Supplementary information

Nitrogen-doped carbon dots increased light conversion and electron supply to improve corn photosystem and yield

Chuanxi Wang [ab], Hanyue Yang[ab], Feiran Chen [ab], Le Yue [ab], Zhenyu Wang*[ab], and Baoshan Xing[c]

[a] Institute of Environmental Processes and Pollution Control, and School of Environment and Civil Engineering, Jiangnan University, Wuxi, Jiangsu, 214122, China

[b] Jiangsu Key Laboratory of Anaerobic Biotechnology, Jiangnan University, Wuxi, Jiangsu, 214122, China

[c] Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003, USA

*Corresponding author: wang0628@jiangnan.edu.cn (Dr. Zhenyu Wang)

Number of pages: 22
Number of texts: 4
Number of figures: 15
Number of tables: 2

Text S1

Sample preparations and measurements

The preparation of CDs and N-CDs: Briefly, CDs were synthesized through putting citric acid (1.0 g, Sigma Aldrich) and ultrapure water (10 mL) into a Teflon-lined autoclave chamber, which was heated at 200 ℃ for 10 h.[1] Thermo-polymerization of melamine (0.13 g, Sinopharm Chemical Reagent Co., Ltd) and ethylene diamine tetraacetic acid (0.25 g, Sinopharm Chemical Reagent Co., Ltd) were initiated (600 ℃) for preparing N-CDs in a tubular oven for 10 h.[2] The carbonized samples were ultrasonicated for 30 min using a CNC ultrasonic machine (150 W, SBL-10DT, 28C017, SCIENTZ, China) to disperse CDs and N-CDs in aqueous solution. Then, the solutions were purified for 48 h with a dialysis bag (MW = 500Da, Solarbio, China), respectively. The CDs and N-CDs were collected by freeze-drying.

TEM measure: CDs (0.1 mg/mL) and N-CDs solutions (0.1 mg/mL) were taken a drop (20 μL) on the carbon supported copper mesh (T10023, Tianld) and dried under infrared light, respectively. Then, the transmission electron microscopy (TEM) images were obtained using a JEM–2100 electron microscope (JEOL, Tokyo, Japan) with an accelerating voltage of 200 kV.

XPS test: The solid CDs and N-CDs (20 mg) were grounded into fine powder, and spread evenly on the aluminum foil. The powder was flattened with a hydraulic press, then cut and uncovered for the XPS test. The XPS spectra were recorded using Thermo Kalpha X–ray photoelectron spectrometer (Nexsa, US) with Mono AlKa source excited by 1486.6 eV. Binding energy calibration was based on C 1s at 284.6 eV.

UV and PL spectra: Three milliliters' solutions (0.1 mg/mL) were taken in the cuvette and measured separately with UV-vis spectrophotometer (Shimadzu, UV–1800, Japan) and F-7000 fluorescence spectrophotometer (Hitachi, Japan).

Fluorescence lifetime: Fluorescence lifetime experiments were conducted with a time-correlated single-photon counting (TCSPC) system, and the results were collected by using the spectrophotometer (Horiba JobinYvon Fluoromax 4C-L, France). Picosecond diode laser was used to as excitation sources (370 and 458 nm excitation). The processes of fluorescence lifetime were monitored through an ordinary microplate reader.

Text S2

CLSM images to observe CDs and N-CDs around chloroplast

The foliar application of CDs and N-CDs were conducted for seven days, and corn leaves were harvested. Chloroplast extraction were carried out according to previous reports.[3] Briefly, washed leaves (2 g) were cut into small pieces, and grounded in 30mL (pH=7.3) sucrose buffer (0.4 M sucrose, 0.01 M KCl, 0.03 M Na_2HPO_4, 0.02 M KH_2PO_4). The solutions were centrifuged at 1000 rpm for 3 min to remove cell debris, and then were centrifuged at 3000 rpm for 5 min to collect precipitation. The collections were resuspended in the above buffer. The chloroplast suspensions were used to observe the entry of CDs and N-CDs around the chloroplast in corn shoots (Figure S9).

Text S3

PCA of six ETR-related variables of CDs and N-CDs

Six variables (light conversion, chlorophyll content, relative gene expression of psbA, activity of ATPase, NADPH production and electron supply) related to electron deliver chain were selected for the analysis. Light conversion was defined as the overlapping shadow area between chloroplast absorptions and PL emission spectra of chloroplast alone, chloroplast/N-CDs mixture, chloroplast/CDs mixture respectively when excited at 365 nm (Figure S7).[4] As a semiconductor material, N-CDs can generate electrons and holes in the light source. In the Figure S12, the PL delay time was used to measure the recombination rate of electron-hole pairs inside the material. The longer the PL time, the better the electron-hole separation efficiency. To a certain extent, it can reflect the ability of electrons in the semiconductor to transfer from the inside to the surface, meanwhile expressing the level of electron supply.[5] It is known that both CDs and N-CDs can improve the 6 variables related to photoreaction. In order to compare the degree of photosynthesis improvement between the two materials and explore the main contribution of N doping, we selected the percentage increase of CDs and N-CDs relative to the control group as the analysis factors (Table S2).

Text S4

Cellular toxicity

Tobacco (BY-2) cells (10^4 cells/150 μL) were cultured for 24 h in an incubator (37 ℃, 5% CO_2), and then for another 24 h in Dulbecco's modified Eagle's medium (20 mL, DMEM). The cells were further incubated for 24 h in DMEM containing the N-CDs at

different doses (0, 1, 5, 10, 50 mg/L). After removing the culture medium, 1 mL of the cells was added into a centrifuge tube, and settled naturally for 10 min for removing the supernatant. The cells were washed three times with phosphate buffer saline (0.1 M PBS, pH 7.2) to remove the supernatant, and a 1 mL of 0.3% (w/v) 2,3,5-Triphenyltetrazolium chloride (TTC) (0.05 M PBS, pH 7.2) was added to resuspend. After incubating for 8 h at 25 °C in the dark, the cells were centrifuged at 6000 rpm to remove PBS. Then 1mL ethanol (95%) was added and the solution was heated in a 60 °C water bath for 15 min. Subsequently, it was centrifuged at 6000 rpm for 5 min, and 200 μL was transferred to a 96-well plate. Then, cell viability was obtained by calculating the ratio of absorbance (485 nm) between the treatment and control group.

Table S1 | Primer sequences used in this study

Primer name	Sequence (5'-3')
M-actin-F	CATGAGGCCACGTACAACTC
M-actin-R	TCATGGCAGTTCATGTATTG
psbA-F	TCGCTGCTCCTCCAGTAGAT
psbA-R	CGCATACCCAGACGGAAACT
ZmSUT4-F	GCTGCAGAATTCGTCTGGAAACTCTTTGTGGGT
ZmSUT4-R	CGGATCCTCGAGCTCCTGTAACTTTTATTCATTGCT

Table S2 | Principal component analysis factors

	Light conversion (%)	**Chlorophyll (%)**	**psbA (%)**	**ATPase activity (%)**	**NADPH production (%)**	**Electronic supply (%)**
N-CDs1	121	11	726	54	115	81
N-CDs2	100	12	1075	49	94	79
N-CDs3	95	25	948	26	120	80
N-CDs4	91	10	1075	36	93	81
N-CDs5	112	12	1304	43	129	80
CDs1	36	15	1133	25	42	18
CDs2	41	7	521	16	27	16
CDs3	45	14	1876	20	30	16
CDs4	33	16	426	16	35	16
CDs5	36	12	438	29	47	17

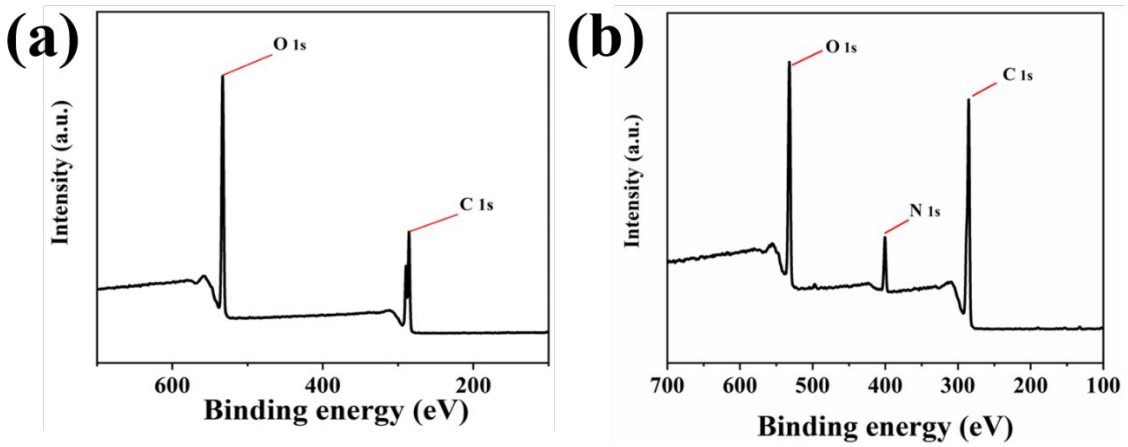

Figure S1. XPS spectra of CDs (a) and N-CDs (b). The presence of N 1s was observed in the spectrum of N-CDs.

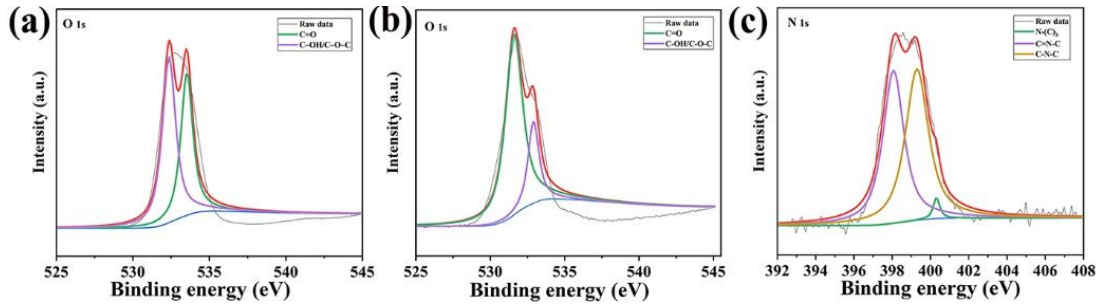

Figure S2. High-resolution XPS spectra. (a) O 1s of CDs, (b) O 1s and (c) N 1s of N-CDs. There was no difference in high-resolution O 1s XPS spectra between CDs and N-CDs. But the N 1s was present only in N-CDs.

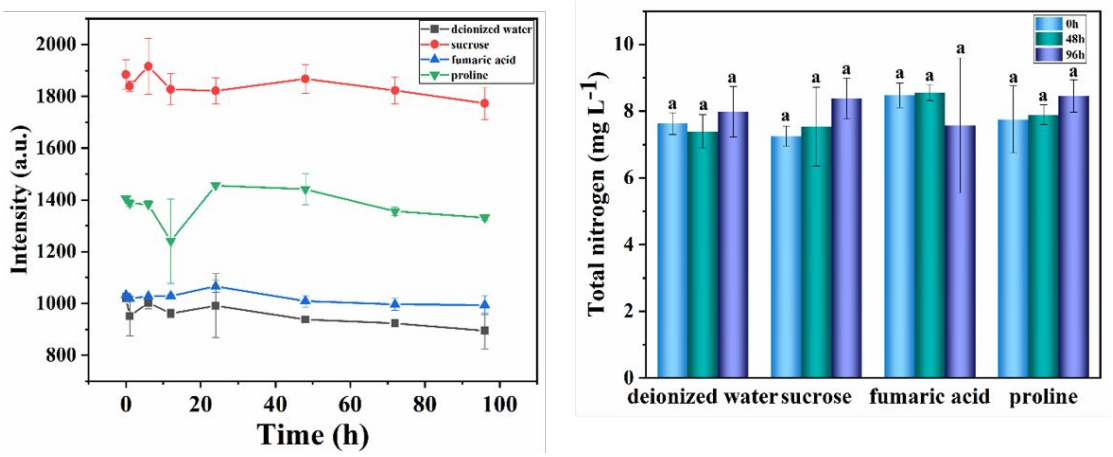

Figure S3. N release of N-CDs during different time points. The fluorescence intensity (365 nm excitation, 420 nm emission) of the solutions at 0, 1, 6, 12, 24, 48, 72, 96 h (left). Total nitrogen content in the solutions at 0, 48, 96 h (right).

Noting: To evaluate whether the nitrogen of N-CDs can be released as nitrogen fertilizer, the decomposition of N-CDs was investigated. The sucrose, fumaric acid and proline represent the dominant sugar, organic acid and amino acid in phloem sap, respectively.[6,7] The fluorescence properties of N-CDs are closely related to their own structure.[8] Thus, the fluorescence of N-CDs and the total nitrogen concentration in the solution were used to illustrate the decomposition of N-CDs (N release). There were no significant fluorescence changes of N-CDs in the 4 solutions (deionized water, sucrose, fumaric acid and proline) during 96 hours, and the total nitrogen concentration in the 4 solutions did not change. These results indicated that the structure of N-CDs were stability, and N-CDs did not release N as a nutrient element for plant growth.

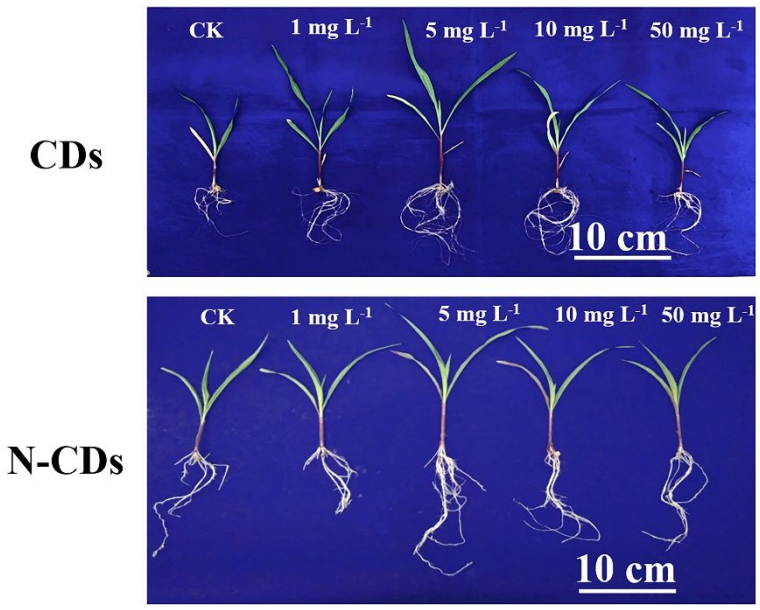

Figure S4. The promoted growth of corn by CDs and N-CDs at various concentrations. The optimal concentration is 5 mg·L^{-1}.

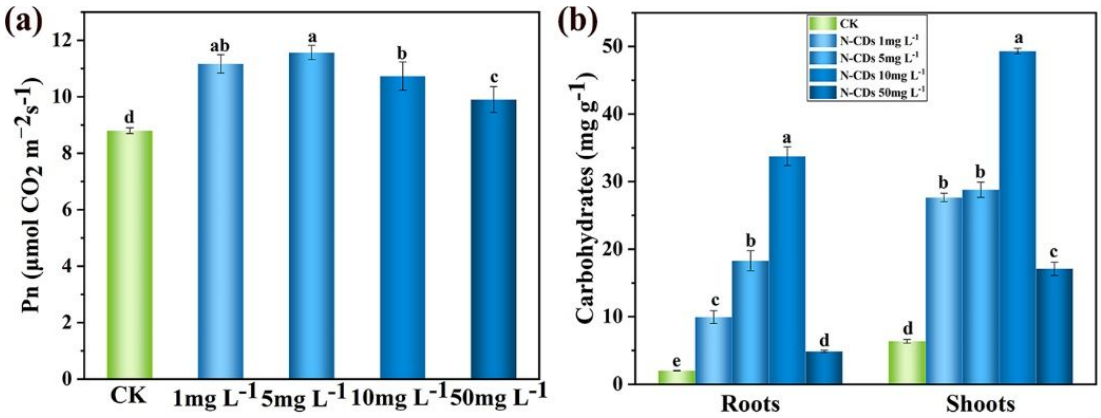

Figure S5. The promoted Pn and carbohydrate content upon exposing to different concentrations of N-CDs (1, 5, 10 and 50 mg·L^{-1}). (a) net photosynthetic rate and (b) carbohydrate content of corn seedlings.

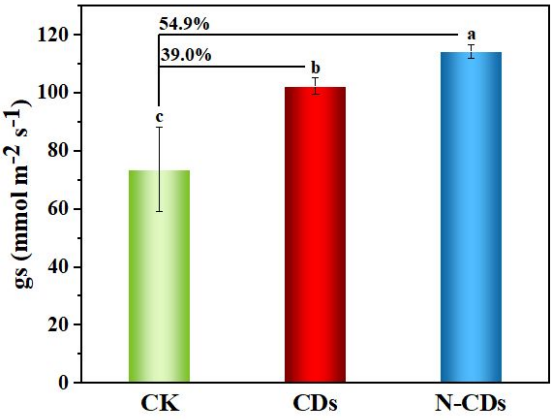

Figure S6. The promoting effects of CDs and N-CDs (5 mg·L^{-1}) in stomatal conductance (gs).

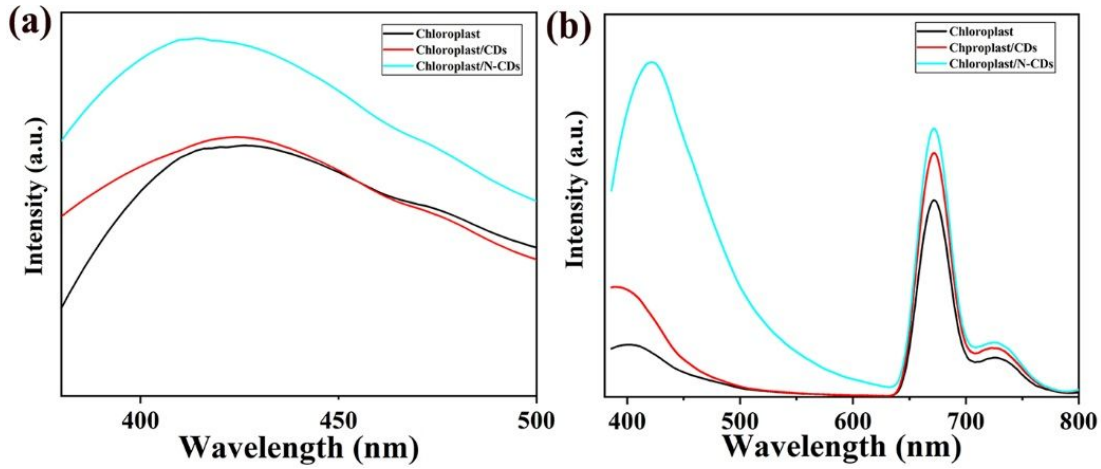

Figure S7. The Ultraviolet-visible absorption and fluorescence spectra of samples. (a) Ultraviolet-visible absorption spectra and (b) fluorescence spectra of chloroplasts alone (black) and chloroplast/CDs (5 mg·L^{-1}/5 mg·L^{-1}) mixture (red), chloroplast/N-CDs (5 mg·L^{-1}/5 mg·L^{-1}) mixture (blue).

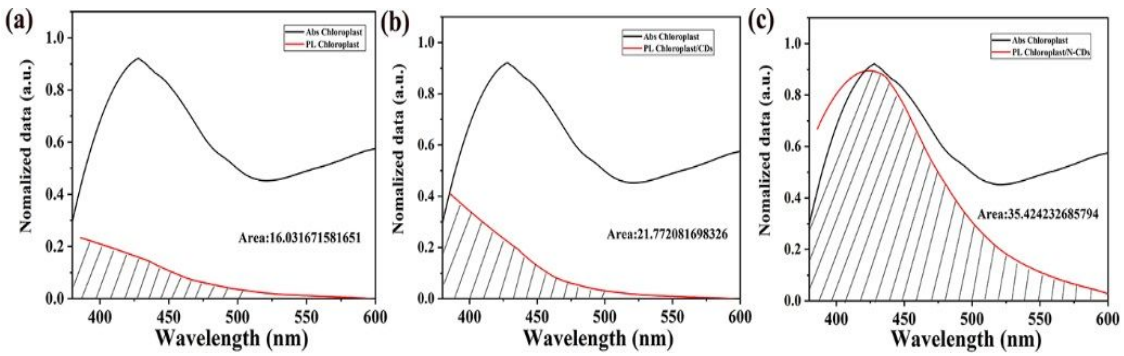

Figure S8. The light conversion efficiency of CDs and N-CDs. Shadow overlap between chloroplast (alone) absorption and fluorescence spectra of (a) chloroplast (alone), (b) chloroplast/CD mixture, and (c) chloroplast/N-CDs mixture. All excited at 365 nm.

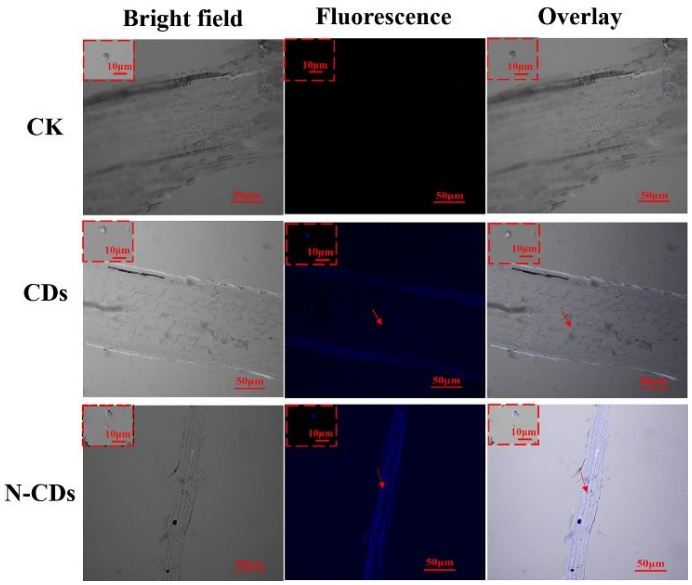

Figure S9. Uptake and distribution of CDs and N-CDs in the corn leaves. Inset images were the isolated chloroplast after applying CDs and N-CDs. These results demonstrated CDs and N-CDs could be taken up and transported around chloroplast.

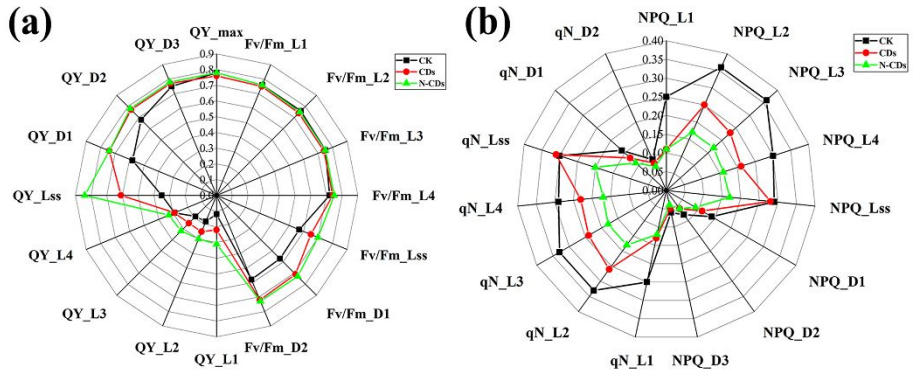

Figure S10. Optical quantum yield parameters (a) and fluorescence quenching parameters (b) after foliar application of CDs and N-CDs at 5 mg·L^{-1}.

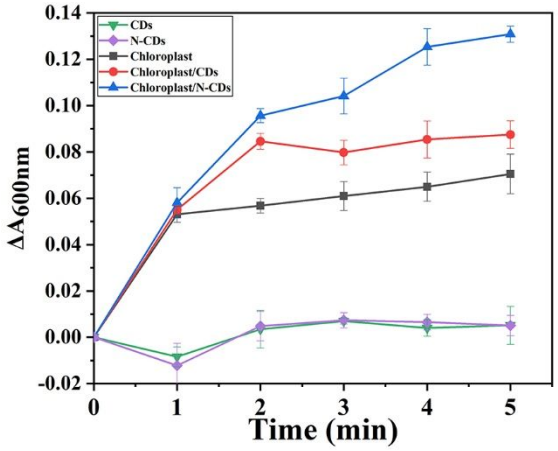

Figure S11. Changes in absorption of DCPIP at 600 nm during the Hill reaction of chloroplast alone (black) and chloroplast/CDs (5 mg·L^{-1}/5 mg·L^{-1}) mixture (red), chloroplast/N-CDs (5 mg·L^{-1}/5 mg·L^{-1}) mixture (blue).

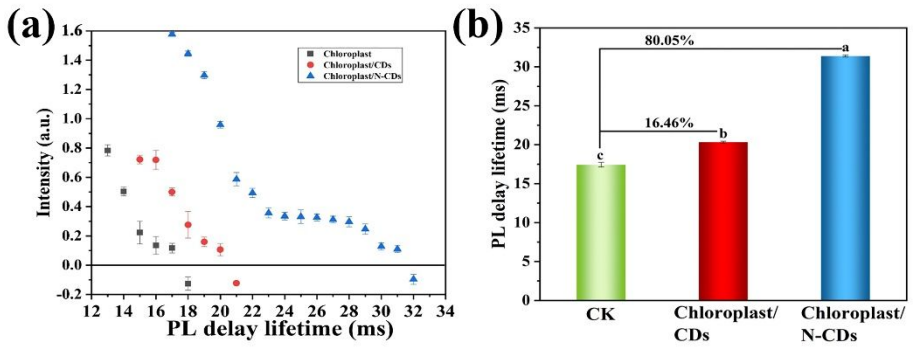

Figure S12. Fluorescence lifetime. (a), delay lifetime and (b), delay time of chloroplast (black), chloroplast/CDs (red) mixture and chloroplast/N-CDs (blue) mixture.

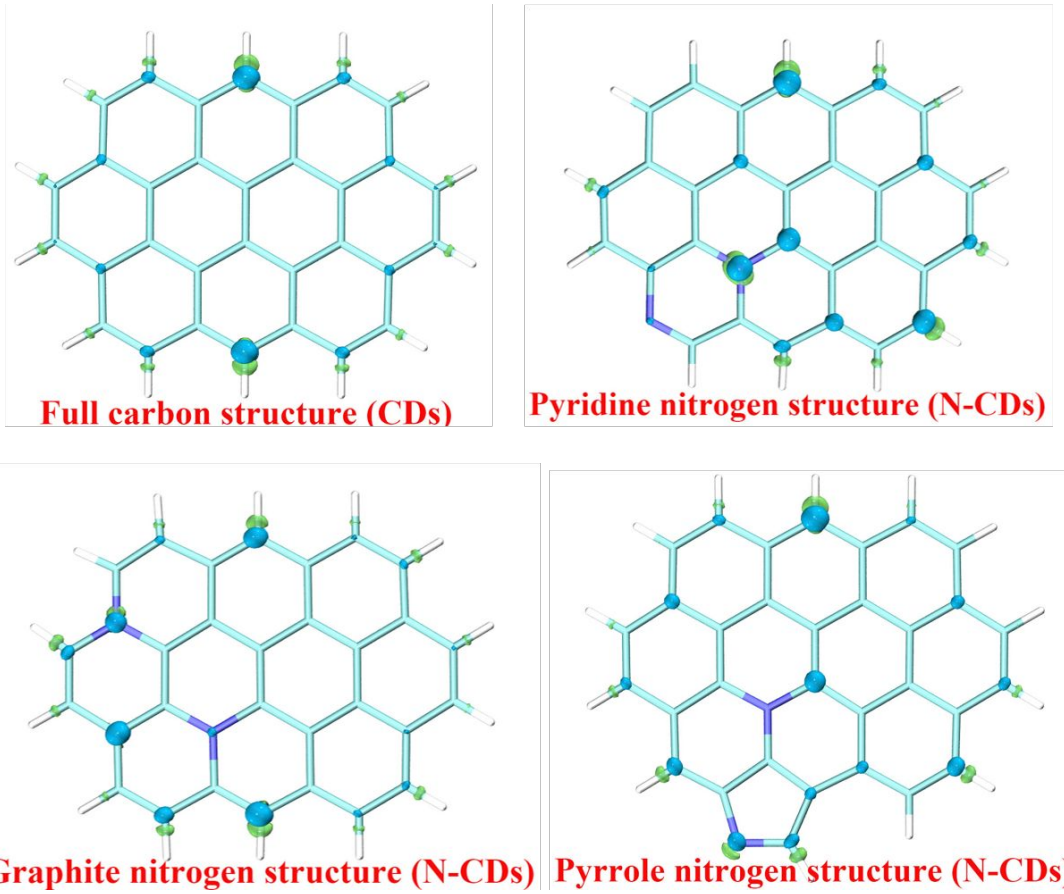

Figure S13. Four different structural models of CDs and N-CDs after ionization: full carbon, pyridine nitrogen, graphite nitrogen, pyrrole nitrogen. The green area and blue area (the electrons are lost) represent increase and decrease respectively in electron density after ionization.

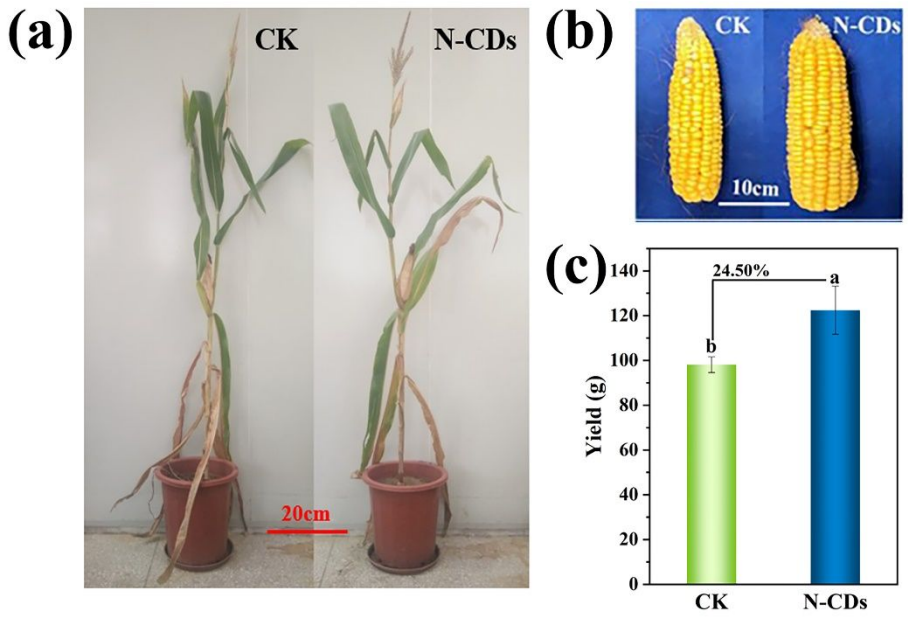

Figure S14. A full life cycle study of N-CDs application on corn. (a) and (b), photographs of corn plants and ears; (c), increased yield.

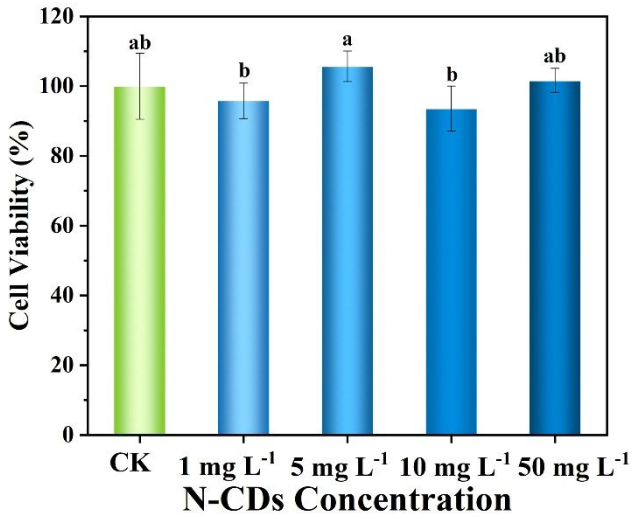

Figure S15. Viability of tobacco cells after 24 h incubation with different concentrations of N-CDs. The result confirmed that the N-CDs had little effect on the cell viability, and low toxicity at the application concentration of this study.

REFERENCES

1. Zhu, S.; Meng, Q.; Wang, L.; Zhang, J.; Song, Y.; Jin, H.; Zhang, K.; Sun, H.; Wang, H.; Yang, B. Highly photoluminescent carbon dots for multicolor patterning, sensors, and bioimaging. *Angew. Chem. Int. Ed*. **2013**, *52*, 3953–3957.
2. Yang, L.; Wu, X.; Luo, L.; Liu, Y.; Wang, F. Facile preparation of graphitic-C_3N_4 quantum dots for application in two-photon imaging. *New J. Chem.,* **2019**, *43*, 3174–3179.
3. Wang, Y.; Li, S.; Liu, L.; Lv, F.; Wang, S. Conjugated polymer nanoparticles to augment photosynthesis of chloroplasts. *Angew. Chem. Int. Ed*. **2017**, *56*, 5308–5311.
4. Li, Y.; Li, W.; Zhang, H.; Liu, Y.; Ma, L.; Lei, B. Amplified light harvesting for enhancing Italian lettuce photosynthesis using water soluble silicon quantum dots as artificial antennas. *Nanoscale*, **2020**, *12*, 155–166.
5. Wu, H.; Li, X.; Tung, C.; Wu, L. Semiconductor quantum dots: an emerging candidate for CO_2 photoreduction. *Adv. Mater*. **2019**, *31*, 1900709.
6. Hijaz, F.; Killiny, N. Collection and chemical composition of phloem sap from *Citrus sinensis* L. Osbeck (sweet orange). *PLoS one*, **2014**, *9*, e101830.
7. Ma, C.; Borgatta J.; Hudson, B.G; Tamijani, A. A.; Torre-Roche, R. D.; Zuverza-Mena, N.; Shen Y.; Elmer W.; Xing B.; Mason, S. E.; Hamers, R. J.; White, J. C. Advanced material modulation of nutritional and phytohormone status alleviates damage from soybean sudden death syndrome. *Nat. Nanotechnol*, **2020**, *15*, 1033–1042.
8. Zhang, S.; Zhang, L.; Huang, L.; Zheng, G.; Zhang, P.; Jin, Y.; Jiao, Z.; Sun, X. Study on the fluorescence properties of carbon dots prepared via combustion process. *J. Lumin*. **2019**, *206*, 608–612.