δ(Ro)/(1–δ(Ro))
Model 4	2.47	ABS/RC, φ(Eo), Eto/CSm, δ(Ro)/(1–δ(Ro))

Note. Refer to Table 1 for definitions of chlorophyll fluorescence parameters. DW leaf: dry weight of leaf. TP: total pigment content.

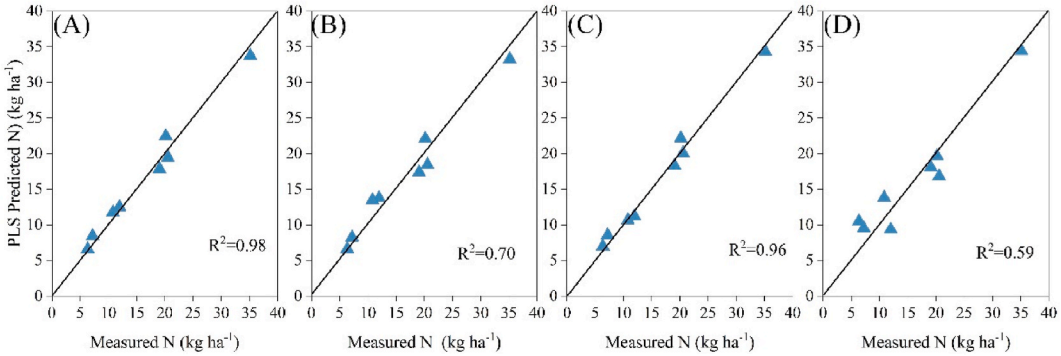

Fig. 7. Regression analysis of the measured of N leaf and the corresponding predicted values from the four partial least squares models formulated in this study. R^2: correlation coefficient. (A) Model 1; (B) Model 2; (C) Model 3; (D) Model 4.

enhance N use efficiency and minimize negative environmental impacts resulting from excessive use of N fertilizers. However, it is important to note that the accuracy of these models can be influenced by specific field conditions, such as crop straw management practices, which underscores the need for local model calibration. By integrating chlorophyll fluorescence with predictive modeling, we offer a novel, time-efficient, and cost-effective alternative to traditional soil sampling and testing methods. This approach provides real-time data to enables the timely adjustment of N fertilization strategies, thus promoting sustainable agricultural practices. Future research should explore the effects of different forms of N fertilizer forms on leaf N status and chlorophyll fluorescence, as well as the long-term implications of using chlorophyll fluorescence technology in diverse agricultural settings.

5. Conclusions

Changes in leaf N and total pigment contents significantly affected yield under different crop straw managements and N application rates. Here, we assessed N leaf non-destructively and found that chlorophyll fluorescence technology can readily provide a viable proxy of N content in maize leaves. Further, we showed that PLSR predictive models generated using ChlF have the potential to detect the N status in fresh maize leaves and can be highly informative about the state of the photosynthetic apparatus under N deficiency. Including both TP and ChlF improved the precision of the model for predicting N leaf. However, when extrapolating these findings to larger scales or uncontrolled experimental conditions, it is crucial to consider specific, local field conditions for model calibration purposes.

CRediT authorship contribution statement

Xiangzeng Meng: Writing – review & editing, Writing – original draft. **Shan Zhang:** Validation, Methodology, Investigation. **Lichun Wang:** Visualization, Supervision, Conceptualization. **Yang Yu:** Data curation. **Sijia Duan:** Investigation. **Yixiang Zhang:** Investigation. **Yanjie Lv:** Writing – review & editing, Funding acquisition. **Yongjun Wang:** Visualization, Supervision, Funding acquisition, Conceptualization.

Ethics statement

Not applicable.

Data availability statement

Data and code will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39601.

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