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Optimized nitrogen application ameliorates the photosynthetic performance and yield potential in peanuts as revealed by OJIP chlorophyll fluorescence kinetics

Pei Guo[1], Jingyao Ren[1], Xiaolong Shi[1], Anning Xu[1], Ping Zhang[1], Fan Guo[1], Yuanyuan Feng[1], Xinhua Zhao[1], Haiqiu Yu[1,2]* and Chunji Jiang[1]*

Abstract

Background Nitrogen (N) is a crucial element for increasing photosynthesis and crop yields. The study aims to evaluate the photosynthetic regulation and yield formation mechanisms of different nodulating peanut varieties with N fertilizer application.

Method The present work explored the effect of N fertilizer application rates (N0, N45, N105, and N165) on the photosynthetic characteristics, chlorophyll fluorescence characteristics, dry matter, N accumulation, and yield of four peanut varieties.

Results The results showed that N application increased the photosynthetic capacity, dry matter, N accumulation, and yield of peanuts. The measurement of chlorophyll a fluorescence revealed that the K-phase, J-phase, and I-phase from the OJIP curve decreased under N105 treatment compared with N0, and W_{OI}, ET_0/CS_M, RE_0/CS_M, ET_0/RC, RE_0/RC, φPo, φEo, φRo, and $\Psi 0$ increased, whereas V_J, V_I, W_K, ABS/RC, TR_0/RC, DI_0/RC, and φDo decreased. Meanwhile, the photosystem activity and electron transfer efficiency of nodulating peanut varieties decreased with an increase in N (N165). However, the photosynthetic capacity and yield of the non-nodulating peanut variety, which highly depended on N fertilizer, increased with an increase in N.

Conclusion Optimized N application (N105) increased the activity of the photosystem II (PSII) reaction center, improved the electron and energy transfer performance in the photosynthetic electron transport chain, and reduced the energy dissipation of leaves in nodulating peanut varieties, which is conducive to improving the yield. Nevertheless, high N (N165) had a positive effect on the photosystem and yield of non-nodulating peanut. The results provide highly valuable guidance for optimizing peanut N management and cultivation measures.

Keywords Electron transport, Nitrogen fertilizer, OJIP, Peanut, PSII performance

*Correspondence:
Haiqiu Yu
yuhaiqiu@syau.edu.cn
Chunji Jiang
jiangchunji2002@syau.edu.cn
[1] College of Agronomy, Shenyang Agricultural University, Shenyang, China
[2] Liaoning Agriculture Vocational and Technical College, Yingkou, China

Introduction

Peanut (*Arachis hypogaea* L.) is one of the most consumed oilseed crops worldwide with high protein content in its seeds [1]. According to statistics, China's peanut production and planting area account for about 40% and 20% of the world's, respectively, making it the world's largest peanut producer [2]. Therefore, a stable

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and sustained increase in peanut yield is essential to ensure food and edible oil security.

Nitrogen (N) is one of the most important elements that influence the growth of plants [3]. A lack of N severely limits crop productivity. Therefore, application of N fertilizers in the field dramatically increases crop yield [4]. Research has proven that appropriate N fertilizer application greatly improves crop growth, photosynthesis, biomass production, yield, and N use efficiency (NUE) [5, 6]. Nevertheless, excessive application of N fertilizers has caused severe N pollution worldwide and decreased crop NUE [7, 8]. The element of N is an important part of photosynthetic organs, and the utilization of nitrogen by crops determines the photosynthetic capacity and function [9]. A change in the N content of crops has a direct impact on the photosynthetic process of leaves, which includes light absorption, electron transfer and energy distribution [10, 11]. Chlorophyll fluorescence is intimately connected to the process of photosynthesis, which has been employed as a reference index to evaluate the relationship between abiotic stress and photosynthesis [12]. This is an important index that allows further reflection on photosynthesis, such as light absorption and energy transformation processes [13–15]. Previous studies have employed the JIP-test in chlorophyll fluorescence determination to analyze the response strategies of crops facing different nitrogen environments and the negative effects of nitrogen deficiency on plant photosystem II (PSII) were reported [11, 16]. Some studies have indicated that photosynthesis, stomatal conductance, photosynthetic pigment content and soluble sugar concentration of crops decrease under N deficiency conditions [17–19]. Specifically, N deficiency caused a slight reduction in the electron acceptor pool size, PSII reaction centre activity and photosynthetic enzyme activity of crop leaves [20]. Appropriate N rates improve leaf PSII performance, which in turn increases photosynthesis and dry matter weight, and greatly contributes to seed yield, especially during the critical period of crop growth (reproductive growth period) [21, 22]. Nevertheless, high N treatments were found to be somewhat beneficial in increasing the isoelectric point (PI) value of soybean, while excessive N application resulted in a significant reduction in the photosynthetic capacity and electron transfer efficiency of summer maize [23].

Legumes can conduct biological N fixation (BNF) through endosymbiotic interactions with bacteria residing in root nodules [24]. Unlike other crops, the main sources of N in legume crops are soil, fertilizer and BNF [25]. In particular, under growing conditions where soil N is deficient, peanut can supply more than 60% of its N requirements from BNF, while under high-yielding conditions it can provide about 40% [26]. The process of BNF is one of the most environmentally friendly and economical sources of N, which can greatly reduce the dependence on fertilizer in peanut production, while saving costs and protecting the environment [4]. During growth, the podding stage is the most vigorous period of peanut vegetative and reproductive growth; it is also the period with the highest N requirement of peanuts [27]. The maximum nodule number and dry weight are reached at the podding stage, which has great potential to provide N to peanuts [25]. However, some studies demonstrated that excessive N applications significantly constrained the BNF of peanuts, resulting in lower NUE, reduced yield, and increased production costs [28–30]. There are notable differences in nodulation characteristics, N acquisition and utilization among different peanut varieties [31].

In our previous studies, we investigated the effect of N fertilizer at different growth stages on nodulation characteristics, dry matter, and N accumulation in different nodulating peanut varieties [25, 32]. We found that peanuts had the highest number of nodules at eighty days after seedling emergence (the podding stage), and the N supply proportions from root nodules were more than half, which played an important role in the formation of peanut pods. This study sought to further investigate the impact of four N application treatments on the photosynthetic physiological response in peanut varieties with different nodulation during the critical period for pod formation. Four peanut varieties were grown in an outdoor pot experiment to investigate photosynthetic and chlorophyll fluorescence characteristics, and the JIP- test was used to analyze the effects of different application rates of N fertilizer on peanut leaves and the photosynthetic electron transport chain.

Materials and methods
Plant material and treatment
An outdoor pot experiment was conducted at the experimental base of Shenyang Agricultural University, Shenyang, Liaoning, China (41°82' N, 123°56' E) in 2022 (Fig. 1A). Peanuts were sown on May 14, 2022 and harvested on September 25, 2022, including the whole growth stages. The region where the test site is located has a temperate semi-humid continental climate, characterised by an average annual rainfall of approximately 878.9 mm and an average annual temperature of 8.7°C (Fig. 1B). The soil type was brown loam, and the soil nutrient status before the test was as shown in Table S1.

Four peanut varieties with different nodulation, i.e., Nonghua 5 (NH5, low nodulation, Shenyang Agricultural
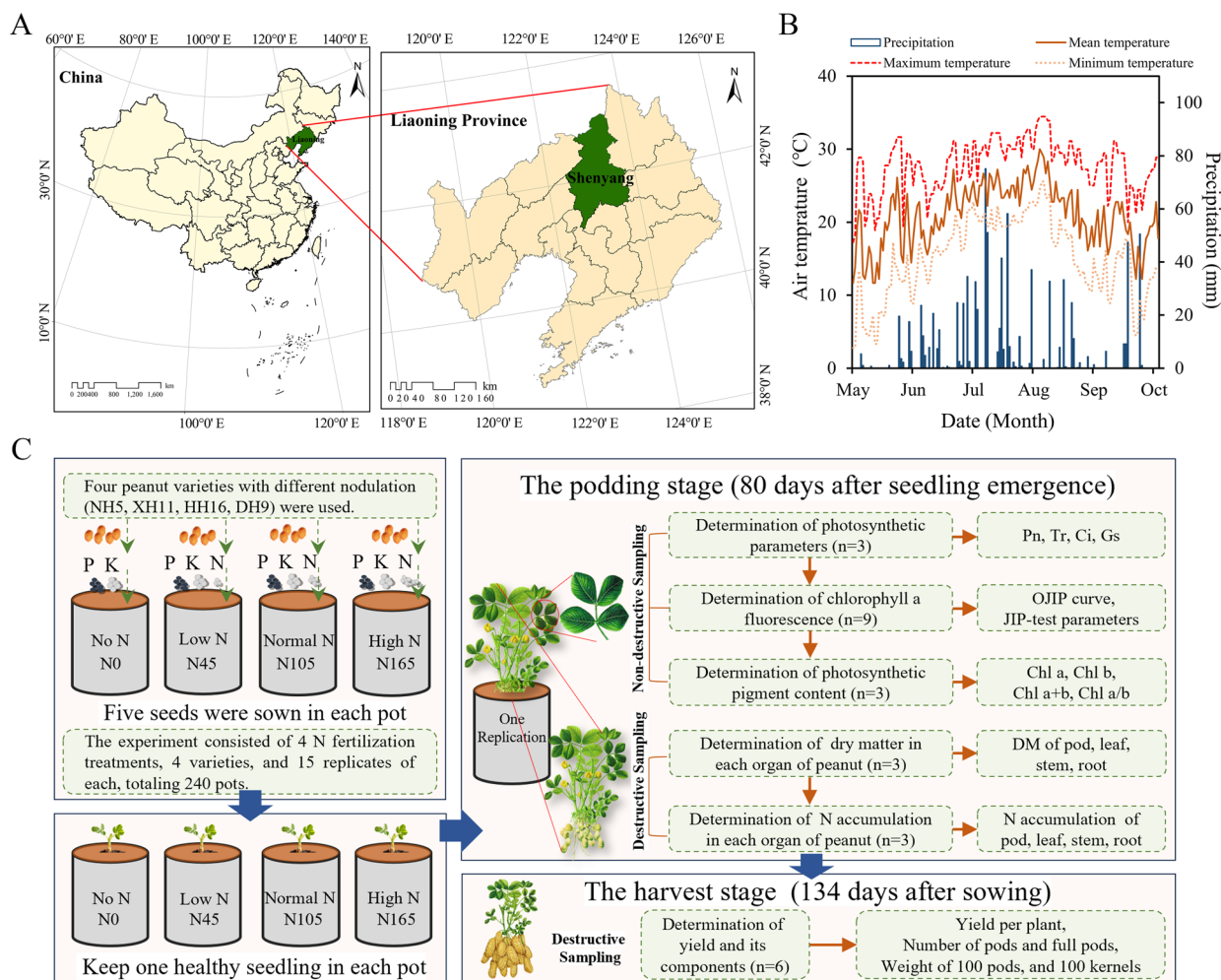
Fig. 1 **A** Map of the study site. **B** Air temperature and precipitation in the growing season of intercropping in 2022. **C** Flowchart showing the study design

University), Xianghua 11 (XH11, high nodulation, Hunan Agricultural University), Honghua 16 (HH16, high nodulation, Oil crops Research Institute, Chinese Academy of Agricultural Sciences) and DH9 (non-nodulation, Shenyang Agricultural University), were used as study materials [25, 32]. Five seeds were sown in each pot, with a height of 40 cm and a diameter of 27cm, and filled with 23 kg soil. Nitrogen (N) fertilizer was added in the form of urea (containing 46% pure N). According to the local farmers' conventional fertilization amount (105 kg N ha^{-1}) and our previous research [25, 32], four N fertilization treatments were utilized: no N fertilizer input (N0), low N fertilizer input (N45: 45 kg N ha^{-1}), normal N fertilizer input (N105: 105 kg N ha^{-1}), and high N fertilizer input (N165: 165 kg N ha^{-1}). In addition, the dose of phosphate (P_2O_5) fertilizer and potassium (K_2O) fertilizer was 6.16 g and 2.31 g per pot, respectively,

applied once as basal fertilizer before sowing. No topdressing was applied in the later stage. After seeding emergence, keep one healthy peanut seedling with consistent growth in each pot. One plant in each pot was regarded as one replication; fifteen replicates were used for each treatment of each variety in this study, with a total of 240 pots (Fig. 1C). The pots were watered according to need with an equal amount of water per pot, normally once or twice a week, except on rainy days.

Measurement items and methods
Photosynthetic parameters
Based on the results of the previous experiments [32], samples were determined eighty days after seedling emergence (at the podding stage) during the critical period of peanut growth. The functional leaves (the third fully expanded leaf counted from the top of

peanut plants) were selected for the assessment of photosynthetic parameters (Fig. 1C). Variations of photosynthetic parameters of peanut leaves, including net photosynthetic rate (Pn), transpiration rate (Tr), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs), were measured using a portable photosynthetic system CIRAS-2 (PP Systems, Hitchin, UK), calibrated as follows: the size of the leaf chamber, 1.75 cm^2 (0.7 cm × 2.5 cm); the light intensity of the internal light source in the leaf chamber, 1200 μmol photons m^{-2} s^{-1}; temperature, 25°C; relative humidity, 70%; and CO_2 concentration, 380 μmol mol^{-1}. All photosynthetic parameters were measured on three replicates per treatment, with each replicate consisting of one pot, each containing one peanut plant.

Chlorophyll a fluorescence

Eighty days after seedling emergence, nine plants from each treatment, without diseases or insect pests and with uniform growth, were selected and labeled as sample plants (4 N treatments × 4 varieties × 9 replicates, totaling 144 pots). A portable plant efficiency analyzer (Handy PEA, Hanstech, UK) was used to determine the fast chlorophyll fluorescence induced kinetic curve (OJIP transient) of the functional leaves of the sample plants. Prior to the start of measurement, plants were acclimated to the light environment, followed by a 30-min period of darkness. The O, K, J, I, and P on the OJIP curve represent the instantaneous fluorescence intensity observed at 0.01, 0.3, 2, 30 and 1000 ms, respectively, and were labelled as F_O, F_K, F_J, F_I, and F_M. The V_t, which represents the relative variable fluorescence at time t, was calculated using the following formula: $V_t = (F_t - F_O)/(F_M - F_O)$. The W_{OJ}, calculated as $W_{OJ} = (F_t - F_O)/(F_J - F_O)$, denotes the K peak. The W_{OI} was calculated as $W_{OI} = (Ft - F_O)/(F_I - F_O)$, which indicates variable fluorescence between steps O and I. The JIP-test parameters $W_K = (F_K - F_O)/(F_J - F_O)$ represent the relative variable fluorescence at the K-step (W_K) to the amplitude $F_J - F_O$. Table S2 provides additional OJIP test parameters used in the current study [14, 15, 33].

Photosynthetic pigment content

After measuring the photosynthetic parameters and chlorophyll a fluorescence, functional leaves of peanuts were taken to measure the photosynthetic pigment content. Photosynthetic pigments were determined spectrophotometrically according to Lichtenthaler [34], including chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a+b (Chl a+b), and chlorophyll a/b (Chl a/b). Measurements were performed in triplicate for each treatment.

Dry matter (DM) and N accumulation

Samples were taken 80 days after seedling emergence, with three plants serving as replicates for each treatment. The peanut plants were uprooted from the pot and washed, and the roots, leaves, stems, and pods were separated. Samples were oven-dried to constant weight at 80℃ and then weighed and pulverized. The N concentration of each peanut organ was measured using the Kjeldahl apparatus. The calculation for N accumulation was based on the multiplication of the concentration value by the dry mass value [35].

Yield and its components

During the harvest stage (134 days after sowing), six plants from each treatment with consistent growth were selected from the remaining sample to assess the yield and its components, including the average yield per plant, number of pods and full pods, weight of 100 pods, and 100 kernels.

Statistical analysis

Statistical analyses and graphing were completed using Microsoft Excel 2021, SPSS 22, Origin 2021b, and GraphPad Prism 8. Analysis of variance (ANOVA) and least significant difference analysis (LSD) were performed on data from at least three independent experiments [32, 36]. A value of $p < 0.05$ was deemed to be statistically significant. All data were expressed as mean ± standard error (SE) (n ≥ 3).

Results

Photosynthetic parameters

Compared with N0, nitrogen (N) application increased the net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs) of different peanut varieties (Fig. 2). The Pn and Tr of NH5, XH11, and HH16 reached the maximum at the N105 treatment, and DH9 exhibited significantly higher Pn and Tr than the other treatments under N165 treatment (Fig. 2A, B). Compared with the N0 treatment, the N105 treatment dramatically enhanced the Pn of XH11 and HH16 by 51.1% and 51.0%, respectively, and no significant difference in Pn of NH5 was found among N treatments (Fig. 2A). The N105 treatment significantly increased the Tr of NH5, XH11, and HH16 by 20.4%, 87.3%, and 55.4% respectively compared with the N0 treatment. However, the Tr of DH9 was not significantly different among N treatments. No significant difference was found between varieties (Fig. 2B). The application of N significantly influenced the intercellular CO_2 concentration (Ci) of NH5 and HH16, reaching

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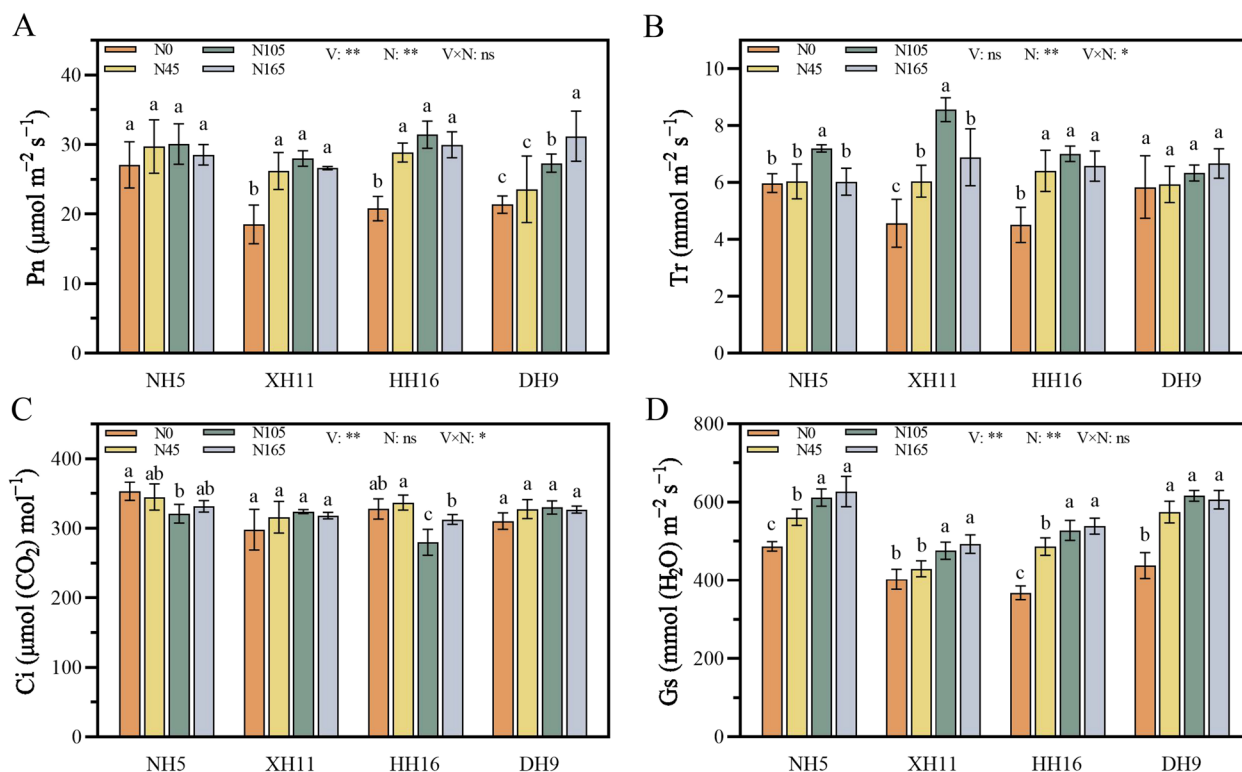
Fig. 2 The photosynthetic parameters of leaves in peanut as affected by different N application rates. **A**, **B**, **C**, and **D** represent net photosynthetic rate (Pn), transpiration rate (Tr), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs), respectively. The different letters indicate the significant differences ($p < 0.05$) among the N treatments within each variety. In multivariate variance analysis, V and N represent the variety and N fertilizer treatment, respectively, * indicates $p < 0.05$, ** $p < 0.01$, and ns indicates not significance. Data from each treatment with three replicates were subjected to analysis of variance (ANOVA), and means were compared by the least significant difference (LSD) test ($p < 0.05$). Values are mean ± standard error (SE) ($n = 3$)

a minimum threshold under the N105 treatment; however, no significant difference was found between XH11 and DH9 (Fig. 2C). The Gs of peanuts increased with an increase in N application, and the Gs under N105 and N165 treatments was significantly higher than that under N45 and N0 treatments (Fig. 2D).

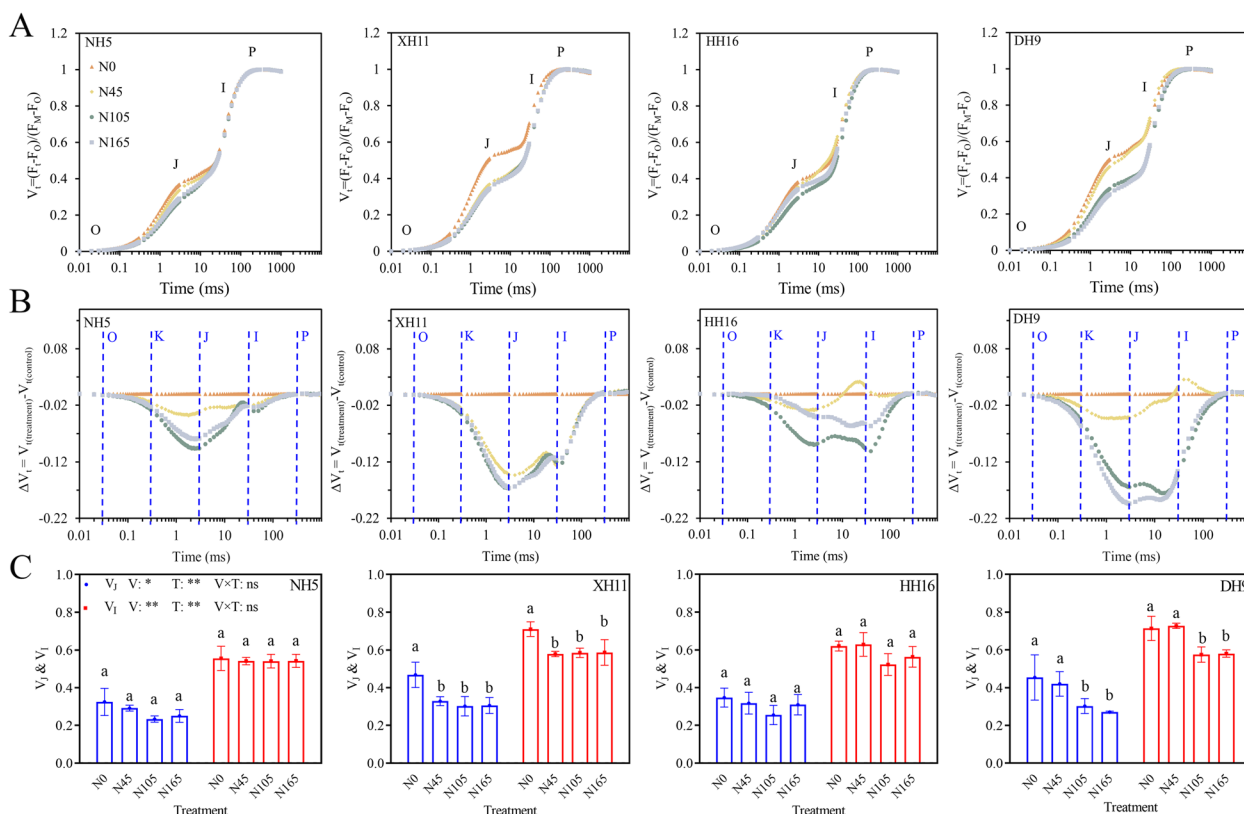**Chlorophyll fluorescence kinetics and the parameters of JIP-test**

The OJIP curves

As shown in Fig. 3A, B, the OJIP curves for the K-phase, J-phase, and I-phase were smaller than N0 under higher N (N105 and N165) treatments, and the peak value of peanut varieties was recorded at J-phase and I-phase. The peaks of NH5 and HH16 in J-phase and I-phase were obtained in the N105 treatment, whereas XH11 and DH9 were lowest in the N165 treatment (Fig. 3B). This demonstrated that N fertilizer on the acceptor side (J step decrease) of photosystem II (PSII) improved the

PSII performance. The relative variable fluorescence at the J-step and I-step (V_J and V_I) of peanuts was smaller relative to that of the N0 treatment under other N treatments (Fig. 3C). N treatment did not significantly affect V_J and V_I in NH5 and HH16 varieties. However, XH11 exhibited a significantly higher value for XH11 in the N0 treatment compared to others treatments. Under N105 and N165 treatments, V_J and V_I of DH9 were significantly decreased compared with N0.

The O-J phase and O-I phase

The O-J on the OJIP curve were standardized (W_{OJ}) and the kinetic difference (ΔW_{OJ}) for chlorophyll fluorescence was calculated (Fig. 4A). With the continued application of N, the K point of nodulating peanut varieties (NH5, XH11, and HH16) showed a trend in which it first increased and then fell, indicating that normal N fertilizer (N105) enhanced the oxygen evolving complex (OEC) activity of peanuts, while high N fertilizer (N165) damaged it. In addition, the K point of DH9 (non-nodulation) gradually decreased with the increase of N application.

Fig. 3 Changes of the relative variables of chlorophyll a fluorescence rise kinetics (OJIP) in four peanut varieties under different N application rates. **A** OJIP curves for chlorophyll fluorescence with normalized [$(V_t = (F_t - F_O) / (F_M - F_O)$]. **B** Kinetic difference (ΔV_t) for chlorophyll fluorescence [$\Delta V_t = V_{t(treatment)} - V_{t (control)}$]. **C** V_J and V_I are the relative variable fluorescence at the J-step and I-step. The different letters indicate the significant differences ($p < 0.05$) among the N treatments within each variety. In multivariate variance analysis, V and N represent the variety and N fertilizer treatment, respectively, * indicates $p < 0.05$, ** $p < 0.01$, and ns indicates not significance. Data from each treatment with nine replicates were subjected to analysis of variance (ANOVA), and means were compared by the least significant difference (LSD) test ($p < 0.05$). Values are mean ± SE ($n = 9$)

Next, we calculated the normalized relative variable fluorescence of K point (W_K) and the difference between control and N treatment (ΔW_K) (Fig. 4B). Among the varieties with nodulation ability, W_K and ΔW_K reached their lowest point under the N105 treatment. And there were no significant differences in W_K or ΔW_K between the other N treatments (N0 and N165) for these nodulating varieties. In contrast, the ΔW_K value in the nonnodulating variety DH9 significantly decreased under the N165 compared to the N0.

Figure 4C illustrates that OJIP curves were normalized by O-I (W_{OI}), with the amplitude of the I-P phase in the $W_{OI} \geq 1$ fraction is indicative of the size of the terminal electron receptor pool on the photosystem I (PSI) receptor side. The graph illustrates that the application of higher N treatments (N105 and N165) increased the size W_{OI}, which was beneficial to improve the efficiency of electron transfer. Meanwhile, the high nodulating variety HH16 exhibited the largest $W_{OI} \geq 1$ fraction under N105,

while the low nodulating variety NH5 did not show differences under N treatments.

Pipeline models of specific energy fluxes

Absorbed photon flux per active PSII (ABS/RC), trapped energy flux per active PSII (TR_0/RC), and dissipated energy (as heat and fluorescence) flux per active PSII PSII (DI_0/RC) of nodulating varieties (NH5, XH11, and HH16) were lowest under the N105 treatment, and there were no significant changes under N treatments in any variety (Fig. 5A). While the ABS/RC, TR_0/RC, electron flux from Q_A^- to the plastoquinones pool per active PSII (ET_0/RC), and electron flux from Q_A^- to the final electron acceptors of PSI per active PSII (RE_0/RC) of XH11 and HH16 (high nodulation) reached the highest under N165 treatment and the ET_0/RC of the two varieties was significantly increased by 33.3% and 15.7% compared with N0, respectively (Table S3). The ABS/RC, TR_0/RC, and DI_0/RC of DH9 (non-nodulation) demonstrated a decreasing trend with increasing N application.

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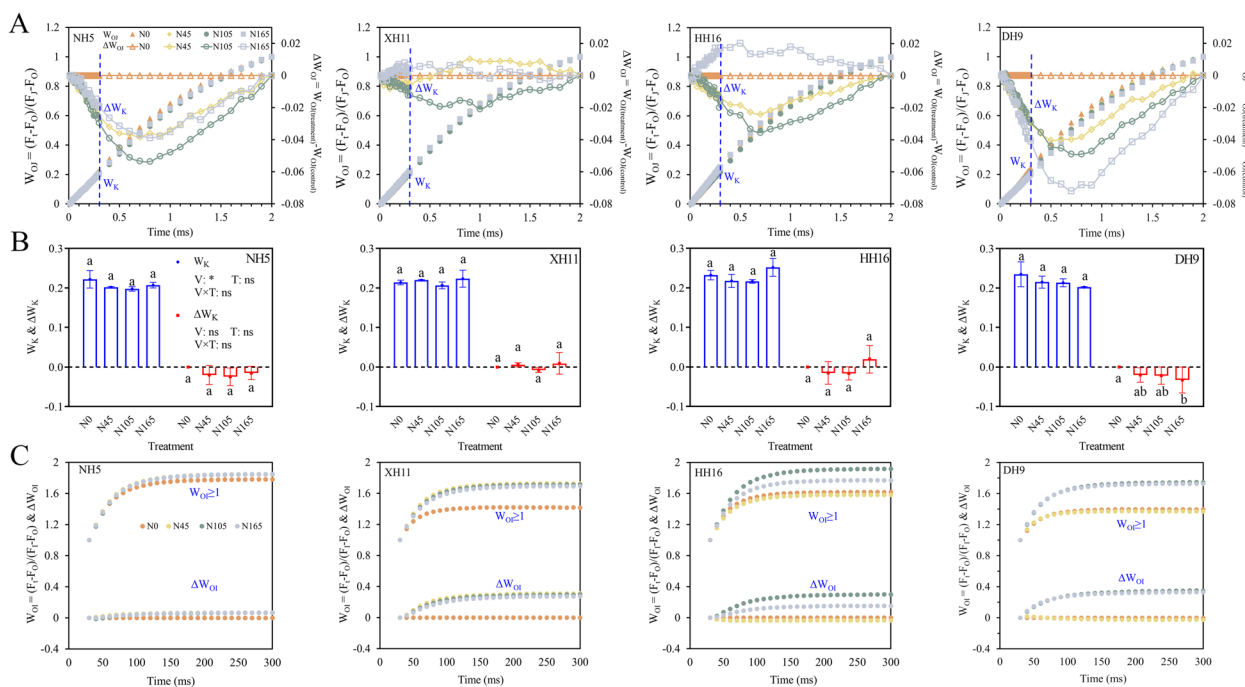
Fig. 4 Changes of the fluorescence O − J phase and O − I phase in four peanut varieties under different N application rates. **A** Variable fluorescence between the steps O and J [$W_{OJ} = (F_t - F_O) / (F_J - F_O)$]; [$\Delta W_{OJ} = W_{OJ (treatment)} - W_{OJ (control)}$]. **B** The ratio of relative variable fluorescence of four peanut varieties at the K-step to the amplitude $F_J - F_O$ as $W_K = (F_K - F_O) / (F_J - F_O)$. $\Delta W_K = W_{K (treatment)} - W_{K (control)}$. The different letters indicate the significant differences ($p < 0.05$) among the N treatments within each variety. In multivariate variance analysis, V and N represent the variety and N fertilizer treatment, respectively, * indicates $p < 0.05$, ** $p < 0.01$, and ns indicates not significance. Data from each treatment with nine replicates were subjected to analysis of variance (ANOVA), and means were compared by the least significant difference (LSD) test ($p < 0.05$). Values are mean ± SE ($n = 9$). **C** Variable fluorescence between the steps O and I [$W_{OI} = (F_t - F_O) / (F_I - F_O)$]; [$\Delta W_{OI} = W_{OI (treatment)} - W_{OI (control)}$]

Furthermore, ET_0/RC and RE_0/RC in the higher N treatments (N105 and N165) were higher compared to those in lower N treatments (N0 and N45), with RE_0/RC being significantly increased by 40.9% and 31.8% under N105 and N165 treatments, respectively, compared with N0 treatment.

Leaf model representing phenomenological energy fluxes

The leaf model of phenomenological energy fluxes per excited cross section of four peanut varieties under different N treatments was established, and the results indicated that compared to N0, higher N treatments (N105 and N165) treatments increased the absorption flux per cross section (ABS/CS_M), trapped flux per cross section (TR_0/CS_M), electron transport flux per cross section (ET_0/CS_M), electron flux reducing end electron acceptors at the PSI acceptor side per cross section (RE_0/CS_M), and amount of active PSII RCs per cross section (t = 0) (RC/CS_0) of XH11, HH16, and DH9, while decreasing thermal dissipation energy flux per cross section (DI_0/CS_M) of NH5, XH11, and DH9 (Fig. 5B). The ET_0/CS_M and RE_0/CS_M of the three nodulating varieties (NH5, XH11, and HH16) were highest in the N105 treatment, and the RE_0/CS_M of the high nodulating varieties (XH11 and HH16) was significantly higher in N105 treatment than under N0 treatment. The ABS/CS_M and TR_0/CS_M of the four peanut varieties had no significant difference between N treatments,

(See figure on next page.)

Fig. 5 **A** Pipeline models of specific energy fluxes per active PSII reaction center (membrane/specific model) for four peanut varieties under different N application rates. **B** Leaf model representing phenomenological energy fluxes per excited cross section for four peanut varieties under different N application rates. **C** Spider plots show normalized values of quantum yields and efficiencies & probabilities in four peanut varieties under different N application rates. The different letters indicate the significant differences ($p < 0.05$) among the N treatments within each variety. Data from each treatment with nine replicates were subjected to analysis of variance (ANOVA), and means were compared by the least significant difference (LSD) test ($p < 0.05$)

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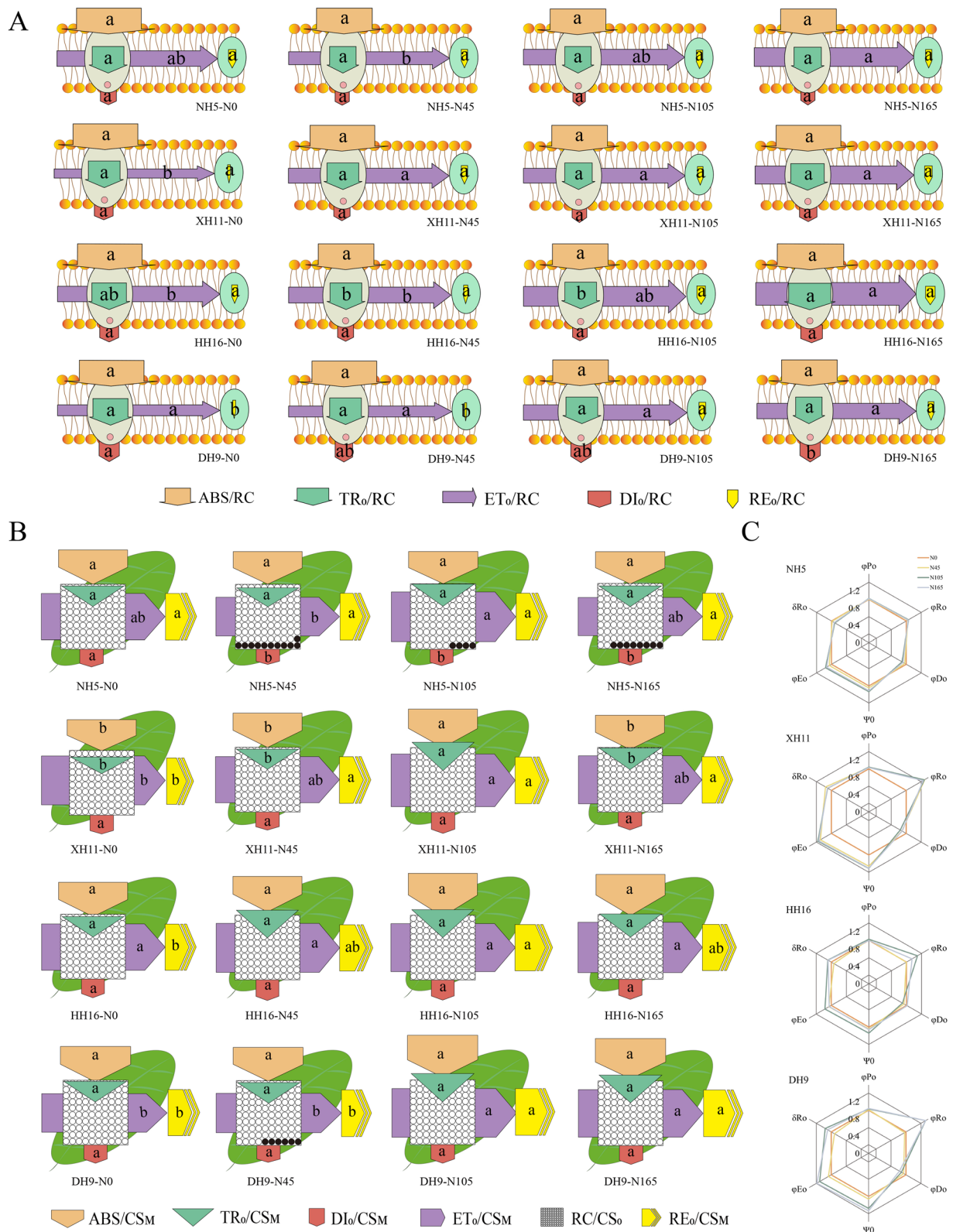
Fig. 5 (See legend on previous page.)

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but there were significant differences between varieties (Table S4).

Quantum yields and efficiencies & probabilities

The N treatment increased maximum quantum yield for primary photochemistry (φPo), quantum yield for electron transport (φEo), quantum yield for reduction of the end electron acceptors at the PSI acceptor side (φRo), and probability that a trapped exciton moves an electron into the electron transport chain Beyond Quinone A (t=0) (Ψ0) and decreased quantum yield (at t=0) of energy dissipation (φDo) in peanut (Fig. 5C). Under N105 treatment, φPo, Ψ0, and φEo of the three nodulating varieties (NH5, XH11, and HH16) reached the maximum values, while φDo reached the lowest value. In contrast, DH9 (non-nodulation) had significantly higher values of φPo, Ψ0, φEo, φRo, and probability that an electron is transported from the reduced intersystem electron acceptors to the final electron acceptors of PSI (δRo) and significantly lower values of φDo compared with N0 under N165 treatment (Table S5). Variations in δRo were significantly different across varieties. The δRo of NH5 decreased with continued use of N, whereas the δRo of XH11, HH16 and DH9 increased with higher N treatments (N105 and N165).

Photosynthetic pigment content in leaves

The applications of N fertilizer increased the photosynthetic pigment content of leaves in the four peanut varieties. There was a significant effect among varieties, N fertilizer treatments, and two-way interaction effects between variety and N fertilizer treatment on the photosynthetic pigment content of peanut leaves (p < 0.01) (Table 1). Photosynthetic pigment contents in leaves of NH5 were significantly higher under N165 treatment compared with other treatments, including chlorophyll a (Chl a), chlorophyll b (Chl b), and chlorophyll a+b (Chl a+b), with an increase of 23.4%, 32.8%, and 26.2%, respectively. The XH11 and DH9 had the highest Chl a, Chl b, and Chl a+b under the N105 treatment, which significantly increased by 26.9%, 28.8%, and 27.4% in XH11 and 37.1%, 41.9%, and 37.7% in DH9, respectively, compared with N0. The HH16 exhibited the highest Chl a, Chl a+b, and chlorophyll a/b (Chl a/b) under the N105 treatment, showing significant increases of 13.7%, 12.6%, and 5.6%, respectively, compared with N0.

Dry matter and N accumulation

The N105 and N165 treatments significantly increased both pod dry matter (Pod DM) and pod N accumulation (Pod N) in the four peanut varieties, and dry matter (DM) and N accumulation in vegetative organs (root, stem, and leaf) also increased to varying degrees (Fig. 6). No

Table 1 The photosynthetic pigment content of leaves in peanut as affected by different N application rates

Variety	Treatment	Chl a (mg g⁻¹)	Chl b (mg g⁻¹)	Chl a + b (mg g⁻¹)	Chl a/b (mg g⁻¹)
NH5	N0	1.71 ± 0.05c	0.58 ± 0.02c	2.29 ± 0.07c	2.94 ± 0.00b
	N45	1.95 ± 0.08b	0.64 ± 0.03b	2.59 ± 0.11b	3.03 ± 0.02a
	N105	1.99 ± 0.06b	0.68 ± 0.03b	2.66 ± 0.09b	2.95 ± 0.04b
	N165	2.11 ± 0.08a	0.77 ± 0.04a	2.89 ± 0.12a	2.74 ± 0.04c
XH11	N0	1.75 ± 0.04c	0.59 ± 0.02b	2.34 ± 0.06c	2.96 ± 0.03b
	N45	1.80 ± 0.07c	0.57 ± 0.03b	2.37 ± 0.09bc	3.16 ± 0.05a
	N105	2.22 ± 0.11a	0.76 ± 0.06a	2.98 ± 0.17a	2.92 ± 0.08b
	N165	1.92 ± 0.04b	0.61 ± 0.01b	2.54 ± 0.05b	3.14 ± 0.02a
HH16	N0	1.83 ± 0.09b	0.64 ± 0.03b	2.46 ± 0.12b	2.88 ± 0.03c
	N45	2.03 ± 0.09a	0.71 ± 0.04a	2.74 ± 0.13a	2.87 ± 0.02c
	N105	2.08 ± 0.05a	0.69 ± 0.03a	2.77 ± 0.08a	3.04 ± 0.04a
	N165	2.02 ± 0.06a	0.68 ± 0.02ab	2.70 ± 0.07a	2.98 ± 0.01b
DH9	N0	1.40 ± 0.01c	0.43 ± 0.01c	1.83 ± 0.01c	3.25 ± 0.03a
	N45	1.61 ± 0.01b	0.50 ± 0.00b	2.11 ± 0.02b	3.25 ± 0.01a
	N105	1.92 ± 0.07a	0.61 ± 0.03a	2.52 ± 0.10a	3.14 ± 0.02a
	N165	1.80 ± 0.10a	0.55 ± 0.05ab	2.35 ± 0.15a	3.25 ± 0.09a
Two-way ANOVA	V	**	**	**	**
	N	**	**	**	**
	V × N	**	**	**	**

The different letters indicate the significant differences (*p* < 0.05) among the N treatments within each variety. In multivariate variance analysis, V and N represent the variety and N fertilizer treatment, respectively, * indicates *p* < 0.05, ** *p* < 0.01, and ns indicates not significance. Data from each treatment with three replicates were subjected to analysis of variance (ANOVA), and means were compared by the least significant difference (LSD) test (*p* < 0.05). Values are mean ± SE (*n* = 3)

significant difference in Pod DM was found among nodulating varieties NH5, XH11, and HH16 under N105 and N165 treatments (Fig. 6A), while Pod N was significantly increased by 18.6%, 19.1%, and 12.1% compared with N165, respectively (Fig. 6B). The DM and N accumulation in DH9 (non-nodulation) reached the maximum under N165 treatment, and Pod DM was significantly increased by 27.1% compared with N105, while the Pod N was not significantly different between the two treatments. Collectively, these data suggest that the N applications, varieties, and interactions between N applications and varieties were significant for N accumulation in various peanut organs.

Yield and its components

Compared with N0, all N treatments increased the single plant yield, pod number per plant, 100-pod weight, and 100-kernel weight among the four peanut varieties (Table 2). The yield and full pod number per plant of NH5, XH11, and HH16 reached the maximum under the N105 treatment, which significantly increased by 26.8%, 27.4%, and 30.4% and 41.2%, 32.7%, and 30.9%, respectively, compared with N0, but showed no significant
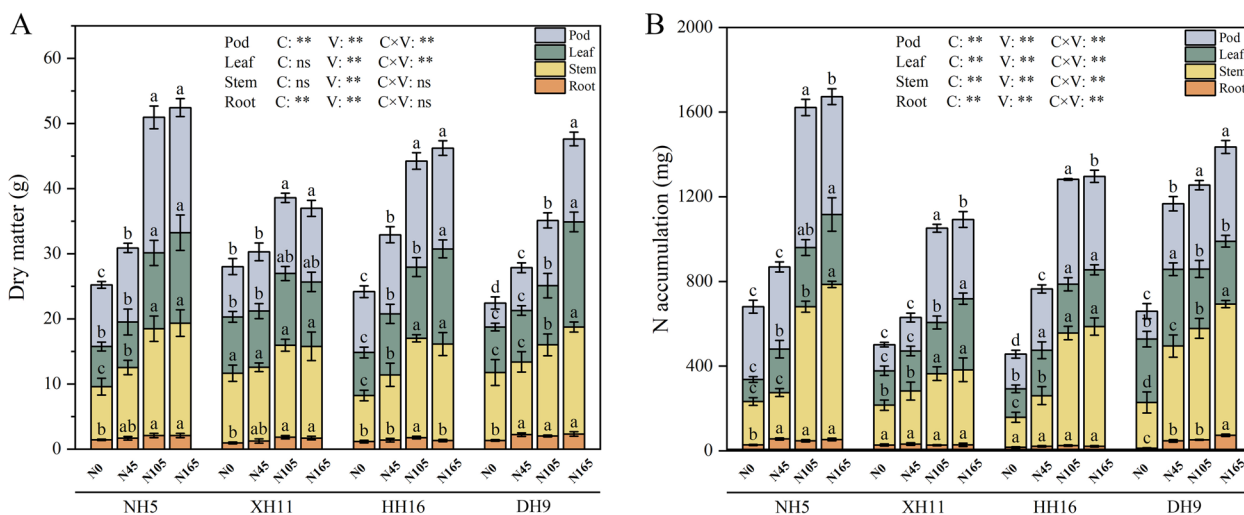
Fig. 6 Dry matter and N accumulation in each organ of peanut as affected by different N application rates. **A** and **B** represent dry matter and N accumulation respectively. The different letters indicate the significant differences ($p < 0.05$) among the N treatments within each variety. In multivariate variance analysis, V and N represent the variety and N fertilizer treatment, respectively, * indicates $p < 0.05$, ** $p < 0.01$, and ns indicates not significance. Data from each treatment with three replicates were subjected to analysis of variance (ANOVA), and means were compared by the least significant difference (LSD) test ($p < 0.05$). Values are mean ± SE ($n = 3$)

Table 2 Yield and its components of peanut as affected by different N application rates

Variety	Treatment	Single plant yield (g plant^{-1})	Pod number per plant	Full pod number per plant	100-pod weight (g)	100-kernel weight (g)
NH5	N0	22.76 ± 1.22b	21.33 ± 1.25b	17.00 ± 0.82c	165.30 ± 6.77b	63.67 ± 1.75b
	N45	23.71 ± 2.45b	23.00 ± 1.63b	19.33 ± 1.25b	170.22 ± 8.03ab	68.73 ± 1.88a
	N105	28.86 ± 2.29a	27.67 ± 2.05a	24.00 ± 1.41a	182.83 ± 9.68a	71.53 ± 2.30a
	N165	28.64 ± 2.25a	27.33 ± 1.70a	23.33 ± 1.25a	185.67 ± 7.36a	72.07 ± 3.74a
XH11	N0	25.10 ± 1.47b	22.00 ± 1.41c	18.33 ± 1.25b	164.17 ± 9.03a	63.03 ± 2.92b
	N45	26.61 ± 1.44b	24.67 ± 1.25bc	21.00 ± 0.82a	166.32 ± 6.21a	65.58 ± 3.08ab
	N105	31.97 ± 1.45a	29.00 ± 2.16a	24.33 ± 2.05a	172.80 ± 7.56a	72.10 ± 3.80a
	N165	31.26 ± 2.69a	27.67 ± 2.05ab	23.00 ± 1.63a	177.40 ± 9.76a	73.12 ± 3.39a
HH16	N0	21.60 ± 2.56b	21.00 ± 2.16b	18.33 ± 1.70b	145.30 ± 9.47a	61.68 ± 3.15a
	N45	24.49 ± 0.65ab	21.33 ± 2.05b	18.33 ± 1.25b	146.67 ± 9.32a	63.72 ± 3.83a
	N105	28.16 ± 1.85a	26.33 ± 1.25a	24.00 ± 1.41a	154.00 ± 5.10a	65.72 ± 3.44a
	N165	28.05 ± 2.48a	28.67 ± 1.70a	22.00 ± 1.41a	149.03 ± 5.61a	66.67 ± 3.06a
DH9	N0	18.23 ± 1.59b	25.67 ± 1.25c	21.67 ± 1.25c	106.41 ± 7.42a	46.62 ± 2.80a
	N45	20.57 ± 1.29b	27.00 ± 2.16c	23.00 ± 1.63c	111.56 ± 9.12a	48.81 ± 3.53a
	N105	23.84 ± 1.12a	35.00 ± 2.45b	30.00 ± 1.63b	119.98 ± 8.22a	49.27 ± 2.86a
	N165	24.47 ± 1.45a	39.67 ± 2.87a	34.00 ± 2.16a	117.32 ± 5.00a	52.31 ± 2.36a
Two-way ANOVA	V	**	**	**	**	**
	N	**	**	**	**	**
	V×N	ns	*	**	ns	ns

The different letters indicate the significant differences ($p < 0.05$) among the N treatments within each variety. In multivariate variance analysis, V and N represent the variety and N fertilizer treatment, respectively, * indicates $p < 0.05$, ** $p < 0.01$, and ns indicates not significance. Data from each treatment with six replicates were subjected to analysis of variance (ANOVA), and means were compared by the least significant difference (LSD) test ($p < 0.05$). Values are mean ± SE ($n = 6$)

difference compared with N165. Non-nodulating variety DH9 exhibited the highest yield under the N165 treatment, with an increase of 39.7% compared with N0.

In addition, NH5 and XH11 had the highest pod number under the N105 treatment, which was significantly enhanced by 29.7% and 31.8%, respectively, compared

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with N0. Meanwhile, HH16 and DH9 had the highest pod number under the N165 treatment, with an increase of 36.5% and 54.5%, respectively. The 100-kernel weight of the four varieties was the largest under the N165 treatment, and no significant differences in 100-pod weight and 100-kernel weight of HH16 and DH9 were observed among N treatments.
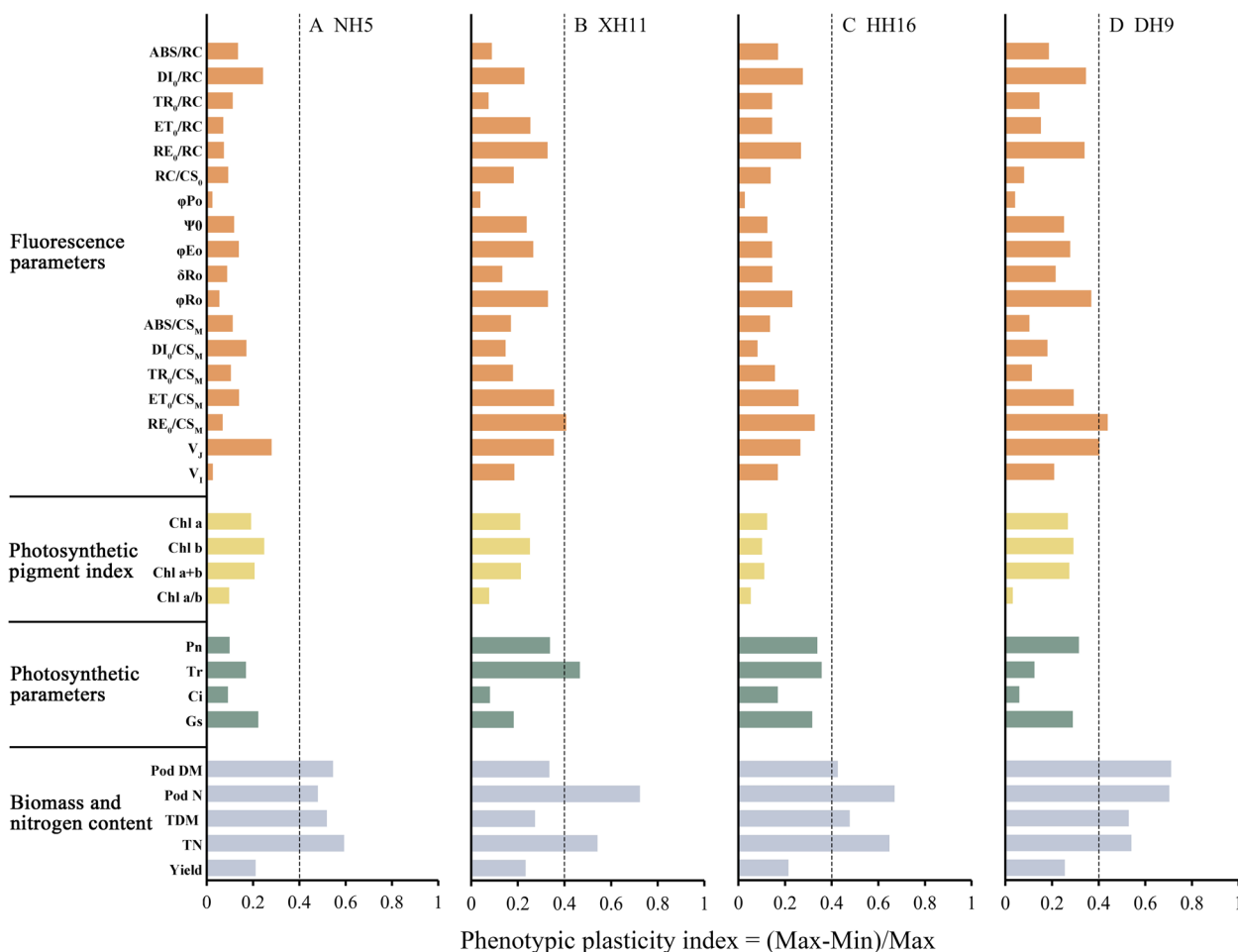
Plant phenotypic plasticity analysis

Analyses of plant plasticity can provide insight into their potential to adapt to changes in the external environment. As illustrated in Fig. 7, the plasticity index of φPo, one of the fluorescence parameters, was the smallest, and it showed stronger correlations with biomass and N content, and variations in plasticity index differed among peanut varieties. In terms of photosynthetic pigments index and photosynthetic parameters, the smallest plasticity index was detected in Chl a/b and Ci, respectively. The plasticity index of biomass and N content of NH5, HH16, and DH9

was higher than 0.4 (Fig. 7A, C, D), while the plasticity index of other indexes of NH5 and HH16 was lower than 0.4 (Fig. 7A, C). The plasticity of fluorescence parameters of the low nodulating variety (NH5) was lower, indicating that it had stable response to N fertilizer. The plasticity index of RE_0/CS_M, Tr, Pod N, and TN of XH11 exceeded 0.4 (Fig. 7B), and the plasticity index of RE_0/CS_M and V_J of DH9 was higher than 0.4 (Fig. 7D). These results demonstrated that different N application rates did not significantly affect φPo, Chl a/b, and Ci of peanut varieties, and the physiological response of peanut varieties to N fertilizer was different.

Correlation analysis

According to the comprehensive analysis among the indexes of four peanut varieties, we then performed a correlation analysis between the traits exhibiting higher plasticity indices (Pn, Tr, RE_0/CS_M, Pod N, and Yield) and the fluorescence parameters with strong responses to N

Fig. 7 Phenotypic plasticity analysis of four peanut varieties with different nodulation under different N application rates. Pod DM, pod dry matter; Pod N, pod N accumulation; TDM, total dry matter; TN, total N accumulation
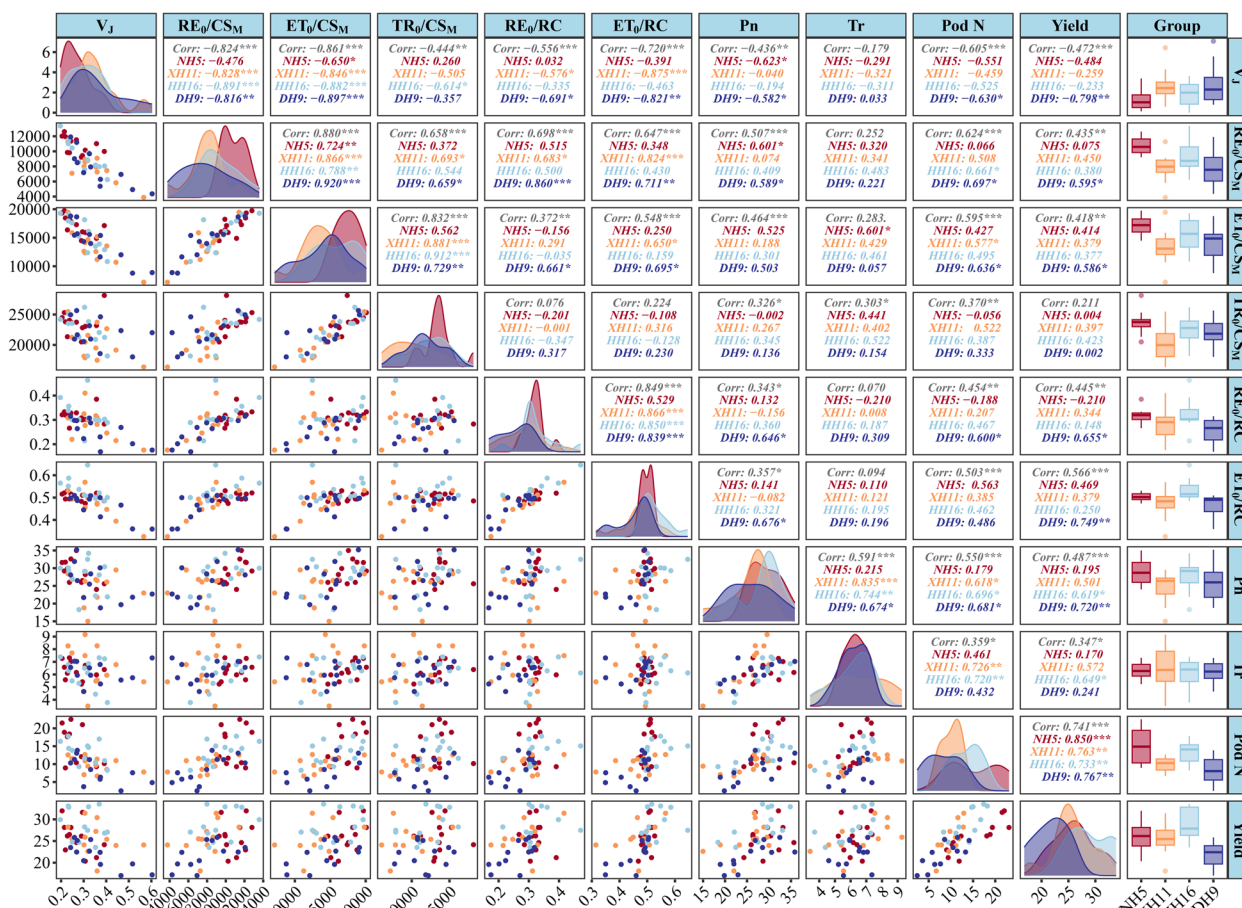
Fig. 8 Correlation analysis between photosynthetic physiological parameters and yield of four peanut varieties with different nodulation under different N application rates

fertilizer application (ET_0/CS_M, TR_0/CS_M, RE_0/RC, and ET_0/RC) (Fig. 8). The Pod N and Pn were significantly positively correlated with other indexes, and peanut yield exhibited a significantly positive correlation with all indexes except TR_0/CS_M. Except Tr, ET_0/CS_M, and RE_0/CS_M were significantly and positively correlated with other indexes, whereas V_J was significantly and negatively correlated with other indexes. Some distinct variations were observed in the correlation between the indices of peanut varieties. No significant correlation with fluorescence parameters indexes was observed for the yield and Pod N of NH5 (low nodulation), Pn and Tr of high nodulating varieties XH11 and HH16 were positively correlated with Pod N. The ET_0/RC, RE_0/RC, RE_0/CS_M, and Pn of DH9 (non-nodulation) were significantly positively correlated with yield, while V_J was significantly negatively correlated with yield, Pod N and Pn. Higher N treatments (N105 and N165) significantly decreased the V_J of DH9, which contributing to the increase its photosynthetic capacity and yield.

Discussion

Nitrogen (N) is a critical element in Calvin cycle enzymes, chlorophyll, and carotenoids, of which the chlorophyll content has a direct effect on the photosynthetic capacity and primary production [37, 38]. Previous studies demonstrated that N application is closely related to the photosynthetic capacity, yield, and agronomic traits [39, 40], and N deficiency can cause the closure of stomata in leaves, blocking photosynthetic electron transfer and ultimately reducing photosystem II (PSII) performance [23, 41]. A lack of N can also reduce chlorophyll content. This can lead to an increase in the allocation of photosynthetic electron transport to photorespiration, thus reducing the progression of photosynthesis, which is not conducive to the accumulation of dry matter (DM) [42]. Our study found that compared with N0 and N45 (lower N), higher N treatments (N105 and N165) increased the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and photosynthetic pigment content of leaves in the four peanut varieties.

Nodulating peanut varieties (NH5, XH11, and HH16) had the highest Pn and Tr in N105 (normal N), while DH9 reached the highest in N165. The Pn of nodulating peanut varieties was lower under the N165 treatment than under the N105 treatment. This may be due to the close arrangement of leaf mesophyll cells caused by excessive N application, which was not conducive to the entry of external CO_2 into the cells, resulting in reduced photosynthetic rate and even toxic effect on crops [7]. Therefore, applying N fertilizer properly can promote chlorophyll synthesis, improve photosynthesis during plant growth, and ultimately increase crop yield.

The OJIP transient detection technology has been widely employed to study the photosynthetic response of plants under abiotic stresses due to its advantages of being non-destructive and easy to use in practical measurements [43]. Since the accumulation of DM is closely related to the change in Pn and photosynthetic fluorescence parameters, the JIP parameters of peanut leaves were assessed. It has been hypothesized that the kinetic curves of OJIP can well reflect changes in the primary photochemical reaction and the photosynthetic function of PSII [44]. The rise of the O-J step on the OJIP curve is correlated with the degree of PSII reaction center closure. Furthermore, the change in fluorescence intensity at the J-step (V_J) can be used as an indicator of the electron transfer from primary acceptor plastoquinone A (Q_A) to secondary acceptor plastoquinone B (Q_B) on the electron acceptor side of PSII [43, 45]. Electrons originating from reduced Phe on the acceptor side of PSII are sequentially transferred to the first Q_A and second Q_B plastoquinone electron acceptors, and subsequently to the mobile pool of plastoquinone within the lipid phase of the thylakoid membrane [46]. In this study, the O-J step and V_J decreased under higher N application (N105 and N165), indicating that N application could improve the openness of the PSII reaction center and electron transfer from Q_A to Q_B on PSII acceptors. Correlation analysis revealed that V_J significantly negatively correlated with Pn and yield, which was consistent with a previous report [47]. The emergence of the K-phase in OJIP is associated with the damage of oxygen evolving complex (OEC) on the donor side of PSII, which plays a crucial role within PSII and is responsible for the cleavage and oxidation of H_2O [48, 49]. The lowest K point of nodulating peanut varieties (NH5, XH11, and HH16) was found under N105 (normal N) treatment. This indicated that peanuts under the N105 treatment exhibited greater stability in their OEC, which was conducive to maintaining PSII activity. As quantified by the ratio of relative variable fluorescence at the K-step to the amplitude $F_J − F_O$ (W_K and ΔW_K), the OEC was damaged under high N treatment (N165), resulting in a

reduced ability to deliver electrons downstream [50]. The ΔW_K of the non-nodulating variety DH9 was the lowest under the N165 treatment and significantly lower than that of N0, indicating that higher application of N may markedly improve the OEC activity on the PSII donor side, thus enhancing the electron transfer efficiency, which is ultimately conducive to yield formation.

A reduction in the secondary electron acceptors Q_B, plastoquinones (PQ), cytochrome and plastocyanin (PC) is related to the J-I step of the curve [51]. The relative variable fluorescence at the I-step (V_I) serves as an indicator of the ability of photosystem I (PSI) and its acceptors to oxidize reduced PQ [52, 53]. The V_I of the four peanut varieties under N105 and N165 treatments was lower than that under N45 and N0 treatments. Standardization of the O-I step (W_{OI}) demonstrated that a higher application of N (N105 and N165) increased the size of the terminal electron acceptor pool on the PSI acceptor side and enhanced electron transfer [54]. The amplitude of the $W_{OI} \geq 1$ of NH5 had no significant difference between the N treatments, indicating that N fertilizer exerted a relatively limited effect on the size of the terminal electron acceptor pool on the PSI acceptor side and the stability of the PSII donor side (K) and acceptor side (J) was stronger, which was conducive to maintaining the influence of N environmental changes on photosynthesis.

Limited N availability disrupts energy transfer within PSII. This occurs through the inactivation of reaction centers, which reduces the efficiency of converting absorbed light energy into usable electrons. Consequently, less excitation energy is captured, ultimately leading to decreased crop yield [53]. Nodulating peanut varieties (NH5, XH11, and HH16) had the lowest absorbed photon flux (ABS/RC), trapped energy flux (TR_0/RC), dissipated energy flux per active PSII (DI_0/RC), and quantum yield (at $t=0$) of energy dissipation (φDo) under N105 treatment, while the electron flux from $Q_A^−$ to the PQ pool per active PSII (ET_0/CS_M), electron flux reducing end electron acceptors at the PSI acceptor side per cross section (RE_0/CS_M), maximum quantum yield for primary photochemistry (φPo), quantum yield for electron transport (φEo), quantum yield for reduction of the end electron acceptors at the PSI acceptor side (φRo), and probability that a trapped exciton moves an electron into the electron transport chain Beyond Quinone A ($t=0$) (Ψ0) were the highest. Moreover, the N105 treatment promoted the transfer of absorbed energy in PSII, reduced the dissipated energy, and was conducive to improving the photosynthetic efficiency [55]. Accumulated studies have shown that when plants are exposed to excess light energy, they initiate a photoprotective strategy, maximizing the use of light energy while increasing

the loss of light energy to maintain normal plant growth [56]. In our study, the absorbed photon flux per active PSII (ABS/RC) of nodulating peanut varieties increased under the N165 treatment, indicating that some reaction centers of peanut PSII were deactivated [57]. The TR_0/RC, electron flux from Q_A^- to the PQ pool per active PSII (ET_0/RC), and DI_0/RC were higher under the N165 treatment than under the N105 treatment, suggesting that leaves may activate the photoprotection strategy and improve the high electron transfer efficiency (ET_0/RC) and heat dissipation (DI_0/RC) ability, which may prevent damage to the photosynthetic mechanism caused by excess light energy and maintain relatively higher photosynthetic capacity [58]. Meanwhile, non-nodulating DH9 was highly dependent on N fertilizer, and the activity of OEC and PSII reaction center increased and the DI_0/RC decreased with an increase in N application, which maintains a high electron transfer efficiency, ensuring high photosynthetic efficiency.

N is a critical component of plant growth and development and it is very important to ensure the balance between nitrogen supply and plant growth needs. A previous study reported a significant correlation between the accumulation of total N and both DM and pod yield in peanuts [59]. In other studies, N deficiency significantly reduced crop height, leaf chlorophyll, DM, and N accumulation [60], but these effects were alleviated by the application of N fertilizer [6]. However, excessive N application can negatively affect crop yield and DM [59]. Our study showed that N application increased DM, N accumulation, yield, and yield components of peanuts, among which the DM, N accumulation, yield, and full pod number per plant of nodulating peanut varieties (NH5, XH11, and HH16) reached the maximum under the N105 treatment. This may be because biological N fixation (BNF) was the main source for meeting N requirements in nodulating peanut varieties in the podding stage [26, 32]. The process of BNF in peanuts requires a certain amount of N initiation, and a small amount of N application is conducive to the formation of root nodules [61]. However, BNF is an energy-consuming process that requires strict control of the number of nodules [62]. To a certain extent, lower N application cannot simultaneously satisfy the BNF and the normal growth and development process of peanuts (N45), while excessive N application (N165) would reduce the
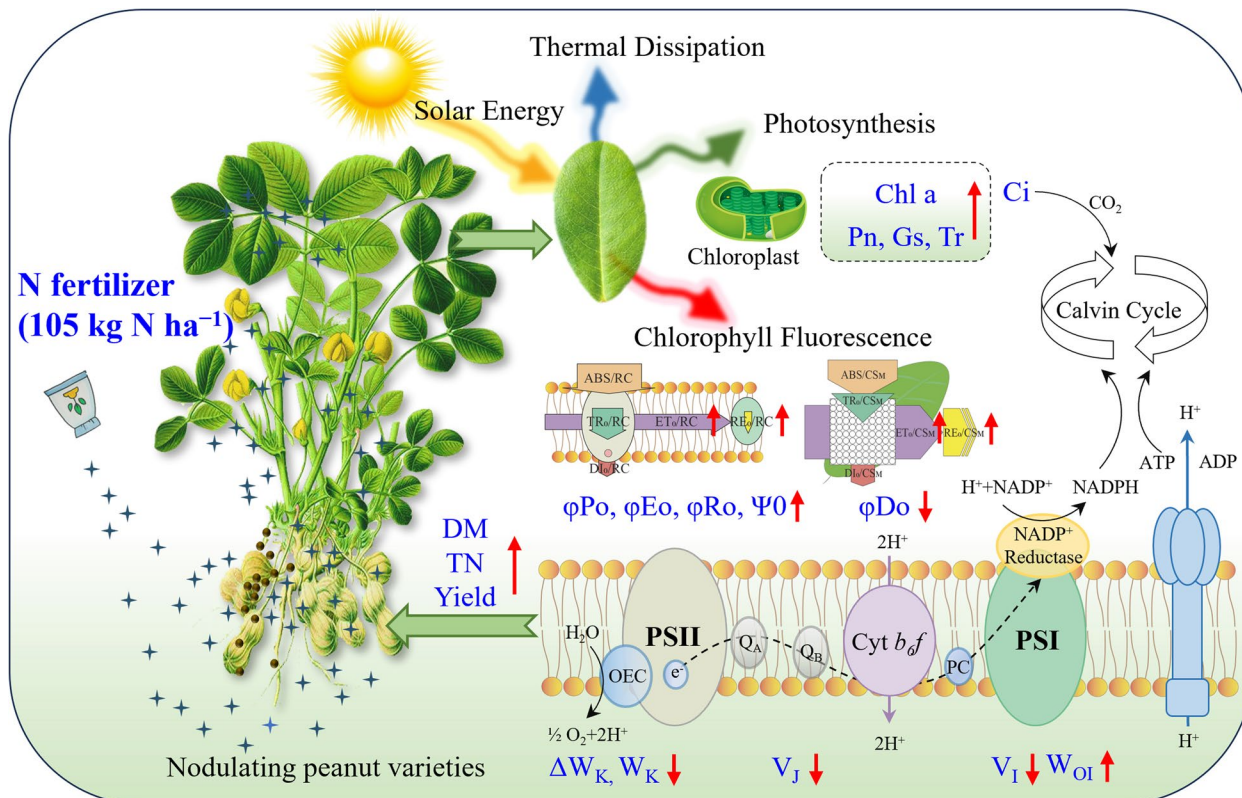
Fig. 9 A model of the adaptive strategy of the peanut photosystem after the application of nitrogen fertilizer. Chl a, chlorophyll a; Pn, net photosynthetic rate; Gs, stomatal conductance; Tr, transpiration rate; Ci, intercellular CO_2 concentration; PSI, photosystem I; PSII, photosystem II; Q_A, primary acceptor plastoquinone A; Q_B, the secondary acceptor plastoquinone B; Cyt b_6f, the cytochrome b_6f complex; PC, cytochrome and plastocyanin; OEC, oxygen evolving complex; DM, dry matter; TN, total N accumulation

number of root nodules [28]. Therefore, in our study, the nodulating peanut varieties had a higher yield potential under the N105 treatment, while the non-nodulating peanut variety (DH9) was more dependent on fertilizer for N sources, and thus reached the highest yield under high N treatment (N165).

The change in plant plasticity is an external representation of the change in its life activities. The plasticity index can directly reflect the ability of plants to adapt to environmental changes, and the higher the plasticity, the greater the potential to adapt to the environment [63]. In our study, the plasticity index of biomass and N content of four peanut varieties were higher, indicating that N fertilizer greatly promoted the growth and yield formation of peanuts. Some differences in the plasticity of various indexes were observed among varieties, among which, the plasticity of fluorescence parameters related to XH11 and DH9 was relatively higher (RE_0/CS_M), indicating that exogenous N fertilizer could promote the photosynthetic electron transfer in the peanut leaves, thus contributing to the improvement of photosynthetic efficiency.

Correlation analysis showed that Pn and Tr had a positive correlation with Pod N accumulation and yield, indicating that the leaf photosynthetic capacity is closely related to peanut yield, consistent with previous findings in rice [40], maize [23], and soybean [64]. The correlation between photosynthetic indices differed among the peanut varieties. Pn was significantly positively correlated with Pod N in high nodulating varieties (XH11 and HH16) and non-nodulating varieties (DH9), while Pn in low nodulating varieties (NH5) was not significantly different between treatments. However, the correlation of Pn with yield was not significant. This may be due to the higher N absorption capacity of NH5, which increases yield by distributing more N to the pods [32]. In addition, correlation analysis also indicated that the trapping of light energy (TR) contributes to the increase in peanut Pn, Tr, and Pod N, and the increase in electron transport flux (ET) and electron transfer to PSI receptor side terminal (RE) improves peanut yield. Xu et al. [55] reached similar conclusions in oilseed rape using different N application rates. Taken together, these results demonstrated that higher activity of PSII RCs, electron transport, quantum yield, and efficiency are conducive to the increase of yield (Fig. 9), providing a foundation for elucidating the interrelationship between peanut photosynthesis and crop yield.

Conclusion

In summary, the present study demonstrates that optimized nitrogen (N) application (N105) can improve the performance of the photosystem II (PSII) donor/acceptor side and the size of the terminal electron acceptor pool of the photosystem I (PSI) acceptor side, enhance photosynthetic electron transport, reduce the dissipated energy, and ultimately promote the photosynthesis efficiency of peanut leaves. Meanwhile, the N105 treatment can improve the photosynthetic pigment content and photosynthetic capacity of peanut leaves, which was conducive to N accumulation and dry matter, and then promote the formation of yield and yield components. However, electron transfer from primary acceptor plastoquinone A (Q_A) to the secondary acceptor plastoquinone B (Q_B) of PSII in nodulating peanut leaves was blocked, the oxygen evolving complex (OEC) was damaged, some reaction centers were deactivated, and photosynthetic capacity decreased under high N application (N165), while the non-nodulating peanut varieties had higher photosynthetic efficiency and yield. This study uncovers the photosynthetic electron transfer response mechanism of peanut varieties with different nodulations under different N fertilizer treatments and provides highly guidance for efficient N fertilizer management in cultivation and production.

Authors' contributions
PG, HY and CJ designed the research. HY, CJ and XZ contributed reagents and materials. PG, JR, XS, AX, PZ, YF and FG conducted the experiments, prepared figures and tables. PG, JR and XS prepared and revised the manuscript. HY and CJ organized and coordinated the whole project.

Availability of data and materials
Data has been included in the manuscript.

Declarations

Ethics approval and consent to participate
The peanut varieties in the current research are not threatened species. The authors declare that we comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction. Experimental research and field studies on plants comply with relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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