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Effects of organic fertilizer nitrogen substitution on non-structural carbohydrates and biological carbon sequestration of *Zanthoxylum armatum*

Abstract: Effective nitrogen (N) management is crucial for enhancing plant photosynthesis, non-structural carbohydrates (NSC) formation, and carbon (C) sequestration in perennial plants. However, the effects of partial substitution of chemical N with organic N on these processes remain poorly understood. A pot experiment was conducted using *Zanthoxylum armatum* to examine seven levels of organic N substitution (0%, 10%, 20%, 30%, 40%, 50%, and 100%) and a no-fertilizer control. Plant growth, photosynthetic pigments, gas exchange, NSC content, and biomass C accumulation were measured. Fertilization enhanced growth, pigment content, and NSC accumulation. Moderate organic N substitution further improved photosynthetic efficiency, stomatal regulation, NSC biosynthesis, and biomass C storage, with regression analysis indicated an optimal substitution rate of 53.3%. The novel finding of this study is that partial organic N replacement can simultaneously enhance photosynthesis, carbohydrate allocation, and biomass C accumulation in *Z. armatum*, while reducing chemical N input under controlled conditions. These results provide mechanistic insight into optimizing organic N fertilization and identify a practical strategy to improve physiological performance and C storage in perennial woody plants.

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Introduction

Nitrogen (N) is of great importance for plant nutrition and N fluxes in ecosystems (Dessureault-Rompré, 2022). The mineral N is traditionally viewed as the main plant-available form (Zhang et al., 2018). However, many species can also absorb soluble organic N, including amino acids, especially in nutrient-poor or cold ecosystems where mineralization is limited (Näsholm et al., 2009; Pallett et al., 2024; Weigelt et al., 2005). In some species, direct uptake of amino acids may contribute obviously to a plant's N budget (Czaban & Rasmussen, 2021). This adaptation highlights an ecological challenge in both managed and natural ecosystems, where excess inorganic N inputs disrupt plant-microbe symbioses and alter carbon-nitrogen coupling (Wu et al., 2024). As a potential solution, integrating both organic and inorganic N sources offers a more sustainable approach. However, the physiological mechanisms behind this integration, particularly in woody perennials and tree species, remain poorly understood.

Globally, anthropogenic N deposition and fertilizer application have more than doubled over the past century, driving soil acidification, eutrophication, and forest nutrient imbalances (Galloway et al., 2021), while numerous studies have examined N dynamics in crops, fewer have explored tree and woody perennial responses to supplemental N (Braun et al., 2024; Högberg et al., 2017; Weinstein et al., 2025). Trees differ markedly from herbaceous crops in their physiological and ecological responses. Broadleaved deciduous trees (e.g., *Populus* and *Betula*) generally exhibit rapid growth and photosynthetic responses to N fertilization due to higher nutrient turnover, whereas evergreen conifers (e.g., *Pinus* and *Picea*) maintain lower but sustained N-use efficiency and photosynthetic rates (Takashima et al., 2004; Warren & Adams, 2004). These contrasting strategies illustrate that N effects on C assimilation depend on leaf habit and life-history traits.

Zanthoxylum armatum, cultivated extensively across South and East Asia, holds important economic, ecological, and ethnobotanical value (Agnihotri et al., 2022). *Z. armatum* is grown on over 108,000 ha, producing approximately 374,000 metric tons annually, generating incomes exceeding USD 40 billion in China (Tian et al., 2022). Its fruits and essential oils are widely utilized in culinary, medicinal, and cosmetic industries due to their distinctive aroma and pharmacological properties (Shah et al., 2025). Among them, *Z. armatum* is notable for its resilience in suboptimal soils and its ecological importance (Pyakurel et al., 2022). However, its sustainable cultivation remains constrained by limited knowledge of nutrient requirements, particularly N, which is fundamental for photosynthesis, carbohydrate metabolism, and biomass accumulation. Moreover, N availability varies considerably across landscapes, from fertile lowlands to nutrient-poor uplands, further complicating its management (Gu et al., 2024; Singh & Shikha, 2017).

In forest ecosystems, organic N from litter and root exudates supports slow but steady nutrient cycling, while inorganic N inputs from fertilization or deposition can disrupt soil microbial balance and carbon (C) sequestration (Huang et al., 2021). The strategic implementation of partial organic fertilizer nitrogen (OFN) substitution has emerged as a practical approach to harmonize productivity with sustainability. The implications of this practice on the biological C sequestration potential of *Z. armatum* remain inadequately investigated. N is essential for photosynthetic processes, carbohydrate metabolism, and biomass accumulation (Gu et al., 2024); its availability within soil matrices is markedly heterogeneous, fluctuating from over 150 mg kg^{-1} in nutrient-rich lowland soils to below 40 mg kg^{-1} in compromised upland environments (Hayashi, 2022). However, the effects of this integration on the growth and C sequestration of *Z. armatum* remain largely unexplored.

Previous studies have demonstrated that integrating organic and inorganic N improves N use efficiency and promotes non-structural carbohydrate accumulation in woody plants such as *Populus tomentosa*, *Quercus mongolica*, and *Acer saccharum* (Chen et al., 2021; Huang et al., 2021; Takashima et al., 2004). However, the growth and physiological responses of *Z. armatum* to N substitution remain poorly understood.

To address this gap, the present study aimed to (1) investigate the effect of different amounts of OFN substitution on growth, photosynthetic performance, carbohydrate metabolism, and biological C sequestration in *Z. armatum*; (2) determine the optimal substitution threshold that increases metabolic efficiency and C storage. By clarifying the importance of integrated N management, this study establishes a foundation for sustainable fertilization strategies that not only improve productivity but also improve climate resilience while reinforcing the ecological and economic value of *Z. armatum*.

Materials and methods

Study area

The fertilization experiment was conducted at Sichuan Agricultural University, Chengdu, China (103°52'E, 30°42'N). The region has a subtropical humid monsoon climate with a mean annual temperature of 15.9 °C and 1,012.4 mm of rainfall.

Experimental design

The uniform and disease-free seedlings of *Zanthoxylum armatum* 'Hanyuan Putaoqing' (average height ≈ 20 cm) were used for a pot experiment conducted under a rain-sheltered greenhouse to avoid precipitation. Each plastic pot (23 cm height × 22 cm diameter) contained 3.5 kg of air-dried soil. The soil was collected and air-dried, then gravel and plant residues were removed, sieved through a 2 mm sieve, and mixed well. Its initial physicochemical properties were as follows: Organic matter 14.76 g kg^{-1}, total N 1.60 g kg^{-1}, total phosphorus (P) 0.98 g kg^{-1}, total potassium (K) 23.58 g kg^{-1}, alkali-hydrolysable N 107.57 mg kg^{-1}, available P 8.19 mg kg^{-1}, available K 70.46 mg kg^{-1}, NO_3^--N 17.22 mg kg^{-1}, NH_4^+-N 6.81 mg kg^{-1}, pH 5.32, sand 48.5%, silt 41.7%, and clay 9.8%.

Eight treatments were designed: One unfertilized control (CK) and seven fertilized treatments with equal total nutrient inputs (1.33 g N, 0.68 g P_2O_5, and 1.52 g K_2O per pot) and varying OFN substitution levels (0%, 10%, 20%, 30%, 40%, 50%, and 100%). The chemical fertilizers were urea (for N), calcium superphosphate (for P_2O_5), and potassium sulfate (for K_2O).

Oil rapeseed cake was selected as the organic fertilizer because it is a regionally available, slow-release organic N source that improves soil structure and microbial activity while supplying moderate levels of P and K. It contained 46.16 g kg^{-1} N, 4.36 g kg^{-1} P, and 14.85 g kg^{-1} K. Any shortfall in P_2O_5 or K_2O was compensated with chemical fertilizers. To reduce N losses, the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) and the urease inhibitor n-butylthiophosphorotriamine (n-BTPT) were applied at a 1:200 ratio with N fertilizer (Zhao et al., 2016). 60% of total fertilizers were incorporated into the soil before transplanting in late January 2021, and the remaining 40% was top-dressed in early July 2021. Pots were maintained at approximately 60% of field water capacity (FWC) throughout the experiment.

Each treatment included three replications, and each replication consisted of five plants (a total of 15 plants per treatment), and the total number of plants in all treatments was 120.

Gas exchange parameters measurement

Gas exchange parameters were measured in mid-July using a portable photosynthesis system (LI-6800, USA) equipped with a red-blue light-emitting diode light source at 9:00–11:00 am under controlled conditions: Photosynthetic photon flux density (PPFD) = 1000 μmol m^{-2} s^{-1}, CO_2 = 400 μmol mol^{-1}, leaf temperature = 25 ± 2 °C, relative humidity ≈ 60%, and airflow = 500 μmol s^{-1}. The third fully expanded leaf from the apex was used to measure net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), and water-use efficiency (WUE = P_n / T_r) (Wu et al., 2024).

Photosynthetic pigments analysis

After gas exchange parameters measurement, the same leaves were sampled for pigments analysis. Chlorophyll a (CHLA), chlorophyll b (CHLB), total chlorophyll (TCHL), and carotenoids (CAR) were determined following the method described by Michopoulos et al. (2021). Samples were extracted with 80% acetone: ethanol (2 : 1, v / v) and measured using a UV-1800 spectrophotometer (Shimadzu, Japan), then CHLA, CHLB, and CAR were calculated (Lichtenthaler & Wellburn, 1983).

Biomass measurement

Plants were harvested in late October 2021. Each plant was separated into root, stem, and leaf. All samples were oven-dried at 105 °C for 2 h and then at 80 °C to a constant weight to determine biomass, and then ground by a fine powder. Total plant biomass (TPB) was calculated as the sum of root, stem, and leaf.

Carbohydrates and carbon content analysis

Sucrose, fructose, glucose, and starch were quantified using the anthrone colorimetric method (Wang et al., 1993). Soluble sugar = sucrose + fructose + glucose; non-structural carbohydrates (NSC) = soluble sugar + starch. C content was determined following (Gong et al., 2012), and total biomass carbon sequestration (TBCS) was estimated by multiplying biomass by C content.

Statistical analysis

One-way ANOVA was conducted using SPSS 26.0, and Duncan's multiple range test ($P < 0.05$) was applied for post-hoc comparisons. Figures were prepared with Origin 2018. Pearson correlation analysis was used to estimate the relationship between the index. The membership function was used to calculate subordinate degree, which was followed by Saba et al. (2022). P_n, WUE, NSC, and TBCS were selected to calculate subordinate degrees and comprehensive evaluation value (CEV). The formula for those four indices to calculate subordinate degree is X(u) = (X − X$_{min}$) / (X$_{max}$ − X$_{min}$). Those four indices were divided into three groups; P_n and WUE belong to the

first group, the other two indices (including NSC and TBCS) belong to the second and third groups. CEV is the total value of the average subordinate degree of the first group plus the subordinate degrees of the second and third groups. The higher CEV represents better improvement to photosynthesis, water use, C formation, and biological C sequestration. SPSS statistics 26 software was also used for fitting a regression model of OFN substitution and CEV to get the optimal OFN level. All data were expressed based on oven-dry weight.

Results

Organic fertilizer enhanced the plant growth indicators

The lowest and highest values for plant height, basal diameter, and total plant biomass (TPB) were observed in the CK and 40% OFN substitution treatment, respectively (Fig. 1a–c). Both the 0% and 10% OFN substitution treatments significantly ($P < 0.05$) reduced root/shoot ratio as compared to the CK, and

the two treatments significantly ($P < 0.05$) lower than the 40% and 100% OFN substitution treatments (Fig. 1d). The 20% OFN substitution treatment recorded the minimum root/shoot ratio (0.416), showing the significant ($P < 0.05$) difference from the highest value observed in the 100% OFN substitution treatment. Growth acceleration became evident from the 20% OFN substitution treatment, and the significant ($P < 0.05$) increases across all growth parameters occurred in the 40% OFN substitution treatment. At this level, plant height, basal diameter, and TPB peaked, and the root/shoot ratio rose to 0.524, which was significantly ($P < 0.05$) higher than those at the 0%–30% OFN substitution levels. The 40% OFN substitution treatment enhanced root biomass by 1.5-fold and stem and leaf biomass by 1.3-fold as compared to the CK (0.484). The 100% OFN substitution treatment had the highest root/shoot ratio (0.585). In contrast, the 40% OFN substitution treatment provided a more proportionate enhancement across roots, stems, and leaves. The root/shoot ratio in the 50% OFN substitution treatment (0.488) showed intermediate values and was not significantly ($P > 0.05$) different from the control. These results
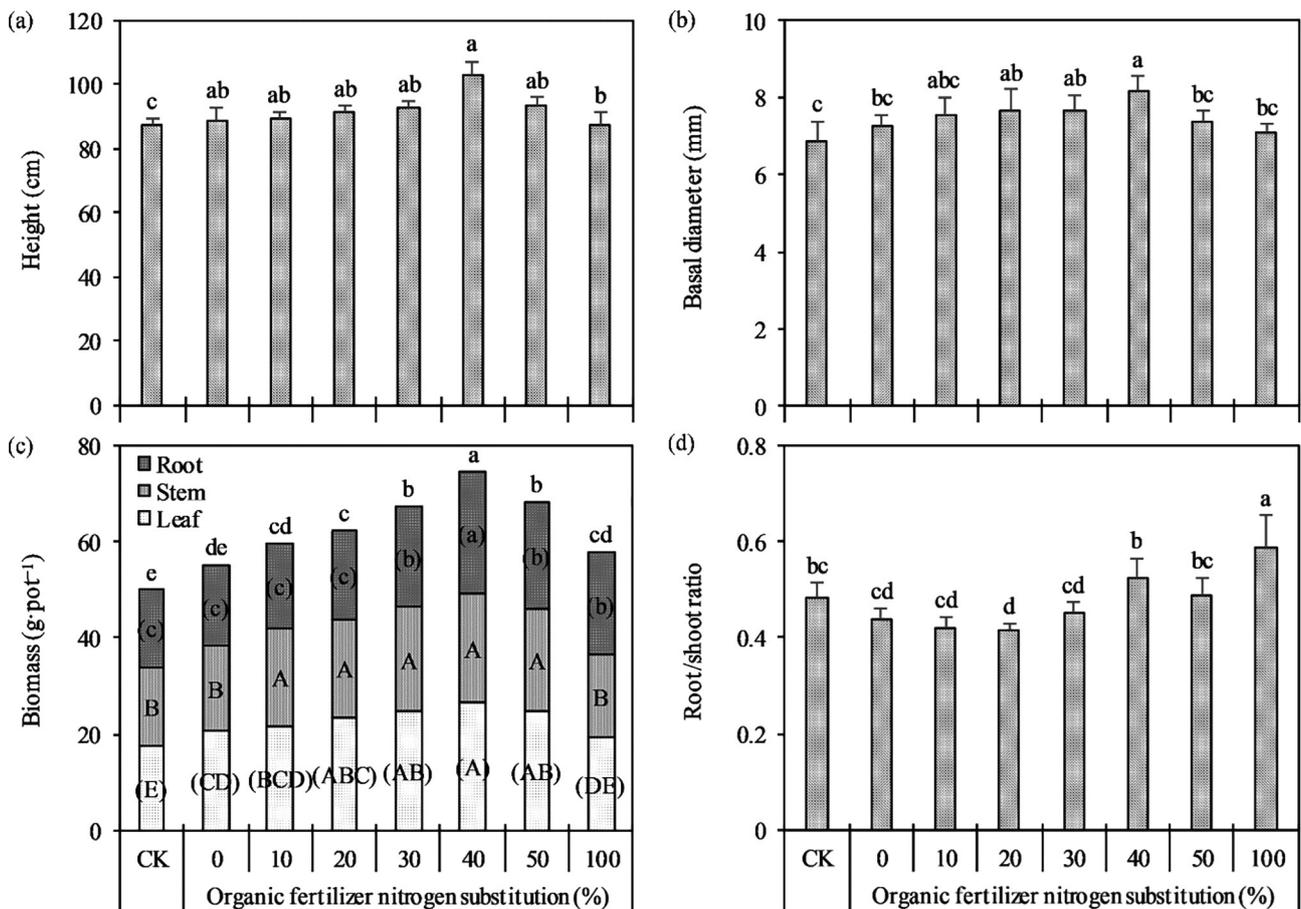

Fig. 1. Plant height, basal diameter, biomass, and root/shoot ratio of Z. *armatum* under the different organic fertilizer nitrogen substitution treatments. Bars with different letters (lower case for plant height, basal diameter, total biomass, and root/shoot ratio; lower case in brackets for root biomass; upper case for stem biomass; and upper case in brackets for leaf biomass) show significant differences at $P < 0.05$

clearly illustrate the progressive increase in biomass and morphological parameters with OFN substitution up to 40%.

Organic nitrogen amendment increased the photosynthetic pigments

Chlorophyll content significantly ($P < 0.05$) increased in all fertilized treatments as compared to the CK, with the maximum total chlorophyll (TCHL, 3.297 mg g^{-1}) observed in the 20% OFN substitution treatment, which was significantly ($P < 0.05$) higher than the CK (2.494 mg g^{-1}) and the 50% OFN substitution treatment (2.727 mg g^{-1}) (Fig. 2a). Chlorophyll a (CHLA, 1.336 mg g^{-1}) showed no significant ($P > 0.05$) difference among the treatments except for the CK, which had the lowest value (1.101 mg g^{-1}) and was significantly ($P < 0.05$) different from all fertilized treatments. Chlorophyll b (CHLB, 2.012 mg g^{-1}) reached its maximum in the 20% OFN substitution treatment, showing a significant ($P < 0.05$) increase over the CK (1.394 mg g^{-1}) and 50% OFN substitution treatment (1.380 mg g^{-1}). Although the 100% OFN substitution treatment displayed elevated CHLA (1.336 mg g^{-1}) and TCHL (3.078 mg g^{-1}) as compared to the CK, these levels were not significantly ($P > 0.05$) different from moderate OFN substitution levels. Overall, moderate OFN substitution levels (10%–40%) significantly ($P < 0.05$) enhanced pigment accumulation, with the highest and most significant ($P < 0.05$) increases in TCHL and CHLB occurring at the 20% OFN substitution level, highlighting this level as the optimal for pigment biosynthesis in *Z. armatum* seedlings. Carotenoids content peaked significantly

($P < 0.05$) in the 10% OFN (0.591 mg g^{-1}) and 30% OFN (0.555 mg g^{-1}) substitution treatments, both significantly ($P < 0.05$) higher than in the CK (0.291 mg g^{-1}) and 40% OFN (0.499 mg g^{-1}) substitution treatments (Fig. 2b).

Impact of organic fertilizer on photosynthesis and water use efficiency

G_s was highest at the 0% OFN substitution level (0.182 mol m^{-2} s^{-1}) and gradually declined with increasing OFN substitution. The CK exhibited the lowest G_s (0.091 mol m^{-2} s^{-1}), which was significantly ($P < 0.05$) lower than all fertilized treatments. Significant ($P < 0.05$) reductions in G_s occurred at the 40% OFN level, where G_s values at 40%, 50%, and 100% OFN were significantly ($P < 0.05$) lower than those at 0% OFN (Fig. 3a). T_r exhibited a similar trend, peaking at the 0% OFN substitution level (3.360 mmol m^{-2} s^{-1}) and then steadily decreasing as substitution increased. The lowest T_r was in the CK (1.631 mmol m^{-2} s^{-1}, $P < 0.05$) (Fig. 3b). P_n remained relatively stable among the 0%–50% OFN substitution levels, ranging from 5.470 to 6.210 μmol m^{-2} s^{-1}, with no significant ($P > 0.05$) differences. The CK had the lowest P_n (1.760 μmol m^{-2} s^{-1}, $P < 0.05$), while the 100% OFN substitution level showed a slight decline (5.650 μmol m^{-2} s^{-1}) as compared to 0% OFN (Fig. 3c). WUE increased significantly ($P < 0.05$) with OFN substitution, from 1.078 in the CK to 2.979 at 100% OFN, with moderate OFN levels (20%–40%) showing intermediate WUE values (2.361–2.781, $P > 0.05$) (Fig. 3d).
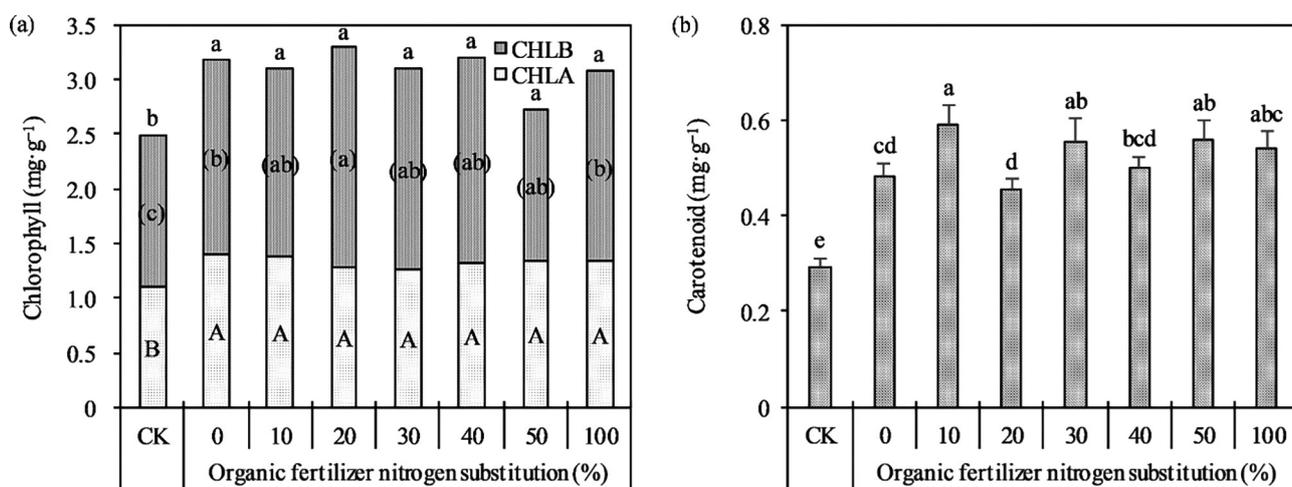

Fig. 2. Chlorophyll and carotenoid content of *Z. armatum* under the different organic fertilizer nitrogen substitution treatments. Bars with different letters (lower case for total chlorophyll and carotenoid content; lower case in brackets for chlorophyll b (CHLB); upper case for chlorophyll a (CHLA) show significant differences at $P < 0.05$
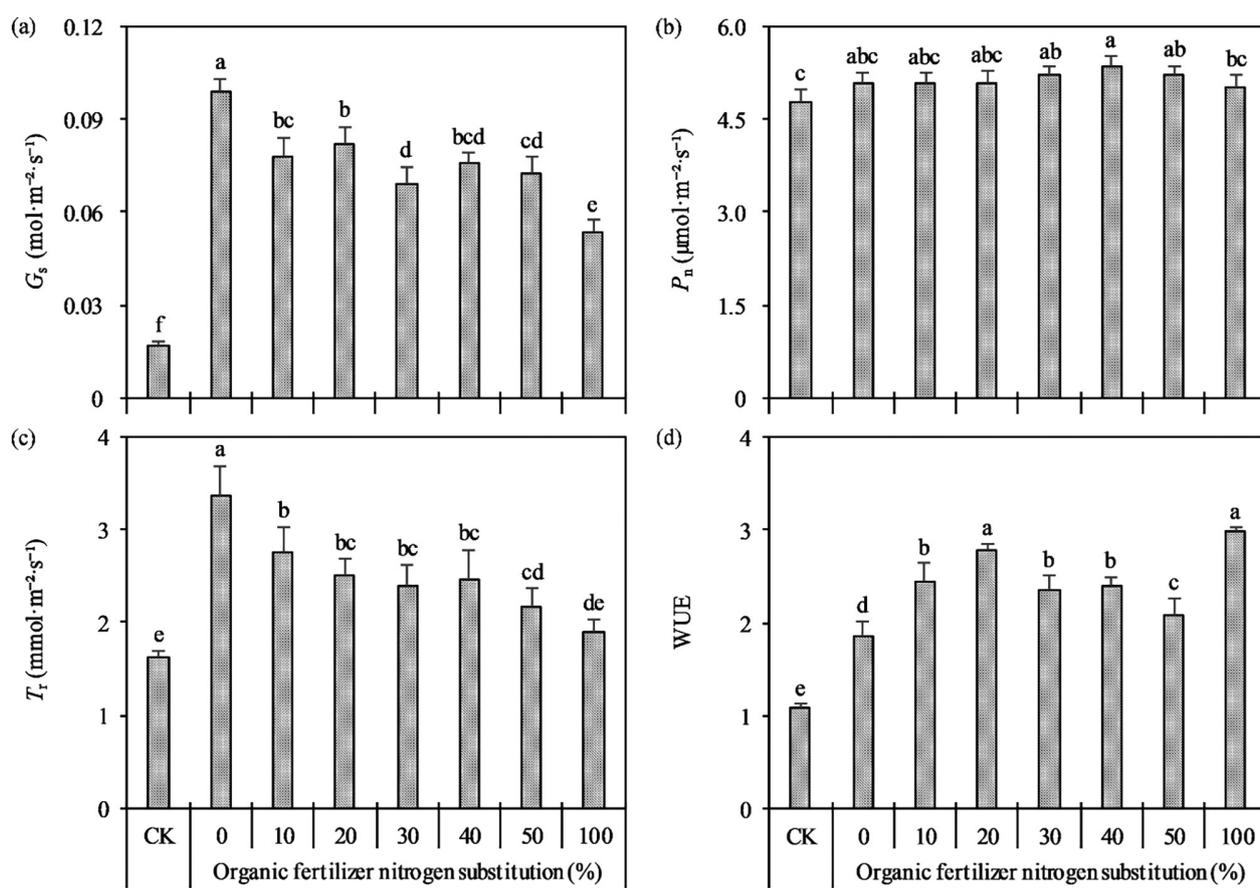
Fig. 3. Leaf gas exchange parameters of *Z. armatum* under the different organic fertilizer nitrogen substitution treatments. Bars with different letters show significant differences at $P < 0.05$. P_n – Net photosynthetic rate, G_s – Stomatal conductance, T_r – Transpiration rate, and WUE – Water use efficiency

Leaf non-structural carbohydrates

Sucrose, fructose, and glucose was 0.939%–1.054% (30% OFN), 0.891%–0.961%, and 2.134%–2.368% among the treatments, respectively; the former two with significant ($P < 0.05$) differences, the latter one with no significant ($P > 0.05$) difference. Soluble sugar content increased significantly ($P < 0.05$), from

Table 1. Correlation of non-structural carbohydrates and biological carbon sequestration with biomass and physiology indexes

	TCHL	CAR	SUC	FRU	GLU	SS	STA	NSC	Pn	WUE	CEV
TPB	0.50*	0.51*	0.61**	0.55**	0.34	0.53**	0.76**	0.73**	0.49*	0.44*	0.87**
TCHL	1.00	0.62**	0.45*	0.44*	0.66**	0.51*	0.34	0.53**	0.80**	0.66**	0.72**
CAR	0.62**	1.00	0.48*	0.40	0.32	0.42*	0.42*	0.50*	0.84**	0.67**	0.73**
SUC	0.45*	0.48*	1.00	0.46*	0.05	0.54**	0.32	0.54**	0.54**	0.52**	0.68**
FRU	0.44*	0.40	0.46*	1.00	0.31	0.31	0.50*	0.45*	0.50*	0.30	0.56**
GLU	0.66**	0.32	0.05	0.308	1.00	0.61**	0.39	0.62**	0.51*	0.26	0.55**
SS	0.51*	0.42*	0.54**	0.31	0.61**	1.00	0.39	0.92**	0.47*	0.40	0.76**
STA	0.34	0.42*	0.32	0.50*	0.39	0.39	1.00	0.72**	0.44*	0.17	0.70**
NSC	0.53**	0.50*	0.540**	0.45*	0.62**	0.92**	0.72**	1.00	0.54**	0.37	0.87**
Pn	0.80**	0.84**	0.54**	0.50*	0.51*	0.47*	0.44*	0.54**	1.00	0.69**	0.78**
WUE	0.66**	0.67**	0.52**	0.30	0.26	0.40	0.17	0.37	0.69**	1.00	0.70**
TBCS	0.50*	0.51*	0.61**	0.53**	0.35	0.55**	0.74**	0.73**	0.49*	0.44*	0.87**
CEV	0.72**	0.73**	0.68**	0.56**	0.55**	0.76**	0.70**	0.87**	0.78**	0.70**	1.00

TPB – Total plant biomass, TCHL – Total chlorophyll, CAR – Carotenoid, GLU – Glucose, SUC – Sucrose, FRU – Fructose, SS – Soluble sugar, STA – Starch, NSC – Non-structural carbohydrates, P_n – Net photosynthesis rate, WUE – Water use efficiency, TBCS – Total biomass carbon sequestration, CEV – Comprehensive evaluation value.
*$P < 0.05$, **$P < 0.01$.

3.981% (CK) to 4.348% (40% OFN), representing a 9.2% increase. The starch content also increased significantly ($P < 0.05$) with OFN substitution, from 0.796% (CK) to 1.005% (40% OFN), representing a 26.2% increase. NSC showed a similar significant ($P < 0.05$) increasing trend, peaking at 5.353% (40% OFN) as compared to the CK (4.777%). These results suggest that moderate-to-high OFN substitution, particularly 40%, substantially enhances starch and NSC accumulation, while sucrose, fructose, and glucose remain relatively stable (Fig. 4, Table 1).

Organic fertilizer amendment elevated the biological carbon sequestration

TBCS increased significantly ($P < 0.05$) with OFN substitution (Fig. 5a). The lowest and highest values was recorded in the CK (21.82 g) and 40% OFN (32.30 g), respectively. C accumulation increased moderately from 0% to 20% OFN and rapidly from 30% to 40%, particularly in roots (from 7.34 g at 0% OFN to 11.12 g at 40%, a 52.1% increase, $P < 0.05$) (Fig. 5b). After 40%, root C decreased slightly, while
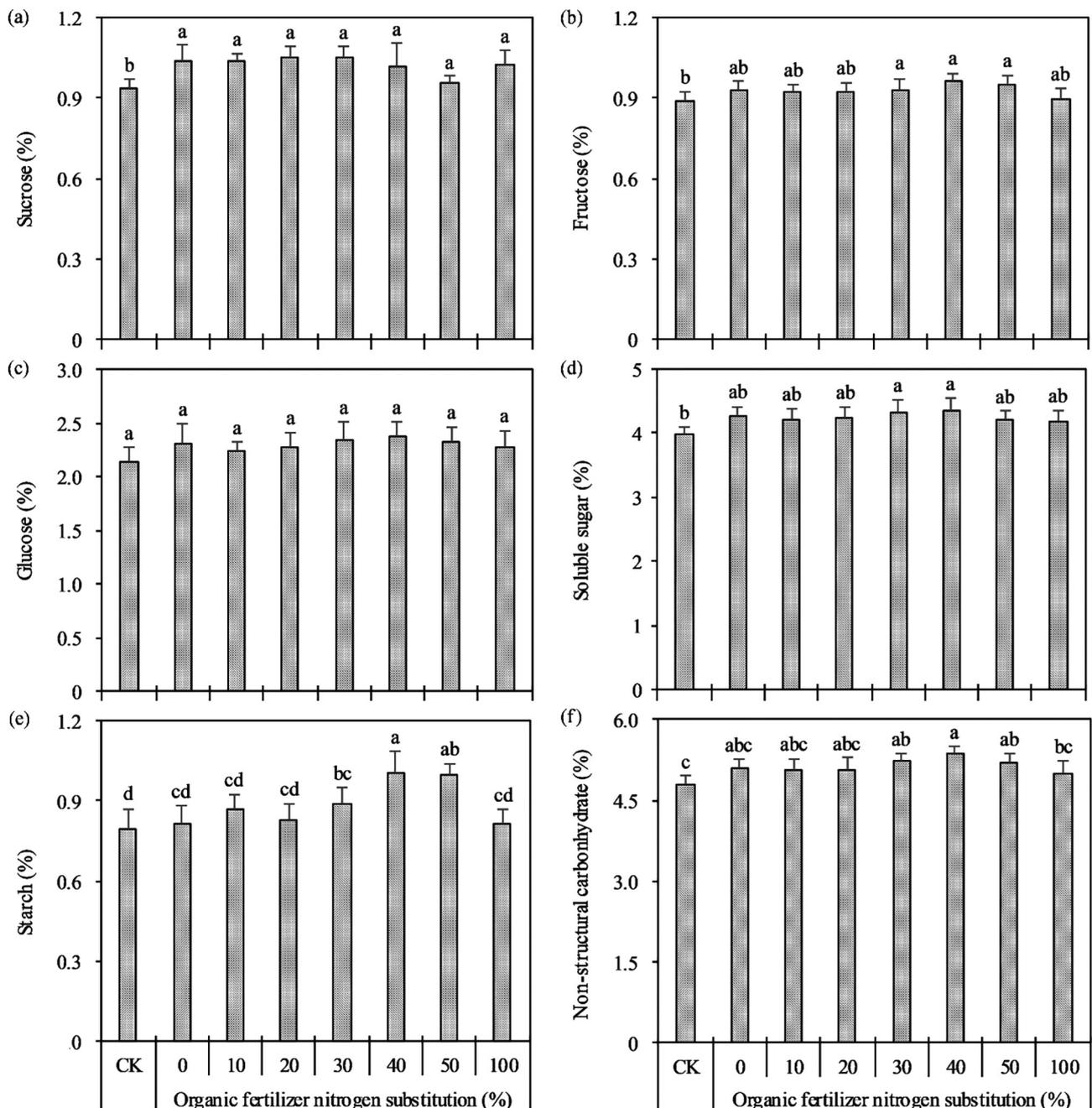

Fig. 4. Non-structural carbohydrates content of *Z. armatum* under the different organic fertilizer nitrogen substitution treatments. Bars with different letters show significant differences at $P < 0.05$
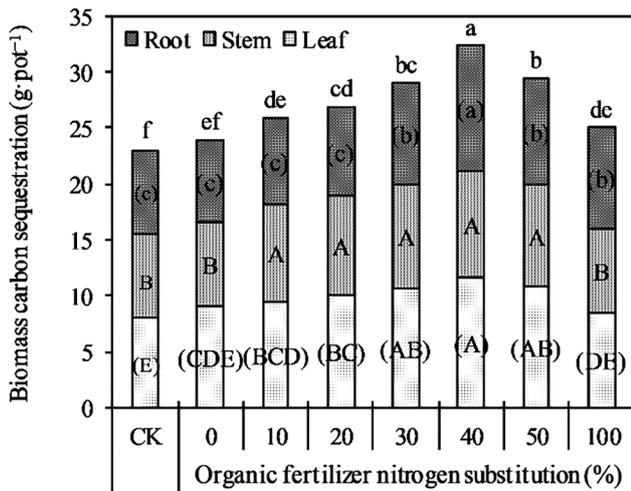
Fig. 5. Biomass carbon sequestration of *Z. armatum* under the different organic fertilizer nitrogen substitution treatments. Bars with different letters (lower case for total biomass carbon (C) sequestration; lower case in brackets for root biomass C sequestration; upper case for stem biomass C sequestration; and upper case in brackets for leaf biomass C sequestration) show significant differences at $P < 0.05$

stem and leaf C stabilized. The 50% OFN treatment maintained high TBCS (29.46 g, $P < 0.05$), but the 100% OFN level declined to 25.15 g (Fig. 5c). The TBCS ranking: 40% OFN (32.30 g) > 50% OFN (29.46 g) > 30% OFN (28.95 g) > 20% OFN (26.77 g) > 10% OFN (25.86 g) > 100% OFN (25.15 g) > 0% OFN (23.85 g) > CK (21.82 g).

Comprehensive evaluation value

The CEV, reflecting the integrated performance of *Z. armatum* under varying OFN substitution levels, exhibited a significant ($P < 0.05$) upward trend with increasing substitution levels. The lowest CEV was observed in the CK (0.360), while the highest value (2.487) occurred at the 40% OFN substitution level. From the 0% to 40% OFN substitution levels, CEV increased steadily and significantly ($P < 0.05$), with the 40% OFN substitution showing the highest significance ($P < 0.05$) level, indicating optimal overall performance. The 50% and 100% OFN substitution treatments followed closely, showing values significantly ($P < 0.05$) higher than the CK, but neither was significantly ($P > 0.05$) different from the other. Intermediate CEV values were found in the 10%, 20%, and 100% OFN substitution treatments, which were significantly ($P < 0.05$) greater than the CK, but significantly ($P < 0.05$) lower than the 30% and 40% OFN substitution treatments. The CK was significantly ($P < 0.05$) lower than all other treatments. Although the CEV slightly declined at the 50% OFN substitution level as compared to the 40% OFN substitution level, it remained significantly ($P < 0.05$) higher than the CK. The relationship between CEV (y) and OFN substitution level (x) was well described by a quadratic regression model: $y = -0.00025x^2 + 0.02601x + 1.61131$ ($R^2 = 0.535$, $P < 0.01$), from the model the optimal OFN substitution level was 53.3% OFN for plant growth, photosynthesis, water utilization, carbohydrates formation, and biological C sequestration of *Z. armatum*.

Correlation analysis

TPB was significantly ($P < 0.01$ or $P < 0.05$) correlated with TCHL, carotenoids, starch, sucrose, fructose, soluble sugar, NSC, P_n, WUE, and CEV, while TPB was not significantly ($P > 0.05$) correlated with glucose; Both TCHL and carotenoids significantly ($P < 0.01$ or $P < 0.05$) correlated with sucrose, soluble sugar, NSC, P_n, WUE, and TPBC; TCHL significantly ($P < 0.01$ or $P < 0.05$) correlated
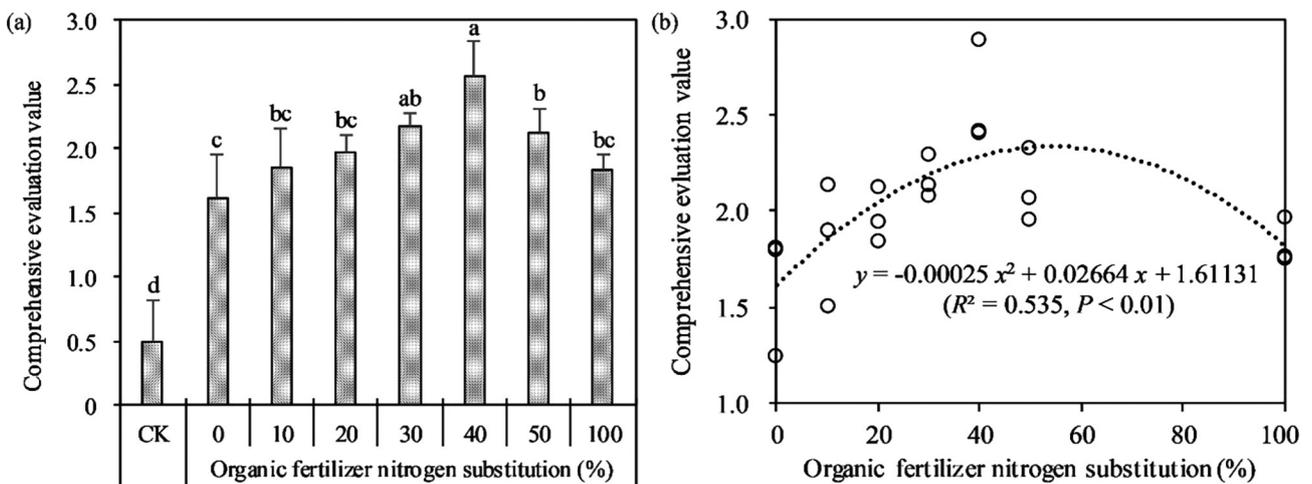

Fig. 6. Comprehensive evaluation value (CEV) of *Z. armatum* under the different organic fertilizer nitrogen (OFN) substitution treatments and the relationship between CEV and OFN substitution level. Bars with different letters show significant differences at $P < 0.05$
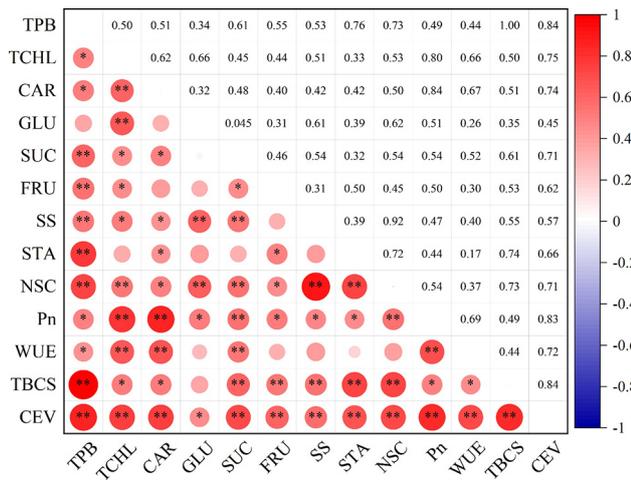
Fig. 7. Correlation of non-structural carbohydrates and biological carbon sequestration with biomass and physiology indexes. TPB – Total plant biomass, TCHL – Total chlorophyll, CAR – Carotenoid, GLU – Glucose, SUC – Sucrose, FRU – Fructose, SS – Soluble sugar, STA – Starch, NSC – Non-structural carbohydrates, P_n – Net photosynthesis rate, WUE – Water use efficiency, TBCS – Total biomass carbon sequestration, and CEV – Comprehensive evaluation value. $*P$ – < 0.05, $**P$ – < 0.01

with fructose and glucose, while carotenoids not significantly ($P > 0.05$) correlated with fructose and glucose; TCHL not significantly ($P > 0.05$) correlated with starch, while carotenoids significantly ($P < 0.01$ or $P < 0.05$) correlated with starch. Starch, sucrose, fructose, soluble sugar, and NSC significantly ($P < 0.01$ or $P < 0.05$) correlated with TPB, P_n, and TBCS; glucose significantly ($P < 0.01$) correlated with P_n, while it did not significantly ($P > 0.05$) correlate with TPB and TBCS. CEV significantly ($P < 0.01$) correlated with TPB, TCHL, carotenoids, sucrose, fructose, glucose, soluble sugar, starch, NSC, P_n, WUE, and TBCS (Fig. 7).

Discussion

Plant growth in response to organic fertilizer nitrogen substitution

Plant growth traits, such as height, basal diameter, and biomass distribution (root/shoot ratio), are critical indicators of nutrient use efficiency (NUE). N is essential for chlorophyll synthesis, amino acid formation, and enzymatic activity, directly influencing plant productivity (Wang et al., 2024). In this study, substituting different amounts of OFN had a significant ($P < 0.05$) effect on plant growth. Rather than restating numerical results, the interpretation here focuses on the developmental patterns associated with substitution levels. Growth increased up to an optimal substitution level (40% OFN), reflecting

improved nutrient synchronization and reduced N loss, while higher substitution levels no longer enhanced development. The enhancement observed at 40% OFN suggests that partial replacement of CFN improved soil nutrient release dynamics and promoted more efficient uptake, leading to greater height, basal diameter, and biomass without over-supplying N. This interpretation aligns with findings in *Brassica napus* and *Camellia oleifera*, where organic substitution improved nutrient availability and root–shoot coordination (Chaukiyal et al., 2013; Wang et al., 2023). These studies similarly attribute improved growth to strengthened root systems under moderate organic inputs, supporting the mechanism observed here. However, our findings disagree with Singh (2018), who reported that higher organic substitution can promote continued growth. The divergence likely arises from differences in soil microbial activity and N mineralization rates, which can vary widely among soil types and organic fertilizer sources. At elevated OFN levels, the slow-release characteristics of organic N may delay mineral N availability (Gutser et al., 2005), creating short-term nutrient gaps that restrict shoot expansion despite continued root proliferation. This pattern was consistent with observations by Panday et al. (2024), who observed disproportionate allocation toward roots at the expense of shoots. Overall, moderate OFN substitution supports balanced morphological development by optimizing nutrient distribution and physiological efficiency.

Leaf pigments in response to organic fertilizer nitrogen substitution

Chlorophyll and carotenoids are central to photosynthesis and growth. Moderate OFN substitution enhanced chlorophyll and carotenoids content, while complete OFN substitution reduced pigments. This trend agrees with Nasar et al. (2022), who reported that organic N promotes chlorophyll biosynthesis. Conversely, excessive CFN application can reduce chlorophyll and carotenoids, likely due to nutrient imbalance or environmental stress affecting pigments stability (Farhan et al., 2024; Oleszkiewicz et al., 2021; Pirastru et al., 2012). The observed decrease in pigments at high OFN may reflect a saturation effect, whereby excess N limits chlorophyll biosynthesis or destabilizes existing pigments. Carotenoids also declined under excessive N, consistent with their role in photoprotection and oxidative stress mitigation (Peralta-Sánchez et al., 2023). Our findings support Verhoeven et al. (1997), who demonstrated that moderate N input improves pigments accumulation, while high CFN causes pigments breakdown due to oxidative stress. The species-specific responses, experimental conditions, and N forms explain

contrasting findings in the literature (Kopsell et al., 2007; Urban et al., 2009). These results highlight the importance of balanced N management for maintaining photosynthetic efficiency and plant health.

Our findings indicate that moderate OFN substitution enhanced chlorophyll and carotenoids, while complete OFN substitution reduced pigments. The observed decrease in pigments at high OFN may reflect a saturation effect, whereby excess N limits chlorophyll biosynthesis or destabilizes existing pigments. Carotenoids also declined under excessive N, consistent with their role in photoprotection and oxidative stress mitigation (Peralta-Sánchez et al., 2023). This supports the need for careful N management. These results highlight the importance of balanced N management for maintaining photosynthetic efficiency and plant health. Moreover, optimal OFN substitution improves chlorophyll and carotenoids, while excessive OFN substitution reduces pigments synthesis.

Leaf photosynthesis, water use efficiency, and carbohydrates in response to organic fertilizer nitrogen substitution

Leaf gas exchange in *Z. armatum* was significantly ($P < 0.05$) influenced by OFN substitution, revealing a trade-off between photosynthetic efficiency and WUE. Stomatal conductance (G_s) and transpiration rate (T_r) were highest in the 0% OFN treatment and declined with increasing OFN substitution, while WUE increased progressively, peaking at the 100% OFN substitution. Net photosynthetic rate (P_n) remained stable up to 30% OFN substitution but declined at higher levels. These findings suggest that excessive N input, whether from OFN or CFN, reduces stomatal activity and photosynthetic capacity due to nutrient imbalance or physiological stress, consistent with the observations of Li et al. (2023). However, higher OFN substitution, especially at 100%, results in reduced chlorophyll and carotenoids. It is plausible that under high N availability, stomata do not need to open fully, allowing plants to reduce transpiration and maintain improved water status, though at the cost of reduced net CO_2 assimilation. Thus, OFN may influence gas exchange by shifting the balance between reduced transpiration (a physiological benefit) and lower C assimilation (a physiological cost). This mechanism aligns with the ecological compensation theory, which explains how plants balance resource trade-offs under varying environmental or nutritional conditions (Baldocchi, 1997). Our study demonstrates that moderate OFN substitution optimizes photosynthetic performance and WUE, whereas excessive substitution limits gas exchange through partial stomatal closure. This outcome agrees with (Hunt et al., 1985; Zhu et al., 2023), who emphasized the necessity of maintaining moderate organic N inputs to sustain both photosynthetic capacity and water regulation efficiency. In contrast, Otoo et al. (1989) reported no significant ($P > 0.05$) photosynthetic decline with increased OFN, highlighting the potential for species- or environment-specific responses. Overall, moderate OFN substitution offers the best balance between photosynthesis, transpiration, and WUE, supporting a physiologically efficient strategy for N management in *Z. armatum*. Leaf carbohydrates metabolism followed a similar trend. Starch and NSC increased at moderate OFN, reflecting efficient N use, while excessive CFN or OFN reduced soluble sugar accumulation. This aligns with previous studies showing that high inorganic N can inhibit carbohydrates biosynthesis, while moderate N promotes balanced partitioning between starch and soluble sugar (Doehlert et al., 1997; Su et al., 2013; Zhang et al., 2021). Future research should observe the long-term impacts of OFN substitution on soil health, interactions with other nutrients (e.g., P and K), and genotype-specific responses, and should explore the role of N stabilizers in enhancing fertilizer proficiency and sustainability.

Organic fertilizer enhanced the carbon sequestration

Moderate OFN substitution enhanced biological C sequestration, particularly in roots, by improving nutrient availability and promoting balanced C allocation rather than merely increasing biomass numerically. The observed increase in root C storage reflects gradual N release, which supports sustained photosynthesis and stimulates microbial activity that facilitates soil C stabilization (Dasgupta & Mahanty, 2024). In contrast, excessive substitution reduced TBCS, likely due to delayed N mineralization and constrained photosynthetic productivity, highlighting the physiological limits of high organic N inputs. These patterns align with Turunen et al. (2004) and Kopáček et al. (2013), who reported that nutrient imbalances can reduce C assimilation under excessive N application. Species-specific differences in N utilization may explain discrepancies with Kumari and Sathish (2020), who suggested that heightened levels of chemical fertilizer nitrogen (CFN) (for example, 50% or more) continued to promote biological C sequestration in select crop varieties. This inconsistency may stem from species-specific differences in N utilization strategies, as certain species demonstrate resilience and derive advantages from elevated CFN levels (Cao et al., 2021; Schulz et al., 2011).

Moderate OFN substitution optimizes both growth and C sequestration, highlighting the importance of balanced N management for improving NUE, plant productivity, and environmental sustainability. These findings also align with Elrys et al. (2022), who highlighted the role of integrated nutrient strategies in sustainable agriculture. Overall, these findings underscore that synchronizing N release with plant demand supports efficient C allocation to both aboveground and belowground tissues. This mechanistic insight underscores the ecological and agronomic benefits of balanced N management, which improves nutrient use efficiency, promotes sustainable growth, and enhances biological C sequestration in *Z. armatum*. Future studies should examine long-term effects on soil nutrient cycling, microbial communities, and genotype-specific N strategies, as well as explore integrated nutrient management approaches to further enhance fertilizer efficiency and C storage.

Conclusion

Moderate OFN substitution enhances performance by coordinating plant growth, physiological efficiency, C allocation, and nutrient utilization. By integrating the CEV, this study provides a novel framework to quantify optimal N substitution in perennial woody plants, linking productivity with TBCS. These findings advance understanding of how partial organic N replacement can simultaneously support plant physiology, metabolic balance, and sustainable fertilization practices. The work offers new insights for optimizing nutrient management in perennial systems, demonstrating the potential for OFN to enhance long-term productivity and ecological sustainability.

Credit authorship contribution statement

Fozia Dost Muhammad: Data curation, Writing-original draft, review & editing. Yuxin Xie: Data curation, Writing-review & editing. Yuanjia Gong: Formal analysis, Visualization, Writing-review & editing. Muhammad Asghar Ali: Writing-review & editing. Shuaijie Lu: Data curation, Writing-review & editing. Wenkai Hui: Supervision, Writing-review & editing. Wang: Funding acquisition, Conceptualization, Project administration, Writing-review & editing. Wei Gong: Formal Analysis, Data curation, Supervision, Writing-review & editing, Validation.

Declaration of competing interest

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

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Data availability

All datasets generated for this study are included in the article.

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