Promotion effect of nitrogen-doped functional carbon nanodots on the early growth stage of plants

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Abstract

The objective of this paper is to study the effects of nitrogen-doped functional carbon nanodots (N-FCNs) on the early growth stage of plants. Hydrosoluble and biocompatible N-FCNs with high content of available N (ammonium and amino groups) and carboxyl groups are synthesized by a super green electrochemical method. N-FCNs universally express good eurytopic influence on different species of plants by inducing seeds germination, promoting root development, biomass accumulation, root cell length, chlorophyll level and transpiration of young seedlings. When functional carbon nanodots without N doping (FCNs) promote tomato and corn seeds germination rate by 92.4% and 76.2% maximally, N-FCNs could further improve the germination rate by about 17.0% and 25.5%. N-FCNs can even significantly raise the green vegetable (pakchoi) yield to 2.1 and 1.4 times on the 18th and 30th day. Leaf chlorophyll content is also increased to 1.36 and 1.55 times compared with FCNs treated group and the control group, respectively. The promotion effect of the nanodots is apparently depended on their composition, nanostructure, as well as plant species and age. Nanoscale structure and abundant hydrophilic functional groups can enable N-FCNs regulating the seed germination and plant growth by promoting the uptake and transportation of water and nutrients. The accumulation and transport of N-FCNs are investigated, which reveals N-FCNs are friendly to cells because they are absorbed and transported through nonprotoplast pathway in plant. As a result, N-FCNs have great potential for horticulture application as a biocompatible nano-medium to regulate both metabolism and early development of plants.

Key words: functional carbon nanodots; nitrogen-doped; early growth regulation; germination; vegetative growth; biocompatible.

INTRODUCTION

Manufactured nanomaterials are widely used in commercial products, such as electronics, textiles, medical products, foods and agrichemicals [1–3]. Nanotechnology has provided a new generation of advanced and effective technological platforms for the modern agriculture and horticulture area. The applications of
nanotechnology in agriculture include fertilizers for promoting plants growth and yield, pesticides for pest and disease management and sensors for monitoring soil quality and plant health [4, 5].

Carbon-based nanomaterials [fullerene, carbon nanotubes (CNTs), graphene, carbon dots, etc.] not only have high surface area, but also are easily to be modified by doping heteroatoms or functional groups. As a result, the fundamental and application research of carbon nanomaterials (CNMs) in the field of environment and agriculture has aroused compelling research interest due to their outstanding chemical and physical properties [6]. It was experimentally demonstrated that CNMs in low doses are able to enhance water absorption, encapsulate and slowly release fertilizers and activate physiological processes (seed germination, vegetative growth, fruit yield, etc.) in plants, making them potentially useful in tissue engineering and agriculture technology [7–10]. CNTs were reported to be able to penetrate tomato seeds coat and significantly promote their germination and growth rates [11]. It was also found that CNTs could penetrate the walls and membranes of tobacco cells and enhance the growth of tobacco cell culture [12, 13]. C60 nanoparticles can be absorbed and transmitted from roots to stem leaves and seeds [14]. Water soluble carbon nanodots can enter inside the plant and enhance the production of cereal crop [15]. Carbon nanoparticles with such penetration ability could deliver different cargoes (water, ions, fertilizers, genes, etc.) into plant cell organelles acting as a potential smart treatment-delivery system in plants [16, 17]. Khodakovskaya et al. [7] also demonstrated that multi-walled CNTs (MWCNTs) could promote flowering rate and fruited rate of tomato by affecting the expression of genes that are essential for cell division and plant development, and MWCNTs also could change soil environment by affecting the composition of soil microbiota. It was also found that moderate concentration (20–30 mg/L) of carbon dots could significantly increase lettuce yield by influencing plant physiological response, such as promote photosynthetic rate, along with increasing root and stem length, biomass and carbohydrate contents [18, 19].

Nitrogen (N) is an essential macronutrient that promotes plant germination, growth, development and productivity [20–22]. Besides its role as an essential nutrient, inorganic and organic N sources play key roles as a signaling molecule that controls genome-wide gene expression as well as impacts both metabolism and development of plants. [23, 24] such as breaking seed dormancy, inducing leaf expansion, regulating lateral root development and coordinating the expression of nitrate-related genes [20, 25, 26]. Arabidopsis thaliana (A. thaliana) seeds exposed to the higher nitrate concentration are less dormant than those exposed to the lower nitrate or just soaked in water, indicating a positive correlation between nitrate and seed dormancy breaking [27]. In addition, this effect is mediated by hormone levels (such as abscisic acid, ABA) reduction which aroused by the induction of the unique gene (such as CYP707A2) associated with hormone catabolism. Based on its tremendous contribution to crops growth, large quantities of nitrogenous fertilizers have been applied in agricultural systems, but the N-use efficiency for crops is very low [28]. Beyond the high economic costs caused by a large quantity of fertilizers, excessive N use in agriculture causes a serious of environmental problems, such as eutrophication and subsequent global acidification, stratospheric ozone loss and greenhouse effect [29, 30]. Functional carbon nanodots (FCNs) with multiple active functional groups can be easily modified by doping other element or chemical groups. If effective N could be doped on FCNs as structure of ammonium, nitro or amino, the N-doped FCNs (N-FCNs) could have active effects on saving nitrogenous fertilizers and promoting plants growth and development as both nutrient and signaling.

In this work, N-FCNs are synthesized as a plant growth regulator by using a very simple one-step electrolytic strategy. N content in materials can be easily adjusted, and this method is green, low cost and scalable without reagent waste. We investigated the potential influence of high hydrosoluble N-FCNs on five plant species including dicotyledonous plants (pakchoi, tomato and soybean), monocotyledon plant (corn) and a dicot model organism A. thaliana. The evaluations focus on the effects of N-FCNs on the development and physiology of plants in seed germination, root elongation, biomass, photosynthetic pigment content, cell morphology as well as the N-FCNs accumulation in plant. Results from this research will help to reveal the plant growth regulation mechanism and biosecurity of N-FCNs, to promote their further practical application in the field of agriculture and horticulture.

RESULTS AND DISCUSSION
Characterization of N-FCNs

Water-soluble N-FCNs were prepared by the electrolytic method using two graphite plates as the electrode and ammonia aqueous solution as electrolyte (Supplementary Fig. S1a). After freeze-drying, the N-FCNs powder is dark gray while FCNs powder is deep brown (Supplementary Fig. S1e). Both of materials are very easily dispersed in water and their aqueous solutions of 1.0 mg ml−1 are shown in Supplementary Fig. S1d. The N content in N-FCNs is very easy to be tuned by changing the ammonia concentration. Elementary analysis of N-FCNs obtained in different ammonia concentration is displayed in Supplementary Table S1. Maximum 8% atomic percentage of N in N-FCNs can be obtained. In the early electrolysis stage, the concentration of N-FCNs increases to 1.26 mg ml−1 at 192 h (Supplementary Fig. S1b), while the solution pH declines from 11.57 to 7.36, and the ion conductivity of the solution increases gradually (Supplementary Fig. S1c). After 168 h, the pH and conductivity of the reaction mixture are stable, suggesting the ions produced by the electrolysis reaction are in equilibrium with that required for the oxidation reaction. Therefore, 168 h is set as the optimal reaction time for the synthesis of N-FCNs.

Transmission electron microscope (TEM) image shows that N-FCNs particles are dots of 4–10 nm (Fig. 1a), larger than FCNs of 2–8 nm (Supplementary Fig. S2a). However, after storing for 30 days in room temperature, N-FCNs are aggregated in fusiform shape with about 100–170 nm in wide and 300–500 nm in length. The large aggregates can recover to the smaller size of 4–10 nm after sonication. High-resolution TEM (HRTEM) observation from the edge of N-FCNs particle indicates that the spindle-shaped large particles are aggregation of nanodots with size of several nanometer, and the crystalline lattice with spacing of 0.21 nm of each N-FCNs nanodots can be clearly observed (Fig. 1c and d).

Raman spectra (Fig. 2a) indicate that the graphene structure of N-FCNs and FCNs has multiple defects because of the abundant functional groups on the surface. The similar /I(D)/I(G) of FCNs and N-FCNs demonstrates that N doping does not alter the graphene structure by replacing the carbon atoms in the carbon ring. Fourier transform infrared (FT-IR) spectra of lyophilized N-FCNs and FCNs (Fig. 2b) show that the main functional groups of N-FCNs are −OH (1360 cm−1), −NH (−NH2, −NH3 3408 cm−1), C=N (1235 cm−1), −COO− (1585 cm−1), O=C−R (around 1700 cm−1), −CH2 (1403 cm−1), C−O−C (1260 cm−1), etc., while FCNs have −COOH,
-COOR, -OH, O=C-R, and C-O-C. ¹H-NMR spectra (Fig. 2c) also show the peak of N-H at 3.39 ppm, proving the existence of amino. From X-ray photoelectron spectroscopy (XPS) in Fig. 2d, N-FCNs contain carbon, N and oxygen elements. The results of high-resolution XPS spectra of N1s (Fig. 2e) and C1s (Fig. 2f) are consistent with the FT-IR and NMR results, revealing the bonding information of N element (-NH-, NH4, and C=N). Compared XPS C1s spectrum of N-FCNs with that of FCNs (Supplementary Fig. S2b), C–OR/C–OH (at 286.7 eV) and COO (at 288.9 eV) peaks disappear and the relative contents of C=O, C-N, sp² C is about 1.8:1.0:5.0. The XPS N1s spectra of N-FCNs can be deconvoluted into two components centered at 400.9 and 399.0 eV, corresponding to the ammonium and amino, respectively, and their content ratio is about 1.4:1. Thermogravimetric analysis (TGA) curve (Supplementary Fig. S3a) shows the apparently reduced weight at about 170°C, which is ascribed to the decomposition of ammonium.

According to the above characterization results, the simulated molecular structures of N-FCNs in front view and top view are shown in Fig. 2h. The crimped geometry may result from interaction of ions (such as reaction of zwitterions), hydrogen bonding interactions and van der Waals force between groups and molecules. The existence of amine and ammonium reveals that both amination reaction and neutralization reaction with acid–base groups may happen in the electrolytic process, which reduce the amount of oxygen-containing groups. The aggregation of N-FCNs nanodots could be attributed to the reduction of oxygen-containing groups, which induce the stronger van der Waals force attraction, hydrogen bonding interactions and ionic interaction between N-FCNs nanodots, resulting in easy aggregation. The simulated individual molecular structure of N-FCNs also presents a fusiform shape, which may determine the final shape of the self-assembled N-FCNs particles.

The optical properties of N-FCNs and FCNs were characterized with visible ultraviolet spectrophotometer (UV–vis) and fluorospectro (FS) photometer. The UV–vis spectrum (Supplementary Fig. S3b) shows that N-FCNs have an ultraviolet absorption band between 200 and 300 nm. In the FS spectra of N-FCNs and FCNs in Fig. 2g, both of the materials have obvious fluorescence emission signal between 500 and 650 nm under the excitation of UV light of 465 nm. Although the fluorescence intensity of fresh N-FCNs (pH 7.8) is much higher than that of FCNs (pH 2.0), the result is reversed when the pH is tuned to 6.8, which is the appropriate culture pH for plant. The change of fluorescence intensity may be determined by the surface chemistry and electron transitions on the materials surface [31]. Based on the fluorescence properties of N-FCNs and FCNs, the distribution of materials in plants can be observed directly through the laser confocal scanning microscope (LSCM).

Plant growth regulation by N-FCNs

N addition in FCNs could change the physiological response of plant. As shown in Supplementary Fig. S4, after 17-day growth under 4 mg kg⁻¹ N-FCNs treatment, the seedlings are developed with obviously longer roots length, larger rosettes and higher fresh biomass with increasing N content in N-FCNs. The difference is not significant when the N atomic percentage increases to 7.81% and 7.88% in the N-FCNs (5 wt.%) group and N-FCNs (8 wt.%) groups, respectively. As a result, moderate amounts of N could induce apparent plant’s metabolic and developmental response, and we select N-FCNs (5 wt.%) to conduct the following experiment.
We found that N-FCNs addition could significantly promote the early vegetative growth of *A. thaliana* especially in seedling growth. N-FCNs-treated plants exhibit larger rosettes and richer, longer roots at the early cultivation time compared with FCNs-treated group and the control group (Murashige & Skoog (MS) only) (Fig. 3a and Supplementary Fig. S4). However, the promotion effect becomes weaker with the time, and the dry mass of N-FCNs-treated individual plant is lower than FCNs treated one at 15 day. Meanwhile, N-FCNs-treated plants are retarded with roots after about 10 days, and exhibit shorter root length than that of FCNs-treated ones when 15 day (Fig. 3b and c). With N doping, the chlorophyll content of N-FCNs-treated plants is always higher than that of control for the whole growth cycle (Fig. 3d). When cultured for 10 days, the promotion effect of N-FCNs on plant growth is the highest with increased dry mass and chlorophyll content by about 26% and 36% than that of FCNs treated group, and by about 64% and 55% than that of control, respectively. Moreover, the phenotype of 10-day N-FCNs treated *A. thaliana* displays deeper green leaves than that of the MS group, indicating much higher chlorophyll content (Supplementary Fig. S5).

The promotion effect of N-FCNs on plant vegetative growth was also proved in pot culture of pakchoi. Comparing the control, FCNs and N-FCNs treatment, pakchoi plants treated by N-FCNs (6.0 mg kg\(^{-1}\) in soil) are the largest in phenotype (Fig. 3f) and have the highest yield on 18 day. The pakchoi yields in N-FCNs group are raised to 2.1 and 1.4 times than control on 18th and 30th day, respectively. The result indicates that N-FCNs have great application potential in market gardening.

When *A. thaliana* seedlings are treated by two different medium orderly, the promotion effect of N-FCNs on seedlings' vegetative growth can be more significant (Supplementary Fig. S6). The *A. thaliana* seeds and their young seedlings were cultivated on respective medium combination including MS/MS, MS/ N-FCNs, N-FCNs/MS, N-FCNs/N-FCNs, N-FCNs/FCNs and FCNs/FCNs groups. By statistic, N-FCNs/FCNs treated seedlings have the highest dry biomass than other groups, about twice of MS/MS group, 1.5 times of N-FCNs/N-FCNs group and 1.2 times of FCNs/FCNs group. The above results prove the specific promotion effect of N-FCNs on plants' early growth. Meanwhile, FCNs have more significantly positive effect on the growth of mature seedlings. As a result, reasonable cooperation...
of N-FCNs and FCNs can exert the best effect on plant growth regulation in agriculture and horticulture.

The specific effect of N-FCNs on plants early growth is speculated to be relative to the composition and structure. On the one hand, the available N doped on N-FCNs is more beneficial to the early vegetative growth of plants. The N element is one of the essential macronutrients and an important signal that has profound impacts on plant growth and development [32]. It was unraveled that the nitrate can act as a signal in regulating genome expression and then results in hormonal changes and root architecture [22, 33]. On the other hand, the large amount of negatively charged –COOH enhances N-FCNs adsorption capacity with roots and nutrients [34]. It means that N-FCNs can act as a bridge between root and nutrients to provide plenty of nutrients for the vigorous growth of plants in seedling stage. In order to understand the mechanism of N-FCNs on plant,
experiments were conducted by comparing the effect of free N nutrient and N-FCNs. In the control experiment, young seedlings were cultivated in 1/2 MS medium and medium with only urea (MS + urea), N-FCNs (MS + FCNs), and mixture of FCNs and urea (MS + urea + FCNs) with equal N content. N-FCNs obviously promote seedlings' growth with significantly higher biomass compared with plants exposed to urea alone and urea and FCNs (Supplementary Fig. S7). The result reveals that N-FCNs have an irreplaceable capability to regulate seedling growth possibly due to their unique nanoscale structure together with N doping.

Seeds germination

To further investigate the eurytopic effect of N-FCNs on plant early growth and development, N-FCNs were proved able to promote seeds germination on both tomato and corn. Figure 3 shows the photographs of shoot and the germination rate of tomato seeds and corn seeds in each group. It is obvious that the seeds germination treated by FCNs and N-FCNs is visible promoted. As radicles expose to FCNs or N-FCNs directly, root is the major organ to confront FCNs or N-FCNs during the seeds germination. Therefore, a significant increase in young roots length of the treated seeds can be observed. When FCNs promote tomato and corn seeds germination rate by 92.4% and 76.2% maximally, N-FCNs could further improve the germination rate by about 17.0% and 25.5% compared with FCNs when 3-day and 2-day, respectively. The results not only prove the universality of N-FCNs to activate the early physiological processes of plants, but also further reveal the regulation effect of N-FCNs on the dormancy and germination of plant seeds.

Certain N-containing compounds have been identified as an exogenously signal molecules by controlling seed dormancy and germination in higher plants, and this signaling may involve interactions with some hormone pathways, like ABA or gibberellins [20, 27, 30]. The water absorption amount of plant seeds during the germination process also affects their germination rate. More absorbed water could activate seeds metabolism and promote germination. The tomato and corn seeds are soaked in different solutions, including pure water (the control group), FCNs solution and N-FCNs solution with concentration of 10 mg L⁻¹. After incubating at 30°C, N-FCNs and FCNs treated tomato seeds absorbed much more water than the control group (Fig. 4e). However, the relative water content in budding corn seeds soaked in different solution has no significant differences. This may because corn seeds are larger than tomato seeds, so their specific surface area is smaller, which leads to the lower sensitivity of corn seeds to N-FCNs and FCNs.

The above results clearly indicate that N-doped N-FCNs can best efficiently affect plants biological activity including seed germination, seedling early development and vegetative growth. The composition and structure of N-FCNs can affect their plant growth regulation capacity, as the synergistic effect of the available N and the unique functional nanostructure can play the more active and effective role in regulating the early growth of plants. First, the available N has the dual function of nutrition and signal. Its regulation process is most probably carried out by inducing hormone synthesis and conversion as an extrinsic signal source at the molecular level during plants early growth stage. Second, N-FCNs can effectively regulate the rhizosphere environment, which might result from the structural specificity including nanoscale size and chemical functionalization. On the one hand, both of –COO⁻ and NH₃⁺ can regulate the rhizosphere pH and microbial environment, and then activate the nutrient ions and provide a suitable external environment for plant growth. On the other hand, N-FCNs possess super adsorption capacity with the functional nanostructure, which enable them act as a bridge between root surface and cargoes, or even as a carrier to promote the uptake of root to water and nutrients. Third, N-FCNs may regulate plants growth by changing their cell protein on genomic level, just as some reported CNMs [35–37]. However, systematic investigation on the mechanism of observed various plant growth regulations based on N doping in CNMs is lacking. Further studies, such as nutrient uptake and proteomic issues, are needed in order to fully understand the complex interaction between N-FCNs and various plant species.

Accumulation and transport of N-FCNs in plant

The LSCM photographs of roots and upper leaves of A. thaliana treated with 4 mg kg⁻¹ N-FCNs or FCNs for 10 days are shown in Supplementary Figs S8 and S9. No obvious fluorescence signal can be observed in the A. thaliana roots of all the groups (Supplementary Fig. S8), which indicates that both N-FCNs and FCNs do not enter the root system, or their concentration in the root is too low to be detected. However, many green fluorescent particles can be seen on the surface of the leaf cells in the entire groups (Supplementary Fig. S9). Moreover, the green fluorescent particles in the N-FCNs and FCNs treatment leaves are significantly more than that in the control. It may be because N-FCNs and FCNs can promote the synthesis of some spontaneous fluorescent substances in plant leaves. Comparing with control, the leaf epidermal cells treated with N-FCNs and FCNs were smaller in shape, and more regular and denser in arrangement, proving the vigorous cell division with N-FCNs and FCNs. N-FCNs and FCNs of 4 mg kg⁻¹ can not only show positive effects on plant growth, but also have extremely small doses in plants. Based on above discussion, the potential damage of N-FCNs and FCNs to the food chain would be very limited in the agriculture and horticulture application.

In order to further study the uptake and transport of plants to N-FCNs and FCNs, it is necessary to enhance the fluorescence intensity of materials in plants and reduce the adsorption-enrichment effect of culture medium to plants. Therefore, a higher concentration (50 mg kg⁻¹) of N-FCNs aqueous solution was applied to the hydroponic soybean seedlings. After 10-day cultivation, the root and leaf in each group was detected by LSCM (Fig. 5). From the LSCM spectra, the green fluorescence in both N-FCNs and FCNs group is obviously stronger in both roots and leaves, while the control shows no fluorescence signal (Fig. 5g and Supplementary Table S2). The average fluorescence intensity in N-FCNs and FCNs treated roots are respectively 2.1 times and 3.6 times than control, which are 2.4 times and 3.2 times in leaves, respectively. N-FCNs and FCNs may be uptake by plant through apoplast pathway, because the fluorescence is mainly distributed in the intercellular space in the root (Fig. 5c and e). Few fluorescence signals are detected in root cell, probably because the cell membrane of these cells is damaged and they lost the protective barrier to nanoparticles. More interesting, N-FCNs and FCNs can also significantly increase the cell length of root (Fig. 5h). In leaves, fluorescent signals are distributed on stomata guard cells (Fig. 5d and f). This may be due to the fact that N-FCNs and FCNs are transported along with water through the duct, and finally accumulate around the stomata. The impetus of water uptake is transpiration pull and capillarity phenomena. In the bright field (Fig. 5b-2, 5d-2, and 5f-2), N-FCNs and FCNs can induce stomatal opening to promote transpiration of plants, and then promote the uptake and transportation of root to cargoes (water, nutrients, ions, nanoparticles, etc.).
Figure 4: Effect of N-FCNs on seeds germination. (a) Tomato shoots of 4 day. (b) Corn shoots of 3 day. (c) Germination rate of corn seeds. (d) Germination rate of tomato seeds. (e) Relative water content of the budding seeds.

Figure 5: LSCM photographs of soybean seedlings treated with high concentration (50 mg kg\(^{-1}\)) of N-FCNs and FCNs. (a, c and e) LSCM photographs of root in control, FCNs and N-FCNs group. (b, d and f) LSCM photographs of leaf in control, FCNs and N-FCNs group. (g) Average fluorescence intensity of roots and leaves in different treatment. (h) Cell length of root in different treatment.
It was proved that CNMs could increase plant growth partly by positively affecting gene expression in plant [9]. Now that N-FCNs and FCNs can be up take into plant, they have potentials to regulate gene expression in plant. The up-regulation of expression of the water channel’s gene and cell wall formation gene provided confirmation that the CNMs were capable to affect plant physiology through up-regulation genes related to plant growth and development [38]. Carbon nanoparticles were proved to upregulate potassium gene expression to enhance K+ accumulation in BY-2 cells, which provided support for improving plant growth by carbon nanoparticles [39].CNTs can induce over-expression of aquaporins (PIP1s and PIP2s) and regulate the concentrations of nutrients in the protoplasts [40].

Khodakovskaya et al. [7] demonstrated that expression of water channel protein (LeAqp2 gene) could be significantly activated in tomato roots by exposure to CNTs. Besides, some genes (NtLRX and CycB) involved in cell division were also upregulated by CNTs in tobacco cells [13]. CNMs can also regulate hormone level through regulation of related genes expression. For example, it was reported that GO had a beneficial influence on root formation in Malus domestica, by increasing the transcript abundance of auxin-efflux-carrier genes (PIN7, ABCB1) and auxin-influx-carrier genes (LAX2, LAX3) [41]. CNTs and C60 promoted the transcription of genes responsible for phytohormone bio-synthesis, thus induced upregulation of the defense-related phytohormones ABA and salicylic acid in treated plants [42]. The penetration and accumulation of single-walled CNTs into Zea mays roots may change the expression of genes and benefit the growth of the seminal root [43]. In addition, carbon nanoparticles increased stress resistance of plants by triggering the up-regulation of genes involved in antioxidant defenses [44]. Graphene increased the expression of PIP1;5 in the shoots and roots, promoting the development of young seedlings under salt stress [45]. Stress response genes were increased in tomato when exposed to CNTs [46]. However, finding a plausible mechanism of how the CNMs activate the expression of certain genes, as well as the effects of FCNs and N-FCNs on plant genes expression, are still open questions and require further experimentation.

**CONCLUSION**

In summary, we demonstrate the one-step electrolytic method to synthesize N-doped carbon dots, which is efficient, controlled and environmental friendly. This nanomaterial has multiple oxygen-containing and N-containing functional groups especially carboxyl and available N (ammonium and amino), resulting in good biocompatibility, hydrophilicity and fluorescence. N-FCNs show significantly plant growth regulation effects of faster germination rates, increased biomass production and green vegetable yield, higher chlorophyll content, longer root cell as well as vigorous transpiration in plants early growth stage. The specific microstructure and chemical functionalization of N-FCNs could regulate the rhizosphere environment, activate plant physiological activity to impinge upon absorption and transport, metabolic, catalysis, growth and developmental responses of plants. What’s more, N-FCNs and FCNs are of good biosafety for plant and food chain, because they are uptake and transported in plant through the extracellular pathway. The observed positive effect of N-FCNs and FCNs on plants regulation could open a new research direction for modern agriculture and horticulture.

**EXPERIMENTAL SECTION**

**Materials**

The graphite plates (purity >99%) were provided by Beijing Xinna International Hi-Tech Material Co., Ltd., Beijing, China. The MS medium was prepared as the formula of MS, 1962 [47].

All the other reagents with analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd., China.

The tomato seeds and corn seeds were purchased from local seeds market. Arabidopsis thaliana (Col-0) seeds were provided by Beijing Xinna International Hi-Tech Material Co., Ltd., Beijing, China. All the seeds are non-GMO.

**Synthesis and characterization of N-doped FCNs materials**

N-FCNs were synthesized in an electrolysis cell at a DC voltage of about 3.0 V by using two graphite plates as electrodes in certain concentration of ammonia aqueous solution (Fig. 1a), which is optimized from our previous methods [48]. Series concentration of ammonia solution was used as the electrolyte and the corresponding N-FCNs samples were labeled as N-FCNs (1 wt.%), N-FCNs (2 wt.%), N-FCNs (5 wt.%), N-FCNs (8 wt.%) by ammonia contents in the reactive solution. Then the sample solution was centrifuged at 10,000 rpm for 15 min and the supernatant was freeze dried to yield N-FCNs powders. By contrast, FCNs samples were synthesized by the similar method using just pure water as the electrolyte, which is modified by Ming’s method [49].

The morphology of N-FCNs and FCNs was characterized by TEM (JEOL-1011, Japan). Elementary analysis was conducted by burning method on Elementar (vario MICRO cube). FT-IR spectra were conducted on Bruker Tensor 37 spectrometer at a range of 400–4000 cm⁻¹ with 16 scans and a resolution of 4 cm⁻¹. N-FCNs or FCNs powders were milled with dried KBr by about 1:49 sample-to-KBr mass ratio. The XPS spectra were recorded with the Escalab 250Xi spectrometer using a monochromatic Al Kα (hv = 1486.6 eV) irradiation source at 75.0 W and 40 eV pass energy. The binding energies were calibrated with the contain carbon (C1s = 284.8 eV).

The MS medium was prepared as the formula of MS, 1962 [47]. The flowering plant A. thaliana (Col-0) seeds were provided by Beijing Xinna International Hi-Tech Material Co., Ltd., Beijing, China. All the seeds are non-GMO.

**Tissue culture of A. thaliana**

The flowering plant A. thaliana is a dicot model organism for much plant biology research [50]. The vernalized A. thaliana...
(Columbia wild type, Col-0) seeds were surface sterilized by 5 wt.% sodium hypochlorite (NaClO) solution, washed three to five times with sterilized water to remove extra NaClO. The seeds were cultivated on half-strength MS agar plates under control conditions or in the presence of N-FCNs or FCNs addition in artificial climate incubator. The climate incubator condition: long day condition (16 h light/8 h dark); light intensity 150 μmol m⁻² s⁻¹; day and night temperature 24/22 °C; and around 50% humidity.

In the first experimental set (EXP-1), A. thaliana seeds were grown in half-strength MS agar medium (1/2 MS medium) supplemented with 4 mg L⁻¹ N-FCNs of different N content. The four experimental groups were labeled as N-FCNs (1 wt.%), N-FCNs (2 wt.%), N-FCNs (5 wt.%) and N-FCNs (8 wt.%) according to N-FCNs sample. Record the fresh weight of seedlings in every group when cultivated for 17 days and compare their phenotype.

The second experimental set (EXP-2) was conducted with the 1/2 MS medium only (group MS) and 1/2 MS medium spiked with 4 mg kg⁻¹ FCNs (group FCNs) or N-FCNs (group N-FCNs). The MS and FCNs groups were used for control groups. All the seedlings cultivated for 5, 10, 15 and 23 days were photographed and gathered statistic of the primary root length. Then subsequently dry the seedlings at 70 °C in an oven until constant weight to determine the dry biomass. Chlorophyll contents in leaves of A. thaliana were determined by spectrophotometry (Details in Supporting Information).

In the third experimental set (EXP-3), the A. thaliana seeds were first germinated on A medium for 10 days, and then transplanted on B medium orderly. After transplanting for 10 days, dried and weighed up the seedlings to compare their growing behavior. The combination of A/B medium include MS/MS groups, MS/N-FCNs groups, N-FCNs/MS groups, N-FCNs/N-FCNs groups, N-FCNs/FCNs groups and FCNs/N-FCNs groups. The four experimental set (EXP-4) was carried on the 1/2 MS medium added by 4 mg kg⁻¹ of N-FCNs (group N-FCNs), urea of equivalent N dose with N-FCNs (group urea), equal amount of urea and 4 mg kg⁻¹ FCNs (group urea + FCNs). The 17-day-old A. thaliana seedlings on different medium were dried and weighed up the dry biomass.

All of the collected statistics were presented as an average of three replicates with standard errors.

Pakchoi cultivation
Each pot (30 cm × 20 cm × 10 cm) was added with nutritive soil of 1.5 kg, then the pure water or materials of 6 mg kg⁻¹ were filled. The water leaking out of the pots should be filled back. Forty-five seeds at uniform spacing are planted in each pot. Thin the seedlings to 15 seedlings of uniform size in each pot when they have grown two true leaves. Three to five repeats were set in each group. All the pot pakchoi were cultivated in the spring wild, with the lowest temperature (night temperature) of 10 ± 6 °C and highest temperature (daytime temperature) of 25 ± 7 °C. The pakchoi seedlings cultivated for 18 and 30 days were harvested and rinsed by running water, then photoed and weighed.

Seeds germination and water absorption
To study the eurytopic influence of N-FCNs, experiments were carried out on the dicotyledous seeds of tomato and the monocotyledous seeds of corn in greenhouse. These seeds were surface sterilized using 70% ethanol before spreading on germination disk with DI water or sample aqueous solution (10 mg L⁻¹ FCNs or N-FCNs), and grouped by CT, FCNs and N-FCNs.

The temperature of accelerating germination was about 30 °C. Germination rates from the first day were recorded. Each experiment was repeated for three times.

Tomato seeds and corn seeds were, respectively, selected with nearly equal sizes and plumpness, and dust was removed by high-pressure air. Subsequently, the seeds were arranged in 50 ml beakers with at a minimum of 50 seeds for each group. In the experiments, pure water (group CT), FCNs solution (group FCNs) and N-FCNs solution (group N-FCNs) solution with concentration of 10 mg L⁻¹ were added into the above beakers. Then the breakers were covered and seeds were incubated at 30 °C for a day, and fresh weight and dry weight of the seeds were weighed up. This experiment was repeated for three times to ensure accuracy.

Detection of materials in plant
The roots and leaves of the A. thaliana seedlings and soybean seedlings of the treatment group containing N-FCNs and FCNs were photoed in bright field and UV light with the LSCM (Zeiss LSM 880). The average fluorescence intensity was analyzed and calculated using Zen Blue software. Similarly, the seedlings in control group were also observed by this method.

Statistical analysis
All of the collected statistics were presented as an average of at least three replicates. The data were statistically analyzed for analysis of variance (ANOVA), and were presented as mean ± standard error. All the statistical analyses were performed using Origin 8.0.

AUTHOR CONTRIBUTIONS
Q.C.: conceptualization, data curation, methodology, validation, visualization and writing—original draft. X.R.: methodology and software. Y.L.: formal analysis. B.L.: project administration. X.W.: supervision. J.T.: supervision. Z.G.: investigation. G.J.: resources. G.M.: writing—review and editing. L.C.: funding acquisition and writing—review and editing.

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CONFLICTS OF INTEREST STATEMENT
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.

SUPPLEMENTARY DATA
Supplementary data are available at Oxford Material Science Journal online.
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