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Nitrogen allocation to biochemical fractions shapes functional traits and N use efficiency in tobacco cultivars

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KEYWORDS

Biomass production, functional nitrogen, nitrogen fractions, nitrogen use efficiency, tobacco cultivars

HIGHLIGHTS

- Two flue-cured tobacco cultivars with contrasting N use efficiency (NUE) were evaluated under three nitrogen application rates.
- Nitrogen allocation to functional fractions significantly influenced biomass and N accumulation.
- The high-NUE tobacco cv. Hongda showed greater allocation to photosynthetic and structural N under low and moderate N input.
- Stepwise regression revealed key nitrogen fractions contributing to cultivar differences in NUE.
- Physiological targets for breeding high-NUE cultivars and optimizing N management across crops were identified.

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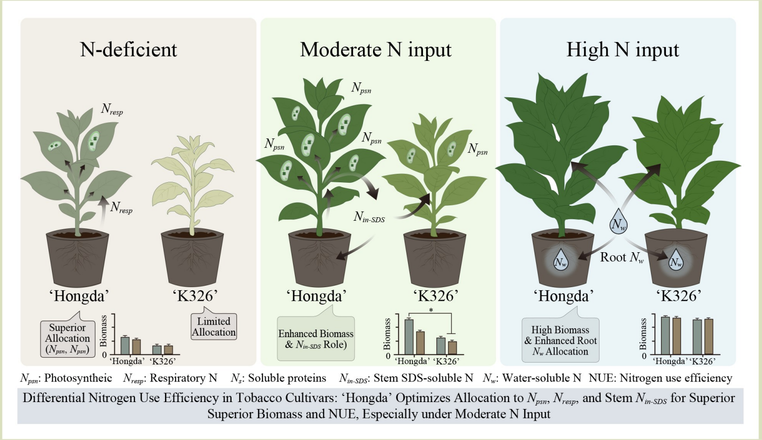
GRAPHICAL ABSTRACT



ABSTRACT

Nitrogen is essential for plant growth, however, excessive application leads to environmental pollution and increased production costs. Improving nitrogen use efficiency (NUE) is therefore critical for sustainable agriculture. This study aimed to elucidate how N fractions and functional allocations jointly regulate N accumulation and biomass production in flue-cured tobacco, with a focus on understanding physiological mechanisms underlying cultivar differences. Two contrasting tobacco cultivars, Hongda (higher NUE) and K326 (lower NUE), were grown under three N application levels: 0 g N per plant (CK), 4 g N per plant (T1) and 8 g N per plant (T2). Biomass and N accumulation were monitored across multiple growth stages. N was divided into four solubility

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fractions: water-soluble (N_w), sodium dodecyl sulfate (SDS)-soluble (N_s), SDS-insoluble ($N_{in\text{-}SDS}$) and non-protein N. These were then further divided into four functional categories: photosynthetic (N_{psn}), respiratory (N_{resp}), structural and storage N. Stepwise regression analysis was used to identify key N components influencing growth. Under N-deficient conditions (CK), greater allocation to N_{psn} and N_{resp} supported continued growth. Under moderate N input (T1), increased stem $N_{\text{in-}SDS}$ contributed to leaf biomass accumulation. At high N input (T2), elevated root N_w enhanced total biomass, reducing genotypic differences. N partitioning into distinct functional forms played a central role in regulating biomass production and overall NUE. The superior NUE of tobacco cv. Hongda was associated with its effective N allocation toward physiologically active pools and organs. These findings provide a physiological basis for optimizing N management and breeding tobacco cultivars with reduced fertilizer requirements.

1 Introduction

Nitrogen is a critical element for plant growth and development, as its accumulation is essential for the synthesis of numerous critical biological compounds, such as amino acids, proteins, nucleic acids, chlorophyll and some plant hormones[1]. N fertilizers are in crop production by supporting the synthesis of essential biological compounds and ensuring high yields. However, in pursuit of maximum output, farmers often apply N in excess of crop demand[2]. While such practices can initially improve yield, they also lead to significant N losses and contribute to environmental problems such as surface water eutrophication, soil acidification, increased greenhouse gas emissions and N deposition[3]. Therefore, improving nitrogen use efficiency (NUE) has become a key objective in sustainable agriculture, aiming to maintain productivity while reducing fertilizer input and minimizing ecological harm.

For years, many agronomic measures have been used in China to improve the NUE. Tamminen et al.[4] increased the recovery efficiency of spinach by 27.6% to 40.8% by applying biochar-based fertilizer. Hu et al.[5] increased the apparent recovery N use efficiency from 25.7% to 42.5% by applying controlled release urea to wheat. Samonte et al.[6] and Lu et al.[7] found that a reasonable proportion of urea and controlled release urea improved NUE by 33.9% compared with bare urea treatment on rice. Nevertheless, Chinese agricultural production still has a far lower NUE than Western countries[8]. Therefore, exploiting the biological potential of crops is a more effective way to increase the NUE of crops by pinpointing the causes of the differences between cultivars[9].

Nitrogen uptake efficiency (NUpE) and N utilization efficiency (NUtE) both contribute to plant NUE[10]. NUpE has been the subject of considerable attention, as cultivars with high NUpE can absorb more N than other cultivars[11]. Several strategies have been applied on roots to improve crop NUpE[12]. However, improving NUtE is more challenging because it involves complex internal metabolic and physiological processes including N assimilation, remobilization and allocation within the plant. These processes are influenced by multiple interacting genetic and environmental factors, making NUtE a more intricate trait to manipulate effectively[13]. Within-leaf N allocation is a crucial mechanism by which plants maximize their biomass[14]. Storage N is mainly used to produce new plant tissues, including structural and photosynthetic components. It may also be used to build defense enzymes, which can be necessary for plant survival[15]. More sodium dodecyl sulfate (SDS)-soluble proteins (N_s) indicate that plants can allocate more N to cell walls to promote both external protection and a means of cell-to-cell communication[16]. Given the complexity of N utilization, studying its dynamics in economically important crops such as flue-cured tobacco is essential to optimize NUE and improve both yield and quality under varying N conditions.

N application is a key cause of the variation in the distribution of the different fractions of N. Increased application of N fertilizer significantly enhances the leaf N content[17]. Leaf area and N utilization significantly increase under N-sufficient conditions compared to N-deficient conditions, whereas photosynthetic NUtE significantly decreases under low N supply[18]. Stored leaf N decreases under low N conditions, resulting in a reduction of the maximum net photosynthetic rate of plants[19].

Flue-cured tobacco is an important cash crop in China, and there are wide variations in NUE and N uptake capacity among cultivars[20,21]. For example, the flue-cured tobacco cv. Hongda consistently has higher NUE than cv. K326 and accumulates more N and biomass under the same fertilizer application regime[22]. Beyond their contrasting NUE performance, these two cultivars are also widely adopted in production due to their distinct agronomic traits: Hongda for its superior leaf quality and is widely used in premium tobacco products, and K326 for its strong stress resistance and broad adaptability. These contrasting characteristics make them ideal model cultivars for studying the physiological mechanisms underlying genotypic differences in N efficiency.

Although genotypic differences in NUE have been previously reported, the physiological mechanisms, particularly the role of N partitioning into different fractions, remain poorly understood. In this study, we addressed this gap by investigating how N allocation contributes to biomass production and N accumulation in these two contrasting cultivars. Specifically, we proposed that the higher NUE observed in Hongda may be associated with more efficient partitioning of N into functional pools that directly support plant growth.

Importantly, we considered that N and biomass accumulation are not only influenced by the total amount of N taken up but also by how N is allocated at specific growth stages. The distribution of N into various fractions during one developmental phase can affect the physiological status and resource use efficiency of the plant in the subsequent stages. Therefore, we designed our study to monitor the dynamics of N partitioning at 25-day intervals, covering key developmental stages. By analyzing the temporal patterns of N allocation and their effects on subsequent biomass and N accumulation, we aimed to identify stage-specific N allocation strategies that contribute to improved NUE. These insights are expected to inform more precise fertilizer application strategies and provide a theoretical foundation for breeding N-efficient tobacco cultivars.

2 Materials and methods

2.1 Experimental site

The field experiment was conducted in Yongping County, Dali Prefecture (25.607° N, 100.268° E) during the tobacco growing season from May to September 2021. The region experiences an average annual precipitation of about 919 mm and a mean annual temperature of 16.7 °C. The soil at the experimental site is classified as loam, with the following baseline physicochemical properties: organic matter, 36.6 $g \cdot kg^{-1}$; total nitrogen, 1.95 $g \cdot kg^{-1}$; total phosphorus, 0.82 $g \cdot kg^{-1}$; total potassium, 17.9 $g \cdot kg^{-1}$; available nitrogen, 233 $mg \cdot kg^{-1}$; available phosphorus, 49.4 $mg \cdot kg^{-1}$; and available potassium, 236 $mg \cdot kg^{-1}$.

2.2 Experimental design and conditions

An open-field pot experiment was conducted to assess the effects of varying nitrogen application rates on the NUE of two flue-cured tobacco cultivars, Hongda and K326. Seedlings aged 48–50 days were transplanted into pots (25 cm × 24 cm) filled with 15 kg of loam soil. Each pot received 1 L of water three times per week to maintain adequate soil moisture. Pots were arranged at a density equivalent to 16,500 plants ha^{-1}, with a spacing of approximately 110 cm × 55 cm[23], simulating field planting conditions.

The experiment employed a completely randomized design with a total of 180 plants, 90 for each cultivar. For each cultivar, 30 plants were assigned to each N treatment, with three biological replicates. Three N application rates were tested: 0 g N per plant (control, CK), 4 g N per plant (T1), and 8 g N per plant (T2). The selected rates reflect typical local fertilizer application practices, where 8 g N per plant represents the recommended rate for K326, and 4 g N per plant is commonly used for cv. Hongda, based on their respective N uptake capacities.

Additionally, each plant received 2.5 g of phosphorus and 10 g of potassium, maintaining an $N:P_2O_5:K_2O$ ratio of 1:1:2.5 based on the T1 treatment. Fertilizers were applied in two stages: 80% of the N and K and all of the P were applied as a basal dose at transplanting, while the remaining 20% of N and K were applied as topdressing 21 DAT. All agronomic practices adhered to the National Standards of the Tobacco Industry in China[24].

2.3 Harvesting and sample analysis

2.3.1 Dry samples

After the tobacco plants were transplanted, samples were collected from the plants at 25, 50, 75, 100 and 125 days after transplant (DAT). After being harvested, the plants were divided into roots, stems and leaves by cutting the plant organs apart from each other. For each treatment, three tobacco plants per replicate were uprooted and destructively sampled at each growth stage. To remove excess soil, the sampled plants were thoroughly rinsed with running water, as described by Hu et al.[25]. The fresh and dry weights of each plant part were recorded before and after curing. To ensure consistency, each plant part was initially dried at 105 °C for 30 min and then at 75 °C for 72 h as part of the curing process[26]. After being thoroughly crushed with a grinder, each dried plant part was sifted through a 2-mm mesh sieve before being crushed and digested with H_2SO_4-H_2O_2. Following the method outlined by the total N content of the digested plant material was determined through continuous flow analysis using an AA3 instrument (Seal Analytical Inc., Southampton, UK).

2.3.2 Fresh samples

Triplicate samples of middle leaves, middle stems and root tips were collected from the plants directly. The samples were rapidly frozen in liquid N for N fractions analysis.

2.4 Nitrogen determination

Continuous flow analysis (AA3, Seal Analytical Inc., Southampton, UK) was employed to determine the total N content in dried plant tissues, as well as N partitioned by fractions in fresh samples.

2.5 Nitrogen fractions

2.5.1 Conceptual framework for nitrogen partitioning

Nitrogen within plant tissues exists in diverse biochemical fractions that serve distinct physiological roles (Fig. 1). To better interpret NUE, nitrogen was classified from two complementary perspectives: (1) solubility and molecular association, and (2) physiological function in photosynthesis, respiration, structure or storage[26].

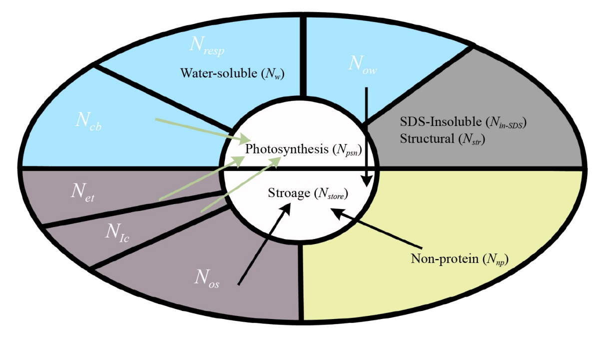

Fig. 1 The model for classifying nitrogen in plants. Nitrogen is categorized into SDS-insoluble nitrogen ($N_{in\text{-}SDS}$), water-soluble nitrogen (N_w), SDS-soluble nitrogen (N_s) and non-protein nitrogen (N_{np}) based on different fractions. $N_{in\text{-}SDS}$ is equivalent to structural nitrogen (N_{str}). N_w is further divided into respiratory nitrogen (N_{resp}) and water-soluble storage nitrogen (N_{ow}), with the remaining N_w after removing N_{cb} and N_{resp}. Carboxylation system nitrogen (N_{cb}) is also included. N_s is divided into electron transport nitrogen (N_{et}) and light capture system nitrogen (N_{lc}), with the remaining N_s, after removing N_{lc} and N_{et}, categorized as water-soluble storage nitrogen (N_{os}). N_{cb}, N_{et} and N_{lc} together combine to photosynthetic nitrogen (N_{psn}), while N_{ow}, N_{np} and N_{os} combine to storage nitrogen (N_{store}).

The first classification includes SDS-insoluble nitrogen ($N_{in\text{-}SDS}$),N_s, water-soluble nitrogen (N_w), and non-protein nitrogen (N_{np}). Functionally, nitrogen was grouped as photosynthetic nitrogen (N_{psn}), respiratory nitrogen (N_{resp}), structural nitrogen, and storage nitrogen, following the LUNA model framework[26].

2.5.2 Determination of nitrogen fractions

Methods for the determination of N partitioning, including the measurement of intermediate physiological indicators such as photosynthetic parameters and chlorophyll content, are detailed in the Supplementary Materials, with appropriate modifications for flue-cured tobacco.

2.6 Statistical analysis

Data were statistically analyzed using SPSS Version 23.0 (IBM, Amarok, NY, USA). Analysis of variance and stepwise multiple regression were performed to assess treatment effects and identify key N-related predictors of biomass and N accumulation. Significance was determined using the least significant difference test based on Tukey's procedure ($p < 0.05$). Model performance was evaluated using the coefficient of determination, F-statistic, root mean square error and corresponding p-values, all calculated within SPSS. All figures were generated using OriginLab 2022 (OriginLab Corporation, Northampton, MA, USA) and finalized in Adobe Illustrator 2019 (Adobe Inc., San Jose, CA, USA).

3 Results

3.1 Biomass and nitrogen accumulation of whole plants in tobacco cvs Hongda and K326 under three N application rates during different growth stages

Significant differences in nitrogen accumulation were observed between Hongda and K326 at different growth stages under varying N application rates (Fig. 2). Under no N application (CK) (Fig. 2(a)), Hongda had accumulated significantly more biomass than K326 at both 50 and 75 DAT ($p < 0.05$). Under the medium N treatment (T1) (Fig. 2(b)), Hongda had accumulated significantly more biomass than K326 at 25, 50 and 75 DAT ($p < 0.05$). Additionally, under T2 (Fig. 2(c)), Hongda had significantly more biomass accumulation than K326 at 75 DAT ($p < 0.05$).

As evident in Fig. 3, Hongda and K326 had different N accumulation patterns over time. Under no N application (CK) (Fig. 3(a)), Hongda had accumulated significantly more N than K326 at 50 DAT ($p < 0.05$). Under medium N treatment (T1) (Fig. 3(b)), Hongda had accumulated significantly more N than K326 during at 25, 50, 75 and 100 DAT ($p < 0.05$). Under T2 (Fig. 3(c)), Hongda had accumulated significantly more N than K326 at 50, 75 and 100 DAT ($p < 0.05$) whereas K326 had greater N accumulation only at 100 DAT. Additionally, Hongda had significantly higher N accumulation at 125 DAT under all treatments ($p < 0.05$).

3.2 Effect of nitrogen on leaf biomass and nitrogen accumulation in tobacco cvs Hongda and K326 under different N application rates during different growth stages

Under low N conditions (CK) (Fig. 4(a)), the relative biomass accumulation of Hongda was significantly greater than that of K326 at 50 DAT ($p < 0.05$). Under medium N treatment (T1) (Fig. 4(b)), Hongda had significantly higher relative biomass accumulation than K326 at 25, 50 and 75 DAT ($p < 0.05$). At 125 DAT, leaf biomass accumulation in Hongda was also significantly greater than in K326 ($p < 0.05$). Under T2 (Fig. 4(c)), Hongda had significantly higher relative leaf biomass accumulation than K326 at 75 DAT ($p < 0.05$). At 125 DAT, leaf biomass in Hongda was still significantly higher than that of K326 ($p < 0.05$).

The relative leaf N accumulation varied between cultivars at

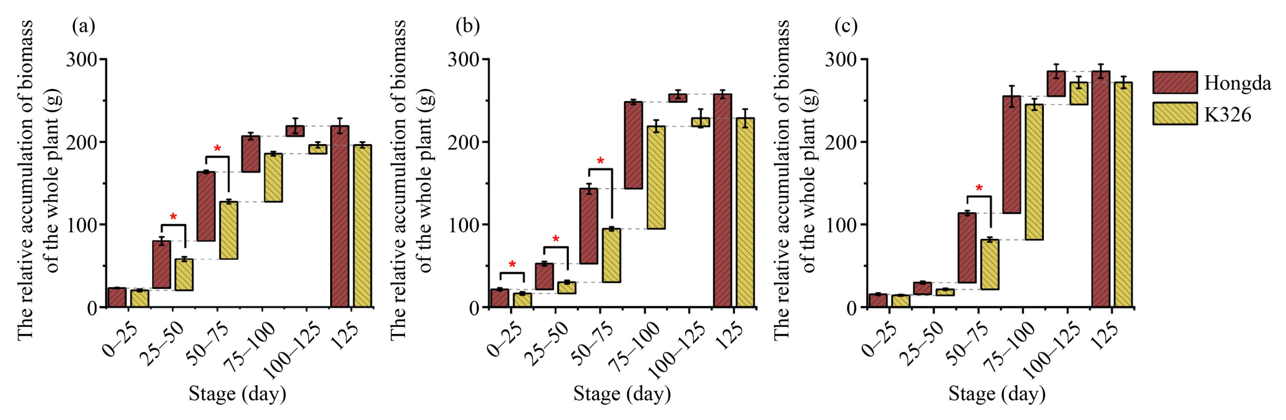

Fig. 2 Relative biomass accumulation of the whole plant in tobacco cvs Hongda and K326 at 25, 50, 75, 100 and 125 days after transplant under three N application rates: (a) CK (0 g per plant), (b) T1 (4 g per plant), and (c) T2 (8 g per plant). Asterisks indicate significant differences between cultivars at $p < 0.05$. Error bars represent the standard error of the mean.

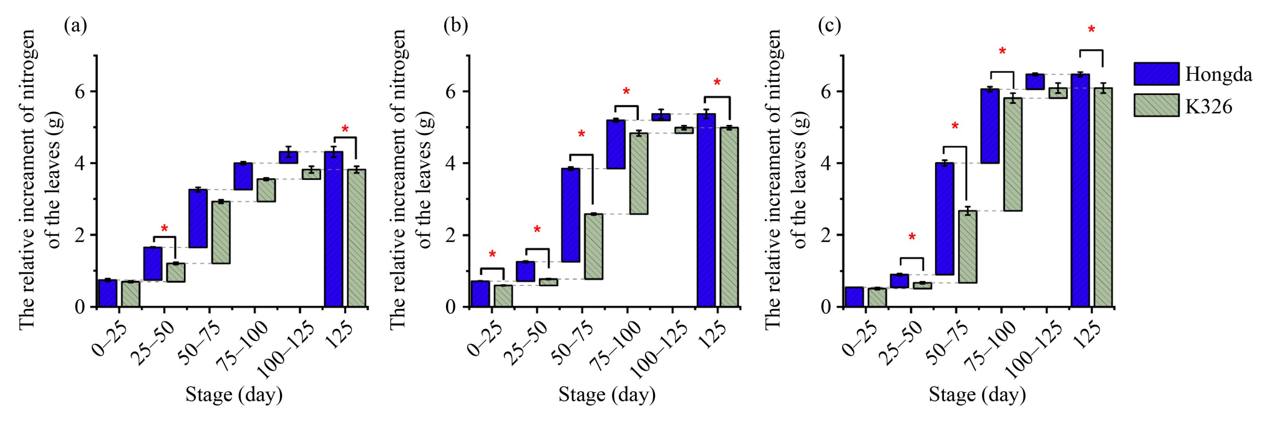

Fig. 3 Relative nitrogen accumulation of the whole plant in tobacco cvs Hongda and K326 at 25, 50, 75, 100 and 125 days after transplant under three N application rates: (a) CK (0 g per plant), (b) T1 (4 g per plant), and (c) T2 (8 g per plant. Asterisks indicate significant differences between cultivars at $p < 0.05$. Error bars represent the standard error of the mean.

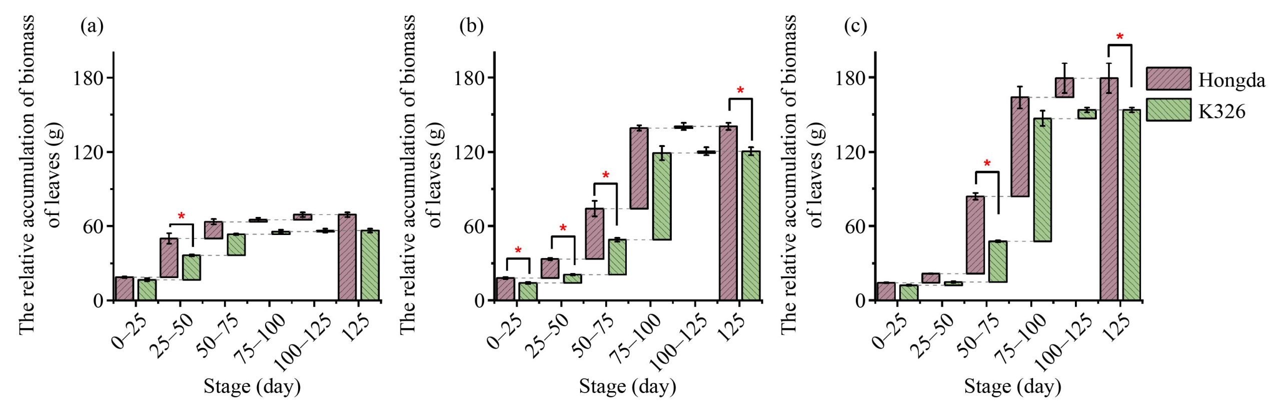

Fig. 4 Relative biomass accumulation of leaves in tobacco cvs Hongda and K326 at 25, 50, 75, 100 and 125 days after transplant under three N application rates: (a) CK (0 g per plant), (b) T1 (4 g per plant), and (c) T2 (8 g per plant). Asterisks indicate significant differences between cultivars at $p < 0.05$. Error bars represent the standard error of the mean.

different assessment times under different N treatments (Fig. 5). Under CK (Fig. 5(a)), Hongda had accumulated significantly more N in its leaves than K326 at 50 DAT ($p < 0.05$). Under medium N application (T1) (Fig. 5(b)), Hongda had accumulated significantly more N in its leaves than K326 at 75 DAT ($p < 0.05$). Under T2 (Fig. 5(c)), Hongda had accumulated significantly more N in its leaves at 75 DAT ($p < 0.05$). Additionally, Hongda had accumulated significantly more N in its leaves than K326 at 125 DAT ($p < 0.05$).

3.3 Stepwise regression analysis of nitrogen fractions distribution in relation to biomass and nitrogen accumulation at different growth stages

Using stepwise regression, various N fractions were identified in flue-cured tobacco plants at different growth stages, along with the subsequent 25-day increase in biomass. Our findings, summarized in Eqs. (1)–(3) (Table 1), revealed that under CK, N_{psn} promoted biomass accumulation. However, tobacco plants distributed more N_s in the leaves, which limited biomass

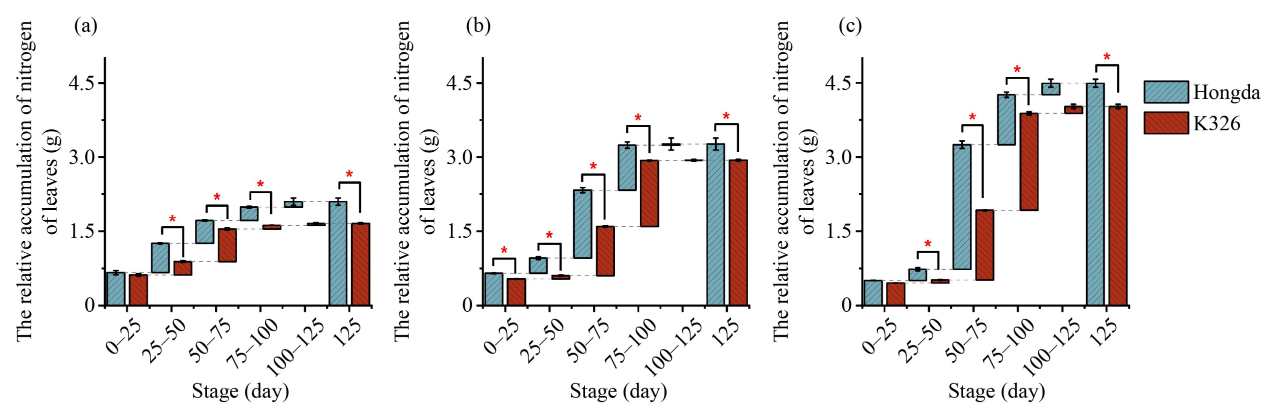

Fig. 5 Relative nitrogen accumulation of leaves in tobacco cvs Hongda and K326 at 25, 50, 75, 100 and 125 days after transplant under three N application rates: (a) CK (0 g per plant), (b) T1 (4 g per plant), and (c) T2 (8 g per plant). Asterisks indicate significant differences between cultivars at $p < 0.05$. Error bars represent the standard error of the mean.

Table 1 Summary of key nitrogen fractions and relative biomass accumulation under different nitrogen treatments

Treatment	The final regression model				Eq.
CK	$B_{\mathrm{RA}} = -14.46 + 4.21 \times N_{psn} - 3.48 \times (N_s \text{ in leaves})$				(1)
	$R^2 = 0.98$				
T1	$B_{\mathrm{RA}} = 32.37 + 359.01 \times N_{lc} - 2.91 \times N_{os}$				(2)
	$R^2 = 0.83$	F = 12.17	RMSE = 22.68	$P = 0.012$	
T2	$B_{\mathrm{RA}} = -18.81 + 19.59 \times (N_w \text{ in roots})$				(3)
	$R^2 = 0.80$	F = 10.78	RMSE = 38.24	$P = 0.017$	
CK	$B_{\mathrm{RA}} \text{ in leaves} = 19.54 - 2.65 \times (N_s \text{ in roots}) + 0.55 \times N_{ow} - 2.25 \times (N_s \text{ in stems}) - 0.51 \times (N_w \text{ in roots})$				(4)
	$R^2 = 0.99$	F = 478.79	RMSE = 0.66	$P = 0.001$	
T1	$B_{\mathrm{RA}} \text{ in leaves} = -74.12 + 342.74 \times N_{lc} + 2.71 \times N_{np} - 2.25 \times (N_s \text{ in stems}) - 0.51 \times (N_w \text{ in roots})$				(5)
	$R^2 = 0.95$	F = 25.32	RMSE = 8.16	$P = 0.005$	
T2	$B_{\mathrm{RA}} \text{ in leaves} = -11.52 + 11.53 \times (N_w \text{ in roots})$				(6)
	$R^2 = 0.95$	F = 25.32	RMSE = 8.16	$P = 0.005$	

Note: CK, 0 g per plant (no nitrogen application); T1, 4 g per plant; T2, 8 g per plant; BRA, relative biomass accumulation; N_{psn}, photosynthetic nitrogen; N_s, SDS-soluble nitrogen; N_{lc}, nitrogen in the light capture system; N_{os}, SDS-soluble storage nitrogen; N_w, water-soluble nitrogen; N_{np}, non-protein nitrogen; $N_{in\text{-}SDS}$, SDS-insoluble structural nitrogen; and N_{ow}, water-soluble nitrogen of storage.

accumulation (Eq. (1)). Under medium N application (T1), the light capture system N (N_{lc}) contributed to increased biomass in the following 25 days. However, the accumulation of SDS-soluble N in storage (N_{os}) negatively affected overall plant biomass (Eq. (2)). UnderT2, more N_w in the roots was associated with greater biomass accumulation (Eq. (3)).

Equations (4)–(6) (Table 1) reveal how the distribution of N affects biomass accumulation in tobacco leaves (Table 1). Under CK, the water-soluble N in storage (N_{ow}) in leaves enhanced leaf production, while N_s in roots, N_s in stems and N_w in roots hindered leaf biomass accumulation (Eq. (4)). Under medium N application (T1), N_{lc}, N_{np} and $N_{in\text{-}SDS}$ in stems contributed to increased leaf production (Eq. (5)). Under T2, N_w in the roots promoted greater leaf biomass accumulation (Eq. (6)).

Through stepwise regression, we found that various N fractions influenced N accumulation in flue-cured tobacco plants at different growth stages over the subsequent 25 days. Our findings, represented by Eqs. (7) and (8) (Table 2), showed that without N application, a higher distribution N_{resp} was beneficial for N accumulation in the whole plant (Eq. (7)). Conversely, additional N distributed as N_{os} under medium N application (T1) restricted N accumulation (Eq. (8)). No significant correlation was observed between the rise in N and its various fractions under T2.

N_w in the leaves under CK promoted relative N accumulation in the leaves (Eq. (9), Table 2)). However, we were unable to establish a clear relationship between N-related accumulation in the leaves and N distribution under both T1 and T2 treatments.

3.4 Differences in nitrogen fractions affecting biomass and nitrogen accumulation between tobacco cultivars at different growth stages under low and medium nitrogen application

Under low N application, key factors influencing the relative accumulation of biomass and N included N_{psn}, N_{ow}, N_{psn} and N_s in leaves, as well as N_s and N_w in roots, and N_s in stems (Fig. 6(a,b)). N_{psn} promoted biomass accumulation in the entire plant and leaves, while N_s limited biomass accumulation. The distribution of N_{psn} in Hongda at 25, 50, 100 and 125 DAT was

Table 2 Summary of key nitrogen fractions and relative nitrogen accumulation under different nitrogen treatments

Treatment	The final regression model				Eq.
CK	$N_{RA} = -0.96 + 0.96 \times N_{resp}$				(7)
	$R^2 = 0.73$	F = 16.57	RMSE = 0.31	$P = 0.007$	
T1	$N_{RA} = 2.25 - 0.17 \times N_{os}$				(8)
	$R^2 = 0.71$	F = 6.22	RMSE = 0.75	$P = 0.047$	
T2	N_{RA} in leaves $= -0.22 + 0.017 \times (N_w$ in leaves)				(9)
	$R^2 = 0.79$	F = 22.85	RMSE = 0.12	$P = 0.003$	

Note: CK, 0 g per plant (no nitrogen application); T1, 4 g per plant; T2, 8 g per plant; N_{RA}, relative nitrogen accumulation; N_{resp}, respiratory nitrogen; N_{os}, SDS-soluble fractions of storage nitrogen; and N_w, water-soluble nitrogen.

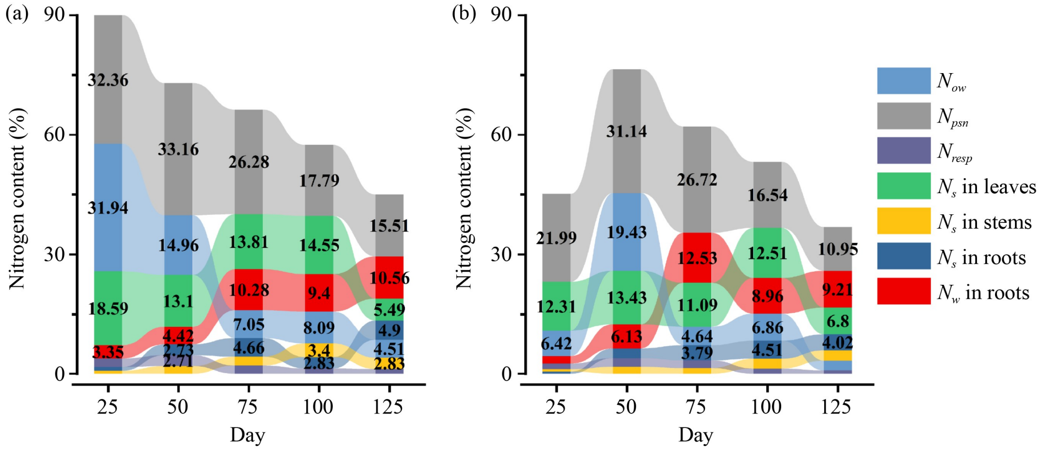

Fig. 6 Accumulation of selected nitrogen fractions in two tobacco cultivars, Hongda (a) and K326 (b), with 0 g per plant at 25, 50, 75, 100, and 125 days after transplant. N_{ow} is the water-soluble fractions of storage nitrogen; N_{psn} is photosynthetic nitrogen; N_{resp} is respiratory nitrogen; and N_s is total SDS-soluble nitrogen.

higher than that in K326. Similarly, the distribution of N_s in leaves was also higher in Hongda than in K326 at 25, 75, and 100 DAT. N_s in roots and stems, as well as N_w in roots, limited relative N accumulation, whereas N_{ow} promoted N accumulation in both the whole plant and leaves. Hongda allocated more N to N_{ow} than K326 at 25, 75, 100 and 125 DAT. Additionally, at 25 and 125 DAT, Hongda allocated more N to N_s in roots and stems, and N_w in roots, compared to K326.

Under T1, N_{lc}, N_{os}, N_{np} and $N_{in\text{-}SDS}$ in stems were key factors influencing the relative accumulation of biomass and N (Fig. 7(a,b)). In Hongda, a higher distribution of N as N_{lc} contributed to increased biomass in the entire plant, while N_{lc}, N_{np} and $N_{in\text{-}SDS}$ in stems enhanced leaf biomass. However, N_{os} limited relative N accumulation in the leaves. At 50, 100 and 125 DAT, K326 allocated more N as N_{lc} and N_{np} in stems, but less N as $N_{in\text{-}SDS}$ in stems.

Although K326 had a higher ratio of N_w in the roots, the N accumulation data showed an opposite trend. N_w in the roots promoted biomass accumulation under T2 (Fig. 8(a,b)). However, the relationship between N accumulation and the various N fractions still requires further investigation.

4 Discussion

Nitrogen is an integral nutrient for plant growth and development[27]. However, excessive N application leads to water eutrophication and economic costs[28]. Researching the mechanism of NUE is important to improve environmental conditions and reduce energy consumption. It has been widely accepted that NUE results from N and biomass accumulation[29]. Thus, understanding why cultivars with high NUE accumulate more N and biomass under the same fertilizer application regime is a key research objective.

By sampling at 25-day intervals throughout the growth period, we systematically quantified the accumulation patterns of various N fractions. Our results demonstrate that the accumulation of specific N fractions significantly influences subsequent N uptake and biomass accumulation. This finding confirms that the dynamic partitioning of N into distinct fractions is a key mechanism underlying variations in NUE.

Previous studies have reported significant differences in NUE among flue-cured tobacco cultivars, attributed to their varying abilities to accumulate biomass and N in both whole plants and leaves under the same environmental conditions, with Hongda exhibiting higher NUE than K326[9]. This study tracked the rise in N and biomass in the entire plant and leaves at 25 day intervals compared to earlier periods under CK (0 g per plant). Hongda accumulated more biomass and N in the whole plant and leaves by 50 DAT. Hongda accumulated more biomass and N under various N treatments at 125 DAT. These results

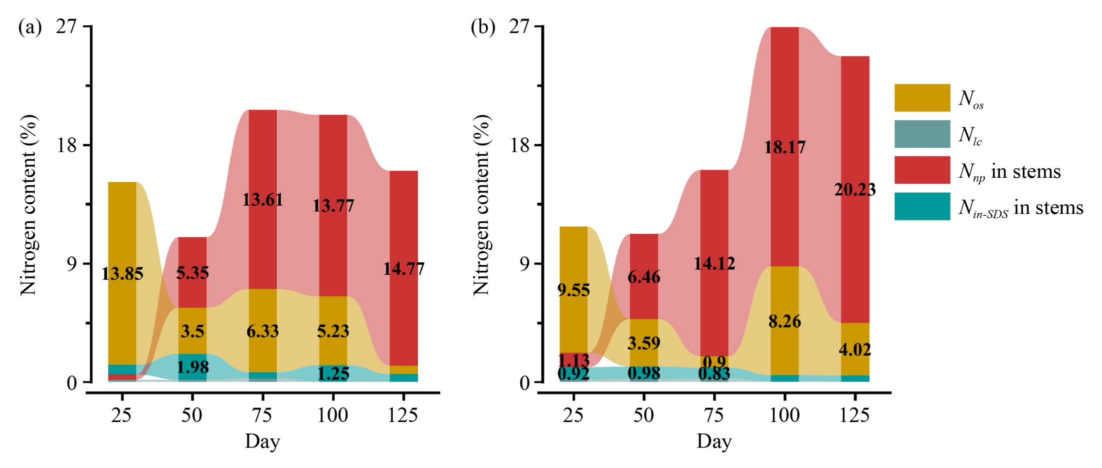

Fig. 7 Accumulation of selected nitrogen fractions in two tobacco cultivars, Hongda (a) and K326 (b), with 4 g per plant at 25, 50, 75, 100, and 125 days after transplant. N_{os} is the SDS-soluble fractions of storage nitrogen; N_{lc} is nitrogen allocated to the light capture system; and N_{np} is the non-protein nitrogen.

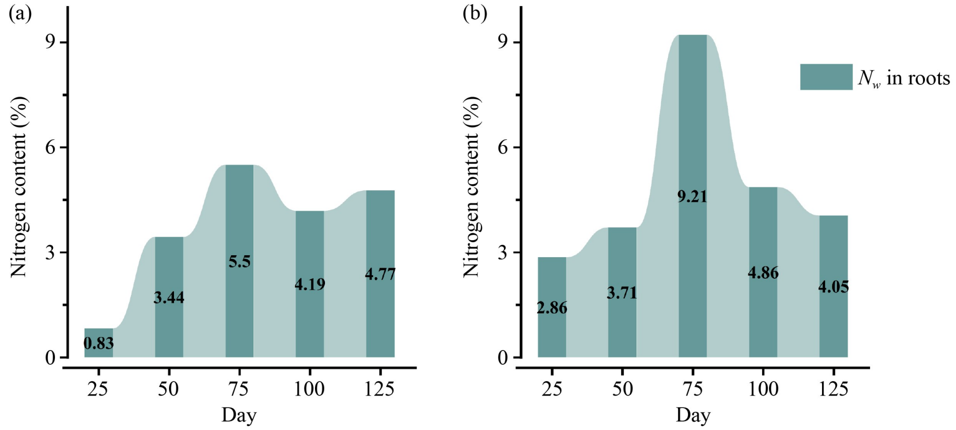

Fig. 8 Accumulation of selected nitrogen fractions in two tobacco cultivars, Hongda (a) and K326 (b), with 4 g per plant at 25, 50, 75, 100, and 125 days after transplant. N_w is water-soluble nitrogen.

indicate that Hongda has stronger biomass and N accumulation abilities than K326, consistent with previous studies[9,30]. Similar to other observations, the biomass and N relative buildup suit an S-model[31]. The relative accumulation rates of N and biomass increased first and then slowed down; under CK, both the increase and slowdown occurred earlier than T1 (4 g per plant) and T2 (8 g per plant), maybe as a result of the inhibition brought on by a high N environment[32].

The distribution of N was highly complex. As the rate of N increased, N_w and N_s in the roots and $N_{in\text{-}SDS}$, N_w and N_s in the stems decreased. The content of the N_{np} in stems and N_w in leaves constantly increased as the rate of N increased. N_w in roots and stems may be related to root respiration[33]. Root respiration provides the driving force for root growth and maintains ion absorption and transport into the xylem[34]. Stem respiration is an important component of an ecosystem carbon budget related to cell growth in the stem[35]. N_s contributes to leaf expansion and photosynthetic capacity in winter oilseed rape[33], and the decrease of N_w and N_s in roots and stems may explain why the high N environment limited the development of roots and stems[12]. $N_{in\text{-}SDS}$ is also structural N. Decreases in $N_{in\text{-}SDS}$ may occur because more N has been absorbed and more N is distributed to metabolic processes and storage functions[33].

N_{np} in stems and N_w in leaves constantly increased as the rate of N delivery increased. N_w in leaves is related to respiration and photosynthesis. Higher N_w content enhances enzymatic activities related to carbon assimilation and energy metabolism, such as Rubisco-mediated carboxylation in the Calvin cycle and mitochondrial respiration for ATP production[36]. Therefore, increased N_w in leaves under higher N input likely reflects the greater allocation by the plant to metabolic functions that support rapid biomass accumulation. Additionally, N_{np}, as part of storage N, may contribute to expanding photosynthetic capacity by supporting the synthesis of structural proteins and enzymes necessary for leaf development[33].

Under different treatments, Hongda and K326 had variable relative accumulations of N and biomass. Takashima et al.[37] reported that photosynthesis rates influenced the rate of biomass and N accumulation. The N accumulation rate relies on the rate of nitrogen uptake by the roots[38]. Many previous studies have discussed the mechanism of biomass and N accumulation[39]. However, how the N distribution in plants affects the rate of biomass production and N accumulation and whether the N distribution determines the variation in biomass production and N accumulation capacities between cultivars still need to be determined.

To determine how N distribution can affect biomass and N accumulation in tobacco, we investigated the link between the distribution of N fractions and the relative accumulation of biomass and N in various periods. The outcome demonstrated that N distribution significantly influenced the relative accumulation of N and biomass under various N conditions.

N_{psn} and N_{ow} promoted biomass accumulation in whole plants and leaves, respectively, without N application (CK). N_{psn} can indicate how intense photosynthesis is. Photosynthesis is the source of plant biomass[40], and higher photosynthesis rate has been shown to help increase the buildup of crop biomass[41]. Storage N benefits leaf expansion in leaf growth[42,43]. Hongda accumulated more biomass in the whole plant and leaves because it delivered more N as N_{psn} and N_{ow} at various times. In addition, Hongda dispersed more N_s in stems and N_w in roots, preventing biomass buildup. These might have contributed to the different accumulations among periods, but they are not the cause of the distinction between Hongda and K326.

Under different N conditions, the primary variables affecting biomass and N accumulation varied. For example, under medium N applications (T1), N_{lc} increased, but N_{os} decreased, biomass accumulation of the whole plant. The leaf biomass accumulation was enhanced by N_{lc}, N_{np} and $N_{in\text{-}SDS}$ in the stems. N_{lc} in photosystems I and II, chlorophyll a/b complexes, proteins used as enzymes in the electron transport chain, and proteins for carboxylation in Calvin cycle enzymes are used to produce ATP and NADPH[44]. The distribution of N in N_{lc} was greater in K326 than in Hongda, which is the reverse of the biomass accumulation outcome. N_{lc} was likely not to be the cause of the difference between Hongda and K326 but rather influenced the buildup of biomass over different periods. $N_{in\text{-}SDS}$ is also structural N in plants. Thus, $N_{in\text{-}SDS}$ promotes stem growth, and stems enhance the leaf biomass by transporting nutrients and water[45]. Hongda allocated more N to $N_{in\text{-}SDS}$ than K326, promoting stem growth and resulting in more biomass accumulation in the leaves. Compared to K326, Hongda allocated more N to $N_{in\text{-}SDS}$, promoting stem growth and increasing biomass accumulation in the leaves. Although the physiological role of N_{np} is still unclear, our results indicate that it may contribute to leaf biomass accumulation among periods rather than to the distinction between Hongda and K326.

Under T2, N_w in the roots can benefit biomass accumulation. N_w includes N_{resp}, which may benefit root respiration. Root respiration is a critical physiological trait involved in root resource acquisition strategies. It is a fundamental metabolic process responsible for growth, ion mobilization and uptake, and cell maintenance through three distinct functions: ATP production, carbon skeleton production and redox balancing[46]. Nevertheless, it was related to biomass accumulation among periods instead of to the reason for the difference in cultivars. The leaf N content is one of the determinants of vegetation productivity[47]. The distribution of N_{resp} benefits N accumulation in tobacco during different periods under CK. Our results are similar to those of previous reports [34] reported that respiration improved along with increased N in leaves. Also, Florian et al.[48] reported respiration with strong and clear links to N metabolism. Hongda distributed more N as N_{resp}, which benefitted respiration, resulting in more N accumulation in the whole plant[49]. N_w in leaves, which promotes N accumulation in leaves, contains N_{resp}, carboxylation system (N_{cb}), and N_{ow}. In addition to N_{resp} and N_{ow}, N_{cb} was positively correlated with the maximum carboxylation rate and the maximum net photosynthetic rate of plants, benefitting biomass accumulation[50], which may result in higher leaf biomass. This could have promoted more N accumulation in the leaves of Hongda compared to K326 under low N environment.

N_{os} is also associated with the coordinates of leaf expansion and photosynthetic capacity[33], indicating that excessive leaf expansion and photosynthetic capacity may consume more N in leaves. However, in our study, N_{os} was related to the N accumulation in leaves between assessment times rather than to the difference between Hongda and K326. Increasing crop NUE can lead to less N input for sustainable productivity or for increasing crop yield and N accumulation while maintaining the same N supply. The physiological mechanism of high NUE cultivars is currently the subject of intense research. Future research should aim to clarify the high NUE mechanism by elucidating the interaction between distinct biomass distributions and N accumulation in high N environments.

These findings offer important implications for improving N management in flue-cured tobacco and potentially other crops. By elucidating how N is differentially allocated into photosynthetic, respiratory, structural and storage fractions, this study identifies key physiological traits associated with high NUE. For example, directing N toward photosynthetic and storage pools in leaves can enhance biomass production without excessive fertilizer input. The superior performance of Hongda under low and medium N conditions highlights the breeding potential of cultivars with efficient N partitioning. Such strategies not only contribute to environmental sustainability but also offer clear economic advantages, reducing fertilizer costs per unit of biomass and improving N return efficiency. Although this research focused on tobacco, the underlying N allocation mechanisms are likely conserved across species, indicating that these insights can inform breeding and nutrient management practices in other N-dependent crops such as maize, rice and wheat.

5 Conclusions

This study demonstrates that the distribution of nitrogen among various fractions is a key determinant of biomass and nitrogen accumulation in tobacco. The high-NUE cv Hongda showed consistently superior performance compared to K326, primarily due to its more efficient allocation of nitrogen into N_{psn}, N_{resp} and N_{ow} fractions under low nitrogen conditions, and into structural nitrogen under medium nitrogen application. These partitioning patterns contributed directly to enhanced nitrogen retention and biomass production.

Our findings highlight key physiological indicators, such as elevated N_{psn} and nitrogen content of $N_{in\text{-}SDS}$, that may serve as valuable selection targets in breeding programs aimed at improving NUE. By promoting more effective internal nitrogen allocation, these traits support higher productivity while reducing dependence on excessive nitrogen application. This can lead to both environmental gains, through reduced nitrogen losses, and economic benefits, including lower input costs per unit of biomass produced.

Although the present study focuses on flue-cured tobacco, the functional roles of nitrogen pools such as photosynthetic and structural nitrogen are conserved across many higher plants. Therefore, the nitrogen partitioning strategies identified here are likely not tobacco-specific but may also operate in other major crops such as maize, rice and wheat. Further comparative studies are warranted to determine the extent to which these mechanisms are conserved across species and to inform broader breeding and management strategies for sustainable, nitrogen-efficient agriculture.

Supplementary materials

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Compliance with ethics guidelines

Guiru Zhao, Shichen Li, Xiaoyao Ding, Xiaoci Peng, Waqar Ahmed, Shaoming Li, and Zhengxiong Zhao declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, we used ChatGPT as an English editing tool to polish the manuscript to minimize grammatical errors. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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