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Stable isotope evidence for biochar nanoparticles as potential nanocarriers boosting nitrogen delivery in soybean

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Abstract

Nano biochar (NBC) is a ubiquitous component of terrestrial ecosystems and plays a pivotal role in rhizosphere dynamics. However, its potential applications in nanobiotechnology and sustainable agriculture remain largely unexplored. In this study, ^{15}N -labeled NBC was synthesized and evaluated as a nitrogen nanocarrier by investigating its uptake, translocation, and transformation in soybean plants. Applied at 5 mg kg^{-1} , NBC significantly enhanced $\delta^{15}\text{N}$ enrichment in nodules, grains, and grain amino acids by 127.8%, 47.9%, and 3.6-fold, respectively, compared with the control, indicating improved nitrogen assimilation. Furthermore, NBC treatment significantly increased the activities of key nitrogen assimilation enzymes and upregulated related gene expression. Consistent with these results, NBC application boosted grain number and weight by 30%–45% and increased amino acid contents by 6.5%–97.1%. Microscopy confirmed the translocation of NBC from roots to leaves. These findings highlight the potential of NBC as a nanocarrier for enhancing nitrogen delivery in soybean.

Keywords: Nano-biochar, Nano-enabled delivery, Nitrogen assimilation, Soybean, Stable isotope tracing

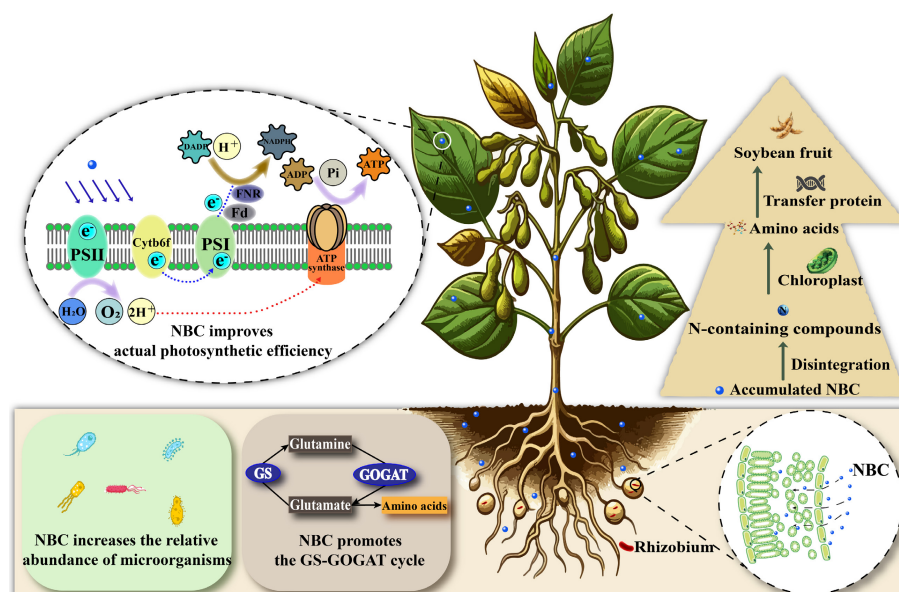
Highlights

- NBC is a promising nanocarrier that boosts nitrogen delivery in soybean, as evidenced by ^{15}N isotope tracing.
- NBC significantly enhanced $\delta^{15}\text{N}$ enrichment in nodules, grains, and grain amino acids.
- NBC significantly increased the activity of key nitrogen assimilation enzymes and upregulated related gene expression.
- NBC can be assimilated by roots and release bioavailable nitrogen to support soybean metabolism.
- NBC offers a sustainable platform for controlled nutrient delivery in precision agriculture.

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Graphical abstract



Introduction

Biochar (BC) is the refractory carbonaceous residue produced naturally or anthropogenically from oxygen-limited pyrolysis of biomass^[1]. BC is commonly considered a complicated combustion continuum from large pieces to dissolved fractions^[2]. Among the mixture, BC with particle sizes smaller than 100 nm, i.e., nano-biochar (NBC), has gained increasing research attention due to its active nature in terrestrial ecosystems^[3]. NBC has high surface reactivity similar to engineered carbon nanomaterials such as graphene oxides (GO) and carbon quantum dots (CQDs) and plays critical roles in many biogeochemical processes, including sorption^[4,5], redox^[6], and heteroaggregation^[7]. Comparatively, NBC possesses higher environmental friendliness and lower cost relative to engineered carbon nanomaterials. Hence, it is essential to explore the potential applications of NBC across various domains such as sorbents, nanofertilizers, and nanopesticides, based on its physicochemical properties and high reactivity.

Nanobiotechnology provides agricultural systems with a great opportunity to become more sustainable, efficient, and resilient in facing climate change^[8,9]. The nanoscale size of engineered nanomaterials enables them to penetrate the vascular systems of plants following root or foliar applications, facilitating new and efficient delivery routes for nutrients and agrochemicals^[10]. The most widely used engineered nanomaterials include carbon-based materials^[11], mineral nanoparticles^[12], and metal oxide nanoparticles^[13]. The delivery efficiency and manner are highly dependent on the exposed concentration, surface charges, and particle size of the nanomaterials^[14]. To our knowledge, no prior studies have investigated NBC as a nanocarrier for crop fertilizers, likely due to concerns about its plant availability and nutrient delivery efficiency. Our previous study demonstrated that NBC preferentially associates with rice roots and subsequently modifies iron plaque composition^[15]. More directly, NBC has been documented to undergo absorption and systemic translocation in wheat and Chinese cabbage plants^[16]. Beyond carbon, heteroatoms such as aromatic nitrogen (N) are consistently identified within NBC molecular structures^[17]. To date, the fate of both NBC and its associated heteroatoms after plant

uptake remains largely unexplored. Based on documented structural transformations of carbonaceous nanomaterials in plant leaves^[18,19], we hypothesize that NBC could serve as a nanoscale platform for the co-delivery of heteroatom-derived nutrients and carbon sources to crops.

N is an essential nutrient for plant growth and development^[20], constituting a significant constraint on crop yield. To satisfy the escalating food demand globally, over 110 Tg of N fertilizers are applied annually to enhance soil fertility, with N fertilizer synthesis consuming 1%–2% of global energy^[21,22]. Enhancing N use efficiency in soybeans (*Glycine max* L.) through nanobiotechnology represents a promising strategy for reducing N fertilizer application while simultaneously increasing crop yields^[23]. Nanomaterials such as molybdenum disulfide and iron oxide nanoparticles^[24] have demonstrated the ability to enhance N utilization and increase soybean yields. In this study, we focus on evaluating NBC as a potential N nanocarrier for soybean cultivation, with specific emphasis on the bioavailability of its heteroaromatic N components.

The primary objectives of this work are: (1) to evaluate the influence of N-enriched NBC on N assimilation, yield formation, and rhizosphere microbial diversity in soybeans throughout their life cycle; and (2) to elucidate the transformation of N-enriched NBC in soybeans and the underlying mechanism of N delivery to the plant. To this end, we synthesized ¹⁵N-doped NBC at two pyrolysis temperatures (400 and 700 °C). Greenhouse pot experiments were conducted to trace the uptake and transformation of ¹⁵N-labeled NBC through analysis of its degradation products and N isotope ratios. The findings will advance our understanding of plant–NBC interactions and underscore NBC's potential as a naturally derived platform for controlled nutrient delivery in nanobiotechnology applications.

Materials and methods

Preparation and characterization of NBC

A mixture of corn stover powder and ¹⁵N-labeled urea (2:1 mass ratio) served as the precursor for NBC synthesis via slow pyrolysis conducted

at 400 and 700 °C^[25]. ¹⁵N-labeled NBC was isolated from the bulk BC via extraction and filtration (0.7 µm). The resulting filtrate was freeze-dried, stored in the dark at 4 °C, and designated as NBC400 and NBC700, respectively, prior to use. The detailed protocol is shown in [Supplementary Text S1](#) in the Supporting Information (SI).

The abundances of ¹⁵N in NBC were determined using an elemental analyzer–isotope ratio mass spectrometer (EA-IRMS; Flash 2000 HT, Thermo Fisher Scientific, USA) at a reaction temperature of 960 °C, with a helium carrier gas flow rate of 120 mL min⁻¹ and a reference gas flow rate of 100 mL min⁻¹. The particle size and surface morphology of NBC were characterized by transmission electron microscopy (TEM) and atomic force microscopy (AFM). The ζ potential and hydrodynamic diameters (*D_h*) in deionized water at different pH values (4, 7, and 10) were determined using a Zeta potential analyzer (Malvern Zetasizer Nano ZS90, UK). Surface chemical compositions were acquired through X-ray photoelectron spectroscopy (XPS, Thermo Fisher, USA). The specific characterization methods are shown in [Supplementary Text S2](#).

Greenhouse experiment

Soil samples were collected from an agricultural field in Tianjin, China (39°23' N, 117°05' E), and their physicochemical properties were characterized prior to soybean cultivation (see [Supplementary Text S3](#) and [Supplementary Table S1](#) for details). All soils used in this experiment were non-sterile, retaining their original soil microbial communities. Soybean seeds (*Glycine max* [L.] Merr. cv. Zhonghuang 13, hereafter abbreviated as *G. max* cv. ZH13) were provided by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, and sterilized with 5% NaClO for 5 min, and subsequently soaked and incubated in deionized water at 25 °C for 3 d. Uniform seedlings were selected and transplanted into pots containing 2.0 kg of soil for subsequent cultivation. Before transplantation, NBC400 and NBC700 were individually mixed into the soil to achieve final concentrations of 1, 5, and 25 mg kg⁻¹, respectively. An untreated soil without any NBC addition served as the control. The pot experiment adopted a randomized block design, with three biological replicates for each group. The transplanted seedlings were cultivated in a greenhouse under controlled conditions: 25 °C during the day, 18 °C at night, 50% humidity, and 70% soil moisture. At the physiological maturity stage (90 d after planting), soybean plants were harvested and stored for subsequent analysis.

Determination of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters in plant leaves, including the actual photosynthetic efficiency of PSII (Δ*F*/*F_m*'), electron transfer rate (ETR), non-photochemical quenching coefficient (NPQ), and the proportion of open PSII reaction centers capable of undergoing photochemical reactions (qP), were assessed before harvest employing a basic modulated chlorophyll fluorometer (Junior-PAM, Zealquest Scientific Technology Co.). The detailed procedure is provided in [Supplementary Text S4](#).

Quantification of total N content in soybean tissues

The total N content in the roots, nodules, leaves, and grains was determined using an elemental analyzer (Flash 2000 CHNS/O) from Thermo Fisher Scientific, USA. The detailed parameters and calculation methodologies are described in [Supplementary Text S5](#).

Determination of amino acid content and N isotope ratios in grains

The sampled grains were subjected to amino acid extraction, purification, and derivatization ([Supplementary Text S6](#)), and the amino acid NPP derivatives were quantified by gas chromatography-mass spectrometry (GC-MS, Shimadzu, Japan) to obtain the content of each amino acid. Amino acid NPP derivatives were separated using a Trace GC Ultra gas chromatograph. The amino acid monomers were then converted to N₂ in oxidizing and reducing furnaces, followed by analysis using a Mat253 isotope ratio mass spectrometer (IRMS, Thermo Fisher, USA) for N isotope ratio determination. Detailed chromatographic and mass spectrometric conditions are given in [Supplementary Text S7](#).

Nitrogen metabolism-related enzyme activity

The activities of glutamine synthetase (GS), glutamate synthetase (GOGAT), and glutamate dehydrogenase (GDH) were determined in freshly collected roots and shoots using assay kits from Beijing Solarbio Science and Technology Co.

Quantitative real-time PCR analysis

Quantitative real-time PCR was employed to analyze the expression of key genes involved in N metabolism, including the GOGAT gene and GS-related genes (*GS1β*, *GmGS1α1*, *GmGS1α2*, *GmGS1β1*, *GmGS1β2*, and *GmGS1γ2*). Detailed methods and information about the internal reference genes and primers can be found in [Supplementary Text S8](#) and [Supplementary Table S2](#).

Soil microbial diversity analysis

High-throughput sequencing, conducted on the NovaSeq-PE250 second-generation sequencing platform from Illumina (California, USA), was employed to scrutinize the soil microbial community's composition. Specific test parameters are detailed in [Supplementary Text S9](#).

Determination of δ¹⁵N in plants

The abundances of δ¹⁵N in the roots, nodules, leaves, and grains of plants were assessed using a Sercon HS-2022 EA-IRMS. Standard substances IAEA-600 (δ¹⁵N = 1‰) and acetanilide (δ¹⁵N = 1.18) were employed. Detailed conversion formulas and δ¹⁵N determination methods are provided in [Supplementary Text S10](#).

TEM imaging of leaf ultrastructure

Soybean leaf segments were fixed in 2.5% glutaraldehyde, dehydrated, embedded in resin, and ultrathin-sectioned to approximately 90 nm. After double-staining with uranyl acetate and lead citrate, the sections were examined using an FEI Tecnai TF20 field-emission TEM equipped with EDS (Oxford Instruments ISIS 300). High-resolution TEM imaging and selected-area electron diffraction (SAED) were performed to analyze the subcellular structure and crystalline features, while EDS was employed to determine the local chemical composition. Lattice spacing measurements were conducted on the acquired HRTEM images using DigitalMicrograph software.

Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) analysis of nodules

In situ, isotope mapping was performed using a NanoSIMS 50L (Cameca, Gennevilliers, France) with a 16 keV Cs⁺ primary ion beam. Secondary ion counts of carbon (¹²C⁻), oxygen (¹⁶O⁻), nitrogen

($^{12}\text{C}^{14}\text{N}^-$ and $^{12}\text{C}^{15}\text{N}^-$), phosphorus ($^{31}\text{P}^-$), and sulfur ($^{32}\text{S}^-$) were measured. Images were processed using OpenMIMS data analysis software from ImageJ (National Institutes of Health, Bethesda, MD, USA). Images were corrected for detector dead time (44 ns) before any other data processing. Plots of $^{15}\text{N} : ^{14}\text{N}$ ratios ($r = ^{15}\text{N}/^{14}\text{N}$ from $^{12}\text{C}^{15}\text{N}^-/^{12}\text{C}^{14}\text{N}^-$) were obtained by dividing the $^{12}\text{C}^{15}\text{N}^-$ counts by the $^{12}\text{C}^{14}\text{N}^-$ counts for each pixel. Ten to fifteen points per sample were analyzed to provide a reliable database for calculating the fate of ^{15}N . Specific test parameters are detailed in [Supplementary Text S11](#).

NBC stability assessment in the soil

To evaluate the structural stability of NBC in the soil, both N-release assays in root exudates and NBC mineralization experiments were conducted. Detailed methods can be found in [Supplementary Text S12](#).

Reactive oxygen species (ROS) accumulation in soybean leaves

ROS in soybean leaves for all treatments and the control were analyzed using electron spin resonance spectrometry (Magnetech ESR5000, Bruker). Specific methods and test parameters are detailed in [Supplementary Text S13](#).

Identification of NBC transformation in soybean leaves

Due to the extremely low concentration of absorbed NBC in soybean leaves, *in situ* monitoring of its transformed products is highly challenging. Therefore, we designed an *in vitro* simulation experiment to analyze the potential transformation pathways of NBC in foliar tissues. Briefly, an aliquot of intracellular fluid extracted from soybean leaves was mixed with NBC (1 mg mL⁻¹) and reacted for 24 h. The reaction was conducted in vials submerged in a thermostated water bath maintained at 25 °C, with simulated sunlight provided by an Atlas SunTest CPS+ solar simulator equipped with a 1 kW xenon arc lamp irradiating the solution from above. The intracellular fluid without NBC

addition served as the control. The degradation products were analyzed by LC-MS equipped with an electrospray ionization (ESI) source. The detailed extraction procedure for intracellular fluids and the mass spectrometer are presented in [Supplementary Text S14](#).

Statistical analysis

Statistical analyses were conducted using one-way analysis of variance in SPSS v26.0. Mean values for each treatment were compared using the least significant difference multiple comparison tests at a significance level of $p < 0.05$. The data are presented as means $\pm$ standard deviation (SD).

Results and discussion

Characterization of NBC

The $\delta^{15}\text{N}$ isotopic signatures ([Supplementary Table S3](#)) demonstrated exceptional ^{15}N enrichment in both NBC400 and NBC700, with values of 150,811‰ and 162,136‰, respectively. These values were three orders of magnitude higher than those of the unlabeled nano-BC controls (137.4‰ and 143.3‰), confirming the successful synthesis of highly enriched ^{15}N -labeled NBC materials. TEM images show that NBC400 and NBC700 were nanosized solids with average particle sizes of 10.1 ± 0.5 nm and 28.9 ± 0.5 nm, respectively ([Fig. 1a](#)). AFM observation suggests that NBC exhibited flake-like morphologies, with thickness (height profile) ranging from 0.38 to 1.75 nm for NBC400 and 0.27 to 1.84 nm for NBC700. The lateral sizes were in the ranges of 47.64–83.53 nm for NBC400 and 69.16–139.7 nm for NBC700, respectively ([Fig. 1b](#)). Surface elemental compositions determined by XPS ([Supplementary Tables S4 and S5](#)) indicate that the contents of C, N, and O were 61.17%, 33.31%, and 5.52% for NBC400, while those for NBC700 were 65.07%, 31.57%, and 3.36%, respectively. High-resolution C1s XPS spectra ([Fig. 1c](#)) show that, besides sp^2 - and sp^3 -hybridized carbons (reflected by the peaks of C–C/C=C), NBC400 and NBC700 contained a high content of oxygen-containing groups (mainly C–OH and C=O, more than 40%), implying their high polarity and surface

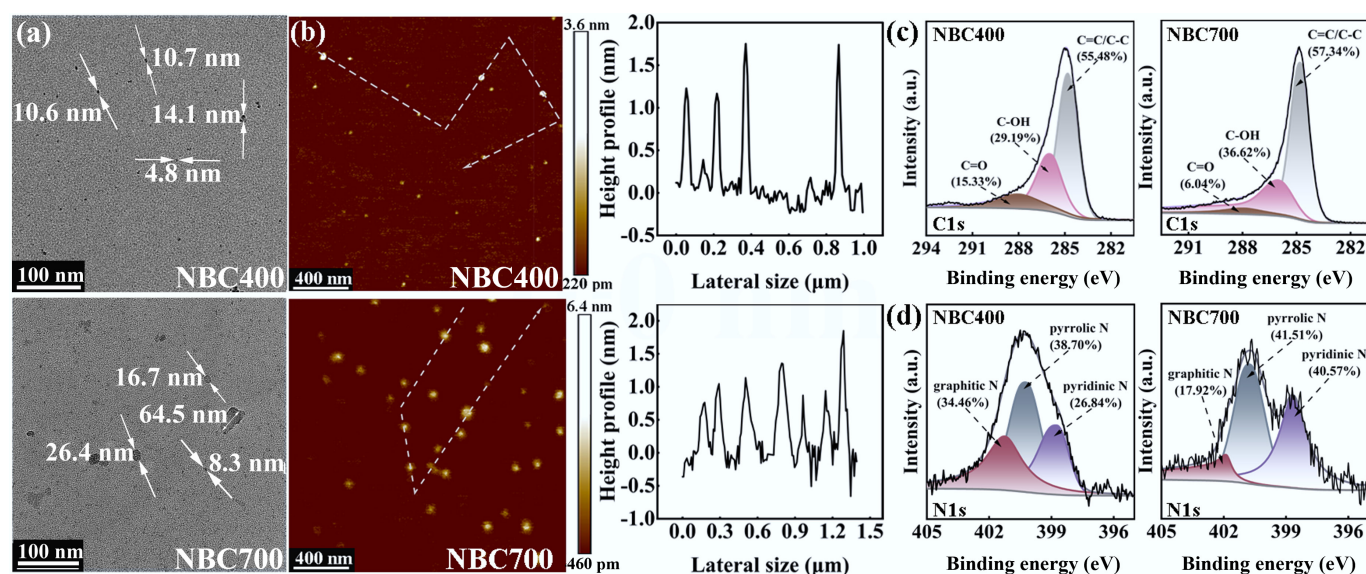


Fig. 1 Characterization of NBC. (a) TEM images of NBC400 and NBC700. (b) AFM images of NBC400 and NBC700. The diameter (lateral size, x-axis) and thickness (height profile, y-axis) analysis of NBC particles along the dotted line in the panel. (c) High-resolution C1s XPS spectra of NBC. (d) High-resolution N1s XPS spectra of NBC.

charge density. The N1s XPS spectra (Fig. 1d) reveal that pyridinic-N and pyrrolic-N constituted the predominant N species in NBC400 and NBC700, collectively accounting for more than 65% of the total N content. These findings demonstrate that NBC400 and NBC700 contained typical heteroaromatic N structures, which are similar to the dissolved black nitrogen separated from the natural environment^[26]. The ζ potentials of NBC remained negative and lower than -25 mV under a wide range of pH conditions (4–10), indicating excellent colloidal stability in aqueous solutions (Supplementary Fig. S1). The D_h of NBC400 and NBC700 ranged from 220 to 342 nm and 396 to 712 nm at different pH levels (Supplementary Figs S2 and S3), suggesting their high potential to be taken up by plant roots.

NBC-enhanced soybean growth and photosynthesis

Impacts of NBC at different dosages (1, 5, and 25 mg kg⁻¹) on the growth and yield of soybean were examined. The application of NBC400 and NBC700 generally enhanced soybean grain number and weight, with the most pronounced improvement observed at the 5.0 mg kg⁻¹ application rate ($p < 0.05$) (Fig. 2a). For instance, the grain numbers were enhanced by 35.0% and 45.0% under the 5.0 mg kg⁻¹ NBC400 and NBC700 treatments, respectively. The root images of soybean visually illustrated that the roots in the 5.0 mg kg⁻¹ NBC treatment developed better and had relatively more root nodules than those of other treatments (Supplementary Fig. S4). The application of NBC400 significantly increased N content in soybean roots and grains compared to the control, with a more pronounced promoting effect than that achieved by NBC700 (Supplementary Table S6). Consistently, NBC application, particularly NBC400, significantly increased the amino acid content in soybean grains ($p < 0.05$; Supplementary Table S7), demonstrating its efficacy in enhancing the crop's nutritional quality. This nano-enabled enhancement of N assimilation and crop yield is consistent with recent reports on other nanomaterials. For instance, Li et al. demonstrated that molybdenum nanofertilizer improved

biological N fixation and soybean yield by delaying nodule senescence and enhancing nutrient uptake^[27]. Compared with engineered nanomaterials, our NBC achieves comparable or even greater promotion (35%–45% increase in grain number) at a very low application rate (5 mg kg⁻¹). These comparisons further support that the observed promotion originates primarily from the nano-size effect of NBC. The superior performance of NBC400 over NBC700 can be explained by its distinct structure. Specifically, NBC400 possesses a higher proportion of edge-site N species, a lower degree of aromatic condensation, and more abundant surface oxygen-containing groups. These properties facilitate ROS-mediated partial decomposition, better aqueous dispersibility, and more efficient root uptake and subsequent N translocation to nodules and grains.

Chlorophyll fluorescence emitted by plants is closely related to chemical processes in photosynthesis^[28]. Thus, the impact of NBC on the soybean photosystem was examined by assessing essential chlorophyll fluorescence parameters, including ETR, $\Delta F/F_m'$, NPQ, and qP. The results suggest that these parameters were generally improved in the NBC treatments, indicative of a promoting effect on photosynthetic activities (Fig. 2b). For example, 1 mg kg⁻¹ of NBC700 increased ETR and $\Delta F/F_m'$ by 22.6% and 27.2% compared with the control.

Enhanced activity of nitrogen assimilation enzymes by NBC

The activities of three key enzymes in the roots and shoots, including GS, GOGAT, and GDH, involved in N assimilation during soybean growth were examined^[29]. GS contributes to glutamine biosynthesis by catalyzing the reaction between NH₄⁺ and glutamate to produce amino acids. GOGAT catalyzes the production of glutamate from glutamine and 2-oxoglutarate. GDH catalyzes the reaction of NH₄⁺ with 2-oxoglutarate to form glutamate^[30]. The NBC treatments exhibited a general promoting effect on the activities of these enzymes both in roots and shoots (Fig. 3a). Specifically, higher dosages of NBC more

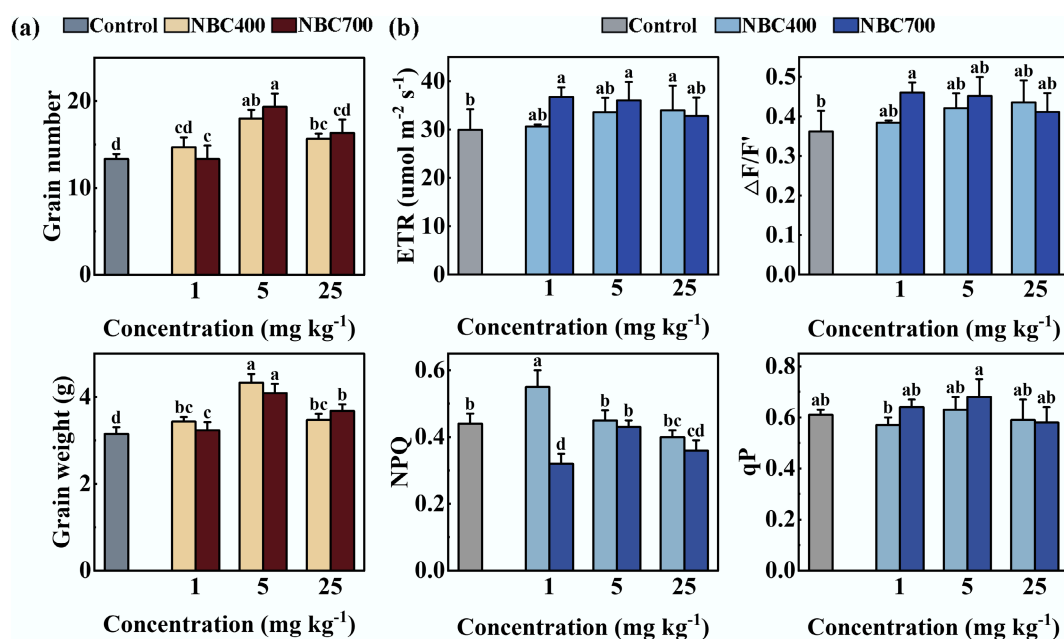


Fig. 2 Beneficial effects of NBC on the growth and photosynthesis of soybean. (a) Grain number and weight; (b) electron transfer rate (ETR), actual photosynthetic efficiency ($\Delta F/F_m'$) of PSII, non-photochemical quenching coefficients (NPQ), and photochemical quenching coefficients (qP). Different lowercase letters indicate significant differences between groups ($p < 0.05$).

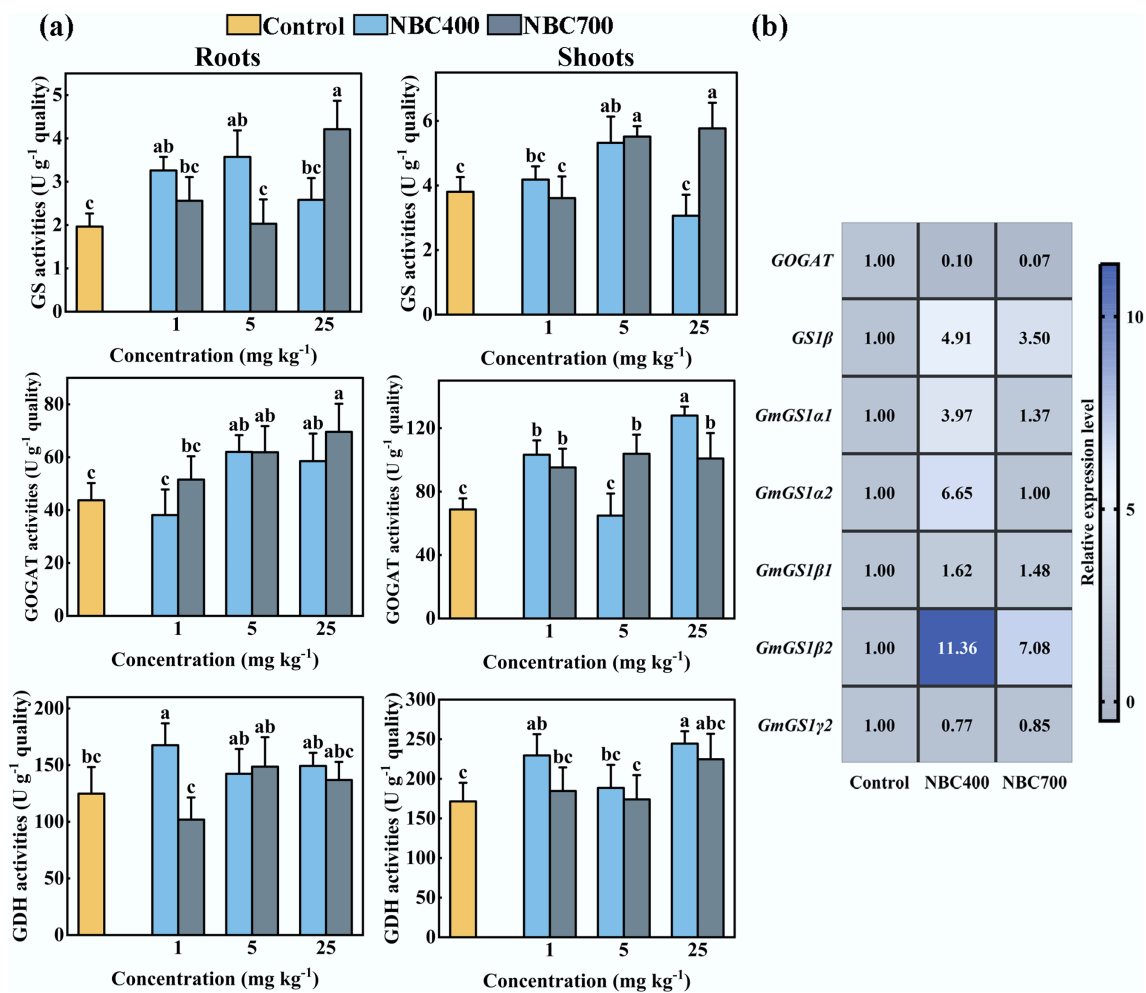


Fig. 3 The effects of NBC on the activity of N assimilation-related enzymes and relative gene expression in soybean. **(a)** The activities of GS, GOGAT, and GDH in the roots and shoots. **(b)** The relative expression of the glutamate synthase gene (*GOGAT*) and the glutamine synthetase gene (*GS*) in plant roots treated with 5 mg kg⁻¹ of NBC. Different lowercase letters indicate significant differences between groups ($p < 0.05$).

significantly enhanced the activities of GS and GOGAT than those of GDH; the NBC dosage-dependent promoting effect on the activity of GDH was not as evident as those for GS and GOGAT. Notably, NBC treatment significantly enhanced enzymatic activities, with GS showing maximal increases of 114% and 51.6% in roots and shoots, respectively. Similarly, GOGAT activity was elevated by 59.1% in roots and 46.6% in shoots. In comparison, GDH exhibited more moderate enhancement, with maximal activity increases of 34.2% in roots and 33.8% in shoots compared to the control. These results reveal that the presence of NBC greatly facilitated the GS-GOGAT cycle, thereby enhancing N assimilation in soybean. The enhanced N assimilation is favorable to plant photosynthesis^[31], which partly accounts for the positive effect of NBC on the photosynthetic performance as discussed above.

To further explore the molecular mechanism of N assimilation enzymatic responses, we investigated the regulation of these enzymes at the genetic level in the presence of 5 mg kg⁻¹ NBC. As depicted in Fig. 3b, the expression of genes related to GS activity (*GS1β*, *GmGS1α1*, *GmGS1α2*, *GmGS1β1*, and *GmGS1β2*, except for *GmGS1γ2*) in the NBC treatment was upregulated from 1.37 to 11.36 times higher than that of the control. The *GmGS1β2* gene encodes a protein with glutamine synthetase activity, which is involved in N

assimilation in soybean nodules. It serves as a key regulatory locus for the genetic enhancement of N metabolism in soybean^[32]. We noted that the *GmGS1β2* gene was the most highly expressed gene (Fig. 3b), which reflected the high potential of NBC in facilitating N assimilation in soybean.

The enhancement of growth, photosynthesis, and enzyme activities by NBC likely involves both indirect and direct pathways: (1) indirect mediation via rhizospheric improvements, where NBC enhances soil conditions and stimulates beneficial microbial activity, thereby supporting plant growth and development; (2) direct uptake and transformation of NBC into soybean root and shoot tissues, where its optoelectronic, physicochemical, and catalytic properties exert nano-specific benefits^[8,9,33]. The structural stability of NBC in soil was assessed by monitoring N mineralization dynamics. No significant differences were observed in $\delta^{15}\text{N}$ abundances of collected gases between NBC-amended and control soils over the two-month experimental period (Supplementary Fig. S5). These findings indicate that NBC exhibits mineralization resistance and is likely to persist in particulate form, facilitating extended interactions with plant roots and soil microbiota throughout the experimental period.

Responses of soil enzyme activities and microorganisms to NBC

Soil nitrate reductase and urease are ubiquitous in agricultural soils and are involved in the geochemical behavior of N^[34]. As shown in [Supplementary Fig. S6](#), NBC treatments did not significantly improve soil nitrate reductase and urease activities during the 90-d incubation. In comparison, the distribution and abundance of rhizosphere soil microorganisms exhibited a more significant response to the presence of NBC than the soil enzymatic activity. Venn diagrams reveal that the incorporation of NBC led to an increase in the distinctiveness of operational taxonomic units (OTUs), with only 1,129 (17.88%) OTUs shared between the treatments and the control ([Supplementary Fig. S7a](#)). Consistently, principal component analysis shows a distinct separation of microbial communities in the rhizosphere as a function of treatment ([Supplementary Fig. S7b](#)). The first principal component explained 69.7% of the differences, with NBC700 exposure showing the greatest disparity. The relative abundance of dominant rhizosphere bacteria was analyzed at the phylum and genus levels, respectively. Both NBC400 and NBC700 (5 mg kg⁻¹) significantly increased the relative abundance of the top 10 bacterial phyla by 0.7–1.4 fold relative to the control ([Supplementary Fig. S7c](#)). Specifically, the relative abundance of *Acidobacteriota* with NBC400 and *Proteobacteria* with NBC700 increased by 5.2% and 15.2%, respectively. *Proteobacteria* are key functional bacteria in the soil and widely involved in the cycles of carbon, nitrogen, and sulfur elements^[35]. All the *Acidobacteriota*, *Actinobacteria*, and *Proteobacteria* could significantly contribute to N transformation in the soil^[36,37]. The relative abundance of *Gemmatimonadota* increased by 16.7% and 3.4% under NBC400 and NBC700 treatments, respectively. *Gemmatimonadota* are plant growth-promoting rhizobacteria that secrete enzymes and metabolites to help solubilize nutrients such as nitrogen, phosphorus, and potassium in the soil. This process makes such nutrients more easily absorbed and utilized by plants, promoting their growth. Additionally, *Gemmatimonadota* activate the immune system in plants, enhancing their resistance^[38].

The incorporation of NBC also significantly increased the relative abundance of the top 10 bacterial genera (e.g., *Flavisolibacter*, *Lysobacter*, *Gemmatimonas*, and *Sphingomonas*) by 0.8–1.6-fold compared with the control ([Supplementary Fig. S7d](#)). *Flavisolibacter* is capable of catalyzing hydrogen peroxide to protect itself and host plants from oxidation^[39]. *Flavisolibacter* can also secrete acid phosphatase, fix carbon dioxide, and participate in ammonia oxidation^[40–42]. *Lysobacter* exhibits strong bacteriolytic and bacteriostatic functions, along with high antagonistic activity against a variety of plant pathogens^[43]. *Gemmatimonas* is one of the dominant taxa found in agroecosystems^[44]. A significant positive correlation between *Gemmatimonas* and soil nutrient composition has been reported in previous studies^[45], and *Gemmatimonas* may be an indicator genus in response to changes in soil nutrient content. *Sphingomonas* has potential roles as biocontrol agents, plant growth promoters, and biological nitrogen fixers^[46]. However, α -diversity analysis showed no significant difference in bacterial diversity between NBC treatments and the control ([Supplementary Fig. S8](#)), indicating that NBC did not remarkably alter rhizosphere microbial diversity. The soil microbial analysis revealed that moderate NBC application exerted negligible impacts on bacterial classification and diversity, demonstrating its low ecotoxicity and excellent environmental compatibility. However, NBC improved the relative abundance of dominant bacterial taxa, thereby promoting nitrogen assimilation and enhancing soybean growth performance.

Uptake and spatial distribution of NBC in soybean

TEM analysis revealed the localization of NBC within leaf mesophyll cells ([Supplementary Fig. S9](#)). Particulate aggregates were observed primarily in intercellular spaces and along cell walls. HRTEM images showed discontinuous lattice fringes, while corresponding SAED patterns exhibited broad, diffuse rings superimposed with discrete diffraction spots. The diffuse rings indicate an amorphous carbon structure, consistent with the short-range ordered, graphite-like microcrystalline architecture characteristic of biochar. Furthermore, EDS mapping confirmed the co-localization of C, N, and O within these particulates, verifying their identity as NBC deposits.

To further elucidate the spatial distribution of ¹⁵N derived from NBC in nodules, NanoSIMS measurement of nodular cells was subsequently carried out. Higher N content especially in the intercellular spaces for the NBC-treated samples was evidenced by the ¹²C¹⁴N⁻ signals ([Fig. 4a](#)). The stronger intensity of ¹²C¹⁴N⁻ signals with NBC400 relative to NBC700 suggests that a higher amount of NBC400 was taken up and accumulated in the nodules, consistent with the stable isotope results ([Fig. 5a](#)). Subsequent measurement of ¹⁵N/¹⁴N ratios ([Fig. 4b](#)) revealed that the intercellular spaces for the NBC-treated samples were highly enriched in ¹⁵N (up to 0.0044), significantly ($p < 0.05$) higher than that of the control (0.0037) ([Fig. 4c](#)). The preferential distribution of NBC within intercellular spaces suggests that a significant portion of the assimilated NBC retains its particulate form within plant tissues. Had extensive decomposition into dissolved nitrogenous fractions occurred, these components would likely have been distributed more uniformly throughout intracellular compartments.

Transformation and fate of NBC in soybean

Values of $\delta^{15}\text{N}$ in the roots, nodules, leaves, and grains were determined ([Fig. 5a](#)). The treatment of NBC400 (5 mg kg⁻¹) increased $\delta^{15}\text{N}$ values in the nodules and grains by 127.8% and 47.9% compared with the control, respectively, and these increases were significantly higher than the enhancement observed in the roots (12.1%). Interestingly, little enhancement of $\delta^{15}\text{N}$ was observed in the leaves of soybeans after exposure to NBC. The isotope tracing results suggest that NBC in the rhizosphere was taken up by soybean roots and preferentially accumulated in the nodules. A portion of the accumulated NBC was translocated from roots to aboveground tissues. The lower $\delta^{15}\text{N}$ in soybean leaves indicates that a majority of the assimilated NBC likely functions as an N reservoir, facilitating nutrient translocation from foliar tissues to developing grains during the reproductive phase to support soybean's physiological requirements. Based on $\delta^{15}\text{N}$ isotopic analysis, the accumulated NBC content in nodules and roots was quantified as 0.05–0.1 mg g⁻¹ (dry weight) for both NBC400 and NBC700 treatments ([Supplementary Fig. S10](#)). We also noted that, although the $\delta^{15}\text{N}$ value of NBC700 was significantly ($p < 0.05$) higher than that of NBC400 in roots ([Fig. 5a](#)), the $\delta^{15}\text{N}$ value of NBC400 was much higher than that of NBC700 in the nodules and grains, indicating the greater transfer efficiency of NBC400 within plant tissues. This large discrepancy might be ascribed to structural differences between low- and high-temperature BCs. With increasing charring temperature, the amorphous carbon in BC is gradually converted into increasingly condensed forms of carbon, leading to a reduced content of oxygen-containing groups and enhanced hydrophobicity of BC. As a result, the morphology of NBC400 contributed more favorably to its absorption by soybean roots compared to that of NBC700, due to its higher hydrophilicity, dispersibility, and smaller D_h in aqueous solution.

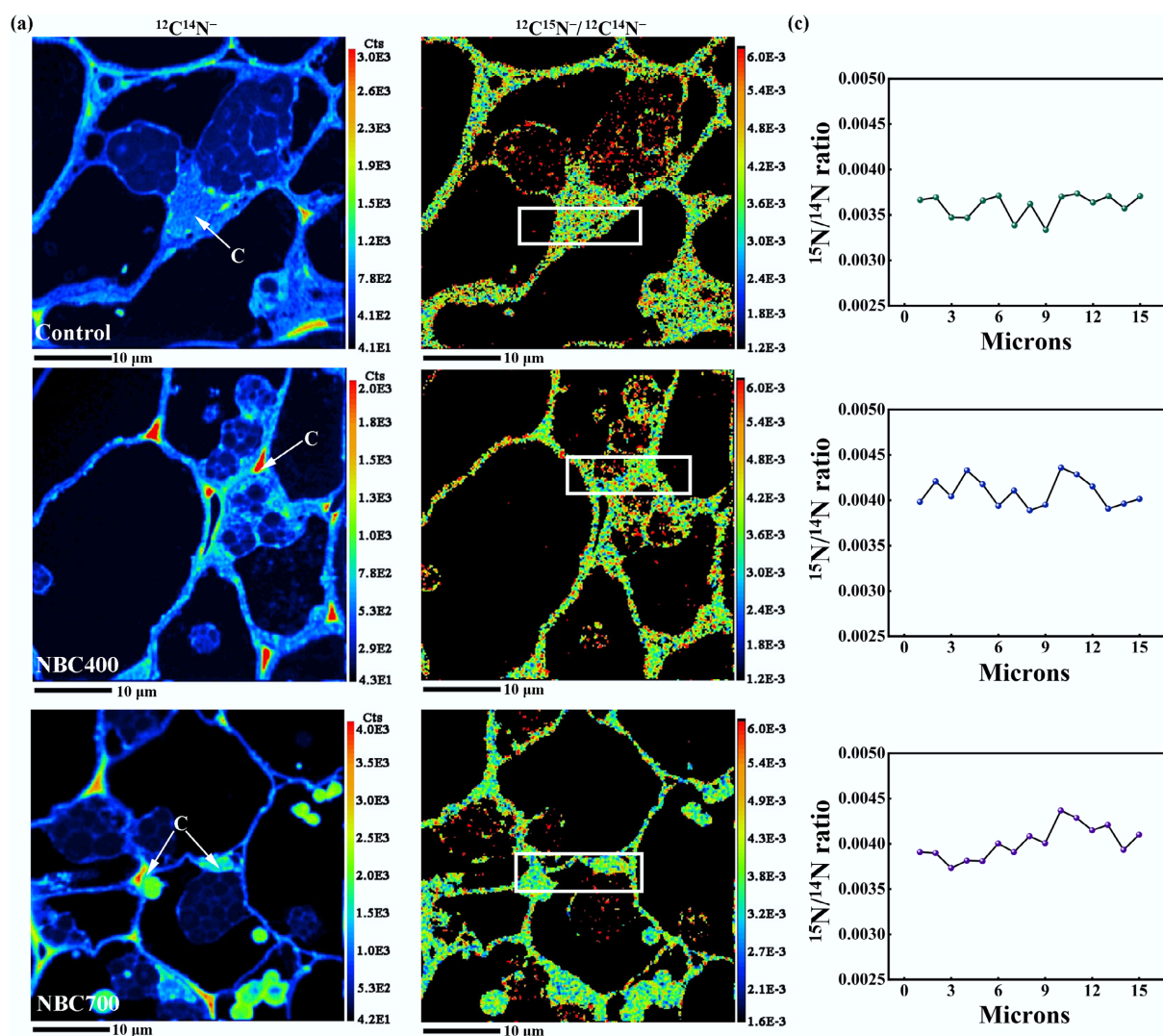


Fig. 4 Nano-scale secondary ion mass spectrometry (NanoSIMS) imaging of soybean nodules in the treatment of NBC400 and NBC700 (5 mg kg⁻¹). (a) $^{12}\text{C}^{14}\text{N}^-$ NanoSIMS images, showing the nitrogen contents in rhizome cells; (b) hue saturation intensity image generated from the NanoSIMS $^{15}\text{N}/^{14}\text{N}$ ratio of the same region, showing the $^{15}\text{N}/^{14}\text{N}$ ratios were enriched for the rhizome cells in the treatments of NBC compared with those for the control; (c) line scan analyses of the selected regions defined in (b) with respect to $^{15}\text{N}/^{14}\text{N}$ ratios. Scale bar: 10 μm for all images.

To further explore the fate of assimilated NBC, the ^{15}N abundance of amino acids in the soybean grains was also examined. It was found that the presence of NBC400 and NBC700 significantly increased ^{15}N abundance of amino acids (Ala, Gly, Val, Leu, Ile, Pro, Asp, Thr, Ser, Met, Glu, Phe, Lys, and Tyr) by 2.3–3.6 times and 1.2–2.3 times compared to the control, respectively (Fig. 5b). We hypothesized that the assimilated NBC underwent partial degradation into smaller fragments and/or nitrogenous compounds during its translocation from root to shoot systems (especially in leaves). These degradation products subsequently participated in plant N assimilation and metabolic processes, potentially serving as additional N sources to support soybean growth and development. To verify the hypothesis, a simulated NBC transformation in soybean foliar tissues was conducted. Intense hydroxyl radical ($\cdot\text{OH}$) signals were detected in mixtures of NBC and intracellular fluid, particularly under illuminated conditions (Fig. 6a). We recently found that NBC is susceptible to photochemical transformation, primarily driven by self-generated ROS^[47]. ROS can also be produced in plant leaves, especially during daylight. These ROS, as byproducts of plant cell

metabolism, actively participate in plant growth and development^[48–50]. The $\cdot\text{OH}$ generated in this study induced partial decomposition of NBC into low-molecular-weight fractions capable of participating in soybean metabolic processes. This conclusion is supported by the identification of analogous degradation products—such as benzoyl cyanide ($\text{C}_6\text{H}_5\text{—CO—CN}$) and hydroxycosylamine ($\text{HO—[CH}_2\text{]}_{19}\text{—NH}_2$)—in both the intracellular fluid incubations and the classical Fenton reaction system ($\text{FeSO}_4 + \text{H}_2\text{O}_2$) (Fig. 6b). Additionally, denitrification products of NBC—including benzoylformic acid ($\text{C}_6\text{H}_5\text{—CO—COOH}$) and 20-hydroxyeicosanal ($\text{HO—[CH}_2\text{]}_{19}\text{—CHO}$)—were identified in the mixture of NBC and intracellular fluid (Fig. 6b). Based on these results, we propose a mechanistic pathway for NBC transformation in soybean foliar tissues (Fig. 6c): $\cdot\text{OH}$ -mediated degradation of NBC produces nitrogen-containing intermediates (e.g., $\text{C}_6\text{H}_5\text{—CO—CN}$ and $\text{HO—[CH}_2\text{]}_{19}\text{—NH}_2$), which are further metabolized through enzymatic denitrification to release bioavailable nitrogen (e.g., NH_4^+) for assimilation via the GS–GOGAT cycle.

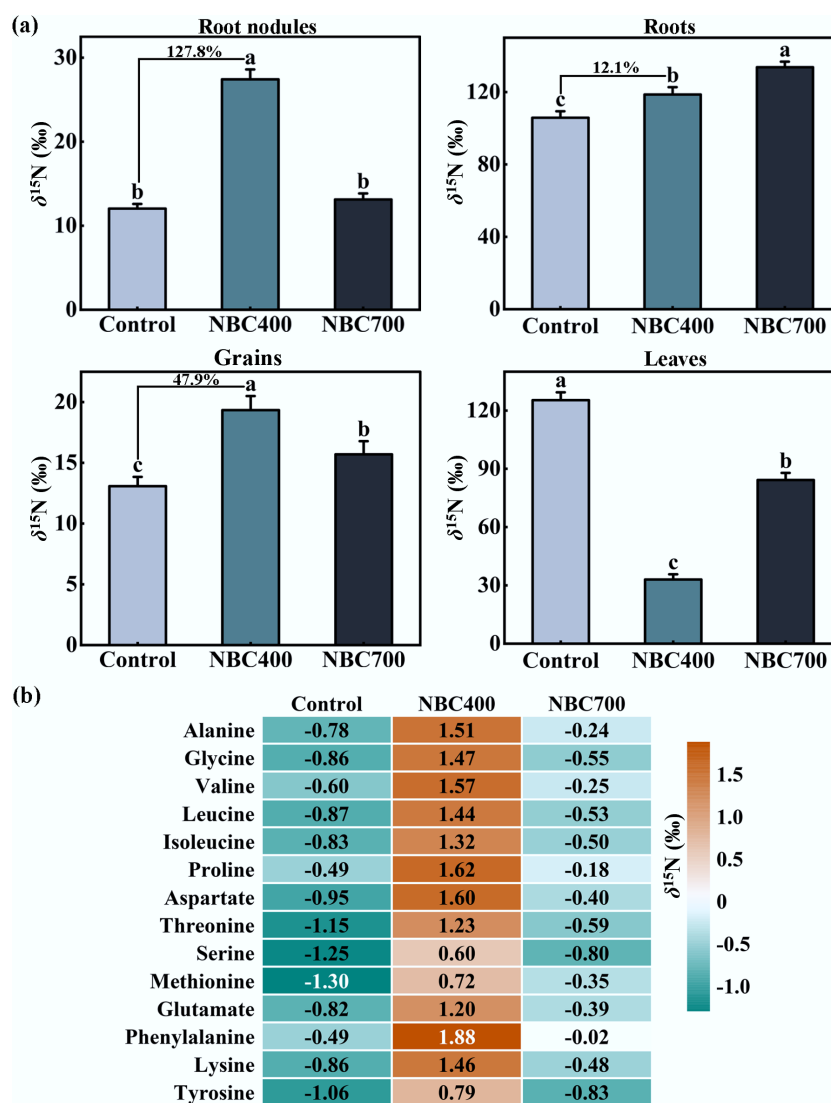


Fig. 5 Accumulation and spatial distribution of ^{15}N in soybean. **(a)** ^{15}N abundance in the roots, nodules, leaves, and grains of soybean; **(b)** ^{15}N abundance in the amino acids of soybean grains. Different lowercase letters indicate significant differences between groups ($p < 0.05$). The application rates of NBC400 and NBC700 were 5.0 mg kg^{-1} .

This study demonstrates that NBC can serve as an efficient nanocarrier to facilitate N delivery from soil to plant tissues and reproductive organs in soybean. The assimilated NBC significantly enhanced photosynthetic performance while upregulating key N assimilation enzymes and associated gene expression, ultimately improving soybean growth and yield. Meanwhile, the application of NBC exhibited minimal adverse effects on soil microbial communities. Beyond N delivery, NBC's unique structure enables the incorporation of additional essential nutrients, including phosphorus, sulfur, and mineral elements, either through feedstock selection or intentional functionalization^[51]. Hence, NBC has significant potential to act as a promising nanofertilizer and/or targeted delivery platform for precision agriculture. Compared with engineered carbon nanomaterials such as GO and SWNTs, NBC offers exceptional advantages in terms of environmental compatibility and the ability to be produced in a large-scale, reproducible, and cost-effective manner. This research highlights the transformative potential of biochar-based nanomaterials in sustainable agricultural practices, providing

an eco-friendly alternative to conventional chemical fertilizers while promoting the development of green nanotechnology solutions.

Conclusions

This study explored the potential of nitrogen-doped biochar nanoparticles as N delivery nanocarriers for soybean. At a soil application rate of 5 mg kg^{-1} , NBC improved photosynthetic performance, upregulated key N assimilation enzymes and related genes, and consequently promoted soybean growth and yield, while exerting negligible impacts on soil microbial communities. Microscopic observations confirmed that NBC can be directly taken up by soybean roots. The unique physicochemical and nano-scale properties of NBC facilitate efficient N delivery and in planta assimilation. Additionally, NBC improved soil physicochemical conditions and stimulated the activity of beneficial microorganisms. Collectively, N-doped biochar nanoparticles exhibit great potential for applications in nano-enabled agriculture as a more effective and low-cost alternative to conventional fertilizers.

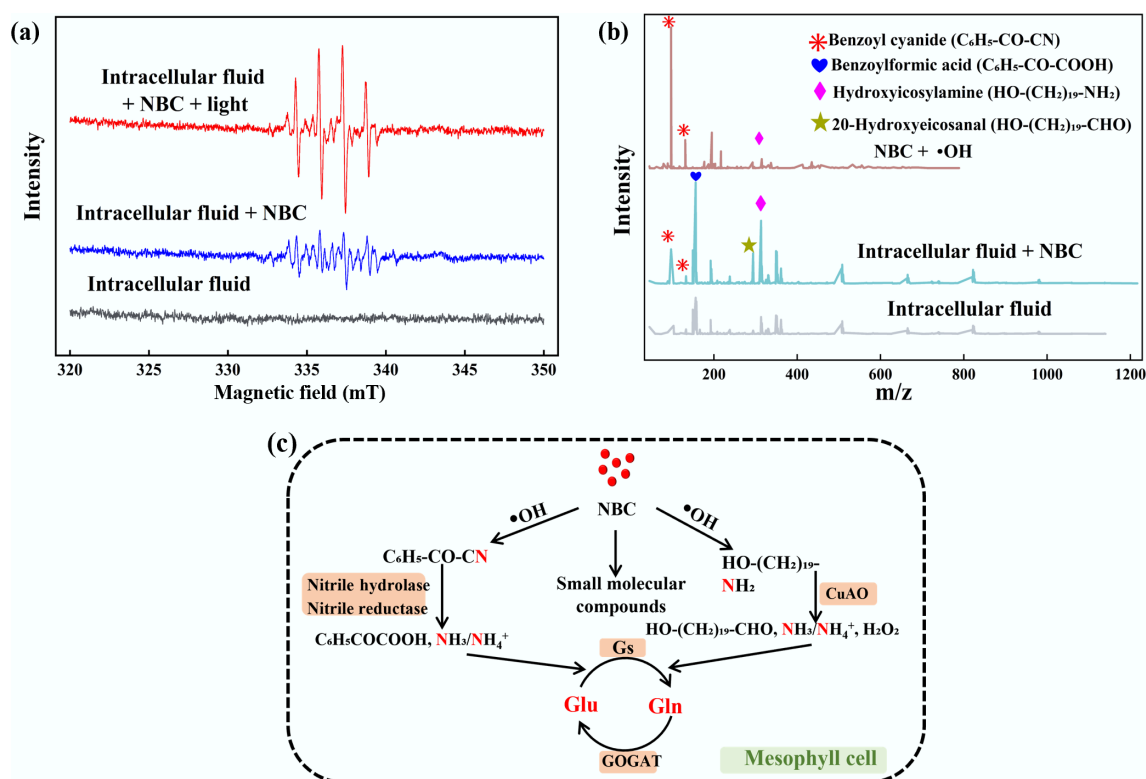


Fig. 6 Transformations of NBC in soybean leaves. **(a)** Hydroxyl radical ($\cdot\text{OH}$) signal intensities in the intracellular fluid extracted from soybean leaves under dark conditions and in intracellular fluid-NBC mixtures with and without simulated sunlight exposure. **(b)** Comparison of NBC degradation products identified by LC-MS in intracellular fluid and the Fenton reaction system. The chemical composition of the intracellular fluid alone was characterized by LC-MS as a control. **(c)** Proposed mechanistic pathway for NBC transformation in soybean foliar tissues. Partial $\cdot\text{OH}$ -mediated degradation of NBC yields nitrogen-containing intermediates, which undergo enzymatic denitrification via nitrile hydratase, nitrile reductase, and copper amine oxidase (CuAO) to release bioavailable nitrogen for assimilation through the GS-GOGAT cycle.

Supplementary information

It accompanies this paper at: <https://doi.org/10.48130/scm-0026-0028>.

Ethical statements

Not applicable.

Author contributions

The authors confirm their contributions to the paper as follows: Zexuan Geng: conceptualization, investigation, data curation, formal analysis, writing – original draft; Heng Zhang: conceptualization, investigation, data curation; Meiyan Wang: investigation, data curation; Jiadong Cheng: investigation, methodology support; Xiaoyi Xu: investigation, data curation; Fei Lian: conceptualization, methodology, funding acquisition, supervision, project administration, writing – review & editing.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

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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References

- [1] Pignatello JJ, Mitch WA, Xu W. 2017. Activity and reactivity of pyrogenic carbonaceous matter toward organic compounds. *Environmental Science & Technology* 51:8893–8908
- [2] Spokas KA, Novak JM, Masiello CA, Johnson MG, Colosky EC, et al. 2014. Physical disintegration of biochar: an overlooked process. *Environmental Science & Technology Letters* 1:326–332

- [3] Lian F, Xing B. 2024. From bulk to nano: formation, features, and functions of nano-black carbon in biogeochemical processes. *Environmental Science & Technology* 58:15910–15925
- [4] Xiao J, Hu R, Chen G. 2020. Micro-nano-engineered nitrogenous bone biochar developed with a ball-milling technique for high-efficiency removal of aquatic Cd(II), Cu(II) and Pb(II). *Journal of Hazardous Materials* 387:121980
- [5] Zhang Y, Fan S, Liu T, Fu W, Li B. 2022. A review of biochar prepared by microwave-assisted pyrolysis of organic wastes. *Sustainable Energy Technologies and Assessments* 50:101873
- [6] Lian F, Gu S, Han Y, Wang Z, Xing B. 2022. Novel insights into the impact of nano-biochar on composition and structural transformation of mineral/nano-biochar heteroaggregates in the presence of root exudates. *Environmental Science & Technology* 56:9816–9825
- [7] Yi P, Pignatello JJ, Uchimiya M, White JC. 2015. Heteroaggregation of cerium oxide nanoparticles and nanoparticles of pyrolyzed biomass. *Environmental Science & Technology* 49:13294–13303
- [8] Lowry GV, Avellan A, Gilbertson LM. 2019. Opportunities and challenges for nanotechnology in the agri-tech revolution. *Nature Nanotechnology* 14:517–522
- [9] Kah M, Tufenkji N, White JC. 2019. Nano-enabled strategies to enhance crop nutrition and protection. *Nature Nanotechnology* 14:532–540
- [10] Li Y, Xu X, Lei B, Zhuang J, Zhang X, et al. 2021. Magnesium-nitrogen co-doped carbon dots enhance plant growth through multifunctional regulation in photosynthesis. *Chemical Engineering Journal* 422:130114
- [11] Xiong JL, Ma N. 2022. Transcriptomic and metabolomic analyses reveal that fullerol improves drought tolerance in *Brassica napus* L. *International Journal of Molecular Sciences* 23(23):15304
- [12] Martin-Ortigosa S, Valenstein JS, Lin VSY, Trewyn BG, Wang K. 2012. Gold functionalized mesoporous silica nanoparticle mediated protein and DNA codelivery to plant cells via the biolistic method. *Advanced Functional Materials* 22:3576–3582
- [13] Yan X, Chen S, Pan Z, Zhao W, Rui Y, et al. 2023. AgNPs-triggered seed metabolic and transcriptional reprogramming enhanced rice salt tolerance and blast resistance. *ACS Nano* 17:492–504
- [14] Wang J, Cao X, Wang C, Chen F, Feng Y, et al. 2022. Fe-based nanomaterial-induced root nodulation is modulated by flavonoids to improve soybean (*Glycine max*) growth and quality. *ACS Nano* 16:21047–21062
- [15] Gu S, Lian F, Han Y, Taherymoosavi S, Mitchell D, et al. 2022. Nano-biochar modulates the formation of iron plaque through facilitating iron-involved redox reactions on aquatic plant root surfaces. *Environmental Science: Nano* 9:1974–1985
- [16] Li Y, Ge C, Cheng C, Wang X, Si D, et al. 2023. Nano-biochar uptake and translocation by plants: assessing environmental fate and food chain risk. *Science of The Total Environment* 905:167012
- [17] Zhang Z, Cui X, Qu X, Fu H, Tao S, et al. 2024. Revealing molecular structures of nitrogen-containing compounds in dissolved black carbon using ultrahigh-resolution mass spectrometry combined with thermodynamic calculations. *Environmental Science & Technology* 58:11998–12007
- [18] Huang C, Xia T, Niu J, Yang Y, Lin S, et al. 2018. Transformation of ^{14}C -labeled graphene to $^{14}\text{CO}_2$ in the shoots of a rice plant. *Angewandte Chemie International Edition* 57:9759–9763
- [19] Jiao Y, Zhu X, Yu F, Xu M, Cai R, et al. 2025. Upcycling trace amounts of biomass waste into flash graphene can boost crop yields by more than a quarter and offer climate benefits. *One Earth* 8(10):101486
- [20] Lyu H, Li Y, Wang Y, Wang P, Shang Y, et al. 2023. Drive soil nitrogen transformation and improve crop nitrogen absorption and utilization - a review of green manure applications. *Frontiers in Plant Science* 14:1305600
- [21] Schroeder JI, Delhaize E, Frommer WB, Guerinot ML, Harrison MJ, et al. 2013. Using membrane transporters to improve crops for sustainable food production. *Nature* 497:60–66
- [22] Hristov AN, Bannink A, Crompton LA, Huhtanen P, Kreuzer M, et al. 2019. Nitrogen in ruminant nutrition: a review of measurement techniques. *Journal of Dairy Science* 102:5811–5852
- [23] Wang Q, Shan C, Zhang P, Zhao W, Zhu G, et al. 2024. The combination of nanotechnology and potassium: applications in agriculture. *Environmental Science and Pollution Research International* 31:1890–1906
- [24] Cao X, Yue L, Wang C, Luo X, Zhang C, et al. 2022. Foliar application with iron oxide nanomaterials stimulate nitrogen fixation, yield, and nutritional quality of soybean. *ACS Nano* 16:1170–1181
- [25] Lian F, Yu W, Wang Z, Xing B. 2019. New insights into black carbon nanoparticle-induced dispersibility of goethite colloids and configuration-dependent sorption for phenanthrene. *Environmental Science & Technology* 53:661–670
- [26] Ding Y, Watanabe A, Jaffé R. 2014. Dissolved black nitrogen (DBN) in freshwater environments. *Organic Geochemistry* 68:1–4
- [27] Li M, Zhang P, Guo Z, Cao W, Gao L, et al. 2023. Molybdenum nanofertilizer boosts biological nitrogen fixation and yield of soybean through delaying nodule senescence and nutrition enhancement. *ACS Nano* 17(15):14761–14774
- [28] Li Z, Chen J, Xu L, Zhang P, Ni H, et al. 2024. Quinolone antibiotics inhibit the rice photosynthesis by targeting photosystem II center protein: generational differences and mechanistic insights. *Environmental Science & Technology* 58:11280–11291
- [29] Oldroyd GE, Murray JD, Poole PS, Downie JA. 2011. The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics* 45:119–144
- [30] Wang Y, Zhang P, Li M, Guo Z, Ullah S, et al. 2020. Alleviation of nitrogen stress in rice (*Oryza sativa*) by ceria nanoparticles. *Environmental Science: Nano* 7:2930–2940
- [31] Li C, Zhao C, Zhao X, Wang Y, Lv X, et al. 2023. Beneficial effects of biochar application with nitrogen fertilizer on soil nitrogen retention, absorption and utilization in maize production. *Agronomy* 13:113
- [32] Morey KJ, Ortega JL, Sengupta-Gopalan C. 2002. Cytosolic glutamine synthetase in soybean is encoded by a multigene family, and the members are regulated in an organ-specific and developmental manner. *Plant Physiology* 128:182–193
- [33] Zhang Y, Wang M, Wang Y, Wang F, Gong Y, et al. 2024. Dissolved black nitrogen: an overlooked active nano-catalyst in the abiotic transformation of chlorophenols by sulfides in the subsurface water. *Biochar* 6:48
- [34] Liu Y, Dai Q, Jin X, Dong X, Peng J, et al. 2018. Negative impacts of biochars on urease activity: high pH, heavy metals, polycyclic aromatic hydrocarbons, or free radicals? *Environmental Science & Technology* 52:12740–12747
- [35] Spain AM, Krumholz LR, Elshahed MS. 2009. Abundance, composition, diversity and novelty of soil Proteobacteria. *The ISME Journal* 3:992–1000
- [36] Ågren GI, Wetterstedt JÅM, Billberger MFK. 2012. Nutrient limitation on terrestrial plant growth – modeling the interaction between nitrogen and phosphorus. *New Phytologist* 194:953–960
- [37] Liu S, Li X, Fu Y, Li P, Qiao J, et al. 2024. Exploring the effects of different fertilizer application durations on the functional microbial profiles of soil carbon and nitrogen cycling by using metagenomics in *Paulownia* plantations in a subtropical zone. *European Journal of Forest Research* 143:955–969
- [38] Haskett TL, Tkacz A, Poole PS. 2021. Engineering rhizobacteria for sustainable agriculture. *The ISME Journal* 15:949–964
- [39] Janarthanan OM, Laycock B, Montano-Herrera L, Lu Y, Arcos-Hernandez MV, et al. 2016. Fluxes in PHA-storing microbial communities during enrichment and biopolymer accumulation processes. *New Biotechnology* 33:61–72
- [40] Lian TX, Wang GH, Yu ZH, Li YS, Liu XB, et al. 2017. Bacterial communities incorporating plant-derived carbon in the soybean rhizosphere in Mollisols that differ in soil organic carbon content. *Applied Soil Ecology* 119:375–383
- [41] Fu H, Liu H, Mao J, Chu W, Li Q, et al. 2016. Photochemistry of dissolved black carbon released from biochar: reactive oxygen species generation and phototransformation. *Environmental Science & Technology* 50:1218–1226
- [42] Yu Z, Li Y, Wang G, Liu J, Liu J, et al. 2016. Effectiveness of elevated CO_2 mediating bacterial communities in the soybean rhizosphere depends on genotypes. *Agriculture, Ecosystems & Environment* 231:229–232

- [43] Bacon CW, Yates IE, Hinton DM, Meredith F. 2001. Biological control of *Fusarium moniliforme* in maize. *Environmental Health Perspectives* 109(suppl 2):325–332
- [44] Banerjee S, Kirkby CA, Schmutter D, Bissett A, Kirkegaard JA, et al. 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biology and Biochemistry* 97:188–198
- [45] Liu C, Zhuang J, Wang J, Fan G, Feng M, et al. 2022. Soil bacterial communities of three types of plants from ecological restoration areas and plant-growth promotional benefits of *Microbacterium invictum* (strain X-18). *Frontiers in Microbiology* 13:926037
- [46] Mehmood MA, Zhao H, Cheng J, Xie J, Jiang D, et al. 2020. Sclerotia of a phytopathogenic fungus restrict microbial diversity and improve soil health by suppressing other pathogens and enriching beneficial microorganisms. *Journal of Environmental Management* 259:109857
- [47] Lian F, Zhang Y, Gu S, Han Y, Cao X, et al. 2021. Photochemical transformation and catalytic activity of dissolved black nitrogen released from environmental black carbon. *Environmental Science & Technology* 55:6476–6484
- [48] Crisp PA, Ganguly DR, Smith AB, Murray KD, Estavillo GM, et al. 2017. Rapid recovery gene downregulation during excess-light stress and recovery in *Arabidopsis*. *The Plant Cell* 29:1836–1863
- [49] Dogra V, Li M, Singh S, Li M, Kim C. 2019. Oxidative post-translational modification of EXECUTER1 is required for singlet oxygen sensing in plastids. *Nature Communications* 10:2834
- [50] Mittler R. 2017. ROS are good. *Trends in Plant Science* 22:11–19
- [51] Santana I, Jeon SJ, Kim HI, Islam MR, Castillo C, et al. 2022. Targeted carbon nanostructures for chemical and gene delivery to plant chloroplasts. *ACS Nano* 16:12156–12173



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