Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Carbon dots can strongly promote the photosynthesis in lettuce (*Lactuca sativa* L.)

Jing Hu,[a] Wenyi Jia,[a] Xinyi Wu,[a] Haiyun Zhang,[a] Yin Wang,[a] Junfeng Liu,[a] Yu Yang,[b] Shu Tao[a] and Xilong Wang[a,*]

[a] Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China

[b] Department of Civil and Environmental Engineering, University of Nevada, Reno, NV 89557, USA

[*] Corresponding author: xilong@pku.edu.cn (X. L. Wang)

Supplementary material

Supplementary material (10 pages) includes: materials and methods, and 7 figures.

Materials and methods

Plant weight measurement

Plants were carefully removed from the incubation system, and rinsed with Milli-Q water. The root and shoot tissues were separated and weighed by a CPA 1003P electronic analytical balance (Sartorius AG., Germany) to obtain fresh weight (FW). Their dry weight (DW) was determined after the tissues were dried for 72 h at 60 ^{o}C.

Measurement of plant physiology

Chlorophyll content. Briefly, 0.1 g of leaf tissues was homogenized in 95% ethanol and all samples were kept in the dark for 14 h to extract chlorophyll from chloroplast. After standing for 30 min, supernatant absorbance was measured at 649 and 665 nm by a Thermo Multiskan GO microplate reader (USA). Chlorophyll content was calculated using the following equations: C_a (Chlorophyll a) = $13.95A_{665}$ - $6.8A_{649}$; C_b (Chlorophyll b) = $24.96A_{649}$ - $7.32A_{665}$; C_T (total chlorophyll) = C_a + C_b = $18.16A_{649}$ + $6.63A_{665}$.[1]

Rubisco enzyme activity. The Rubisco test kit includes one solution (solution one) and four reagents, including reagent one (liquid), reagent two (powder), reagent three (powder) and reagent four (powder). The reagent one was used for preparation of other three reagents. The working solution was a mixture of reagent two and three with a volume ratio of 1:1. Firstly, 0.1 g leaves were homogenized in an ice bath with 1 mL solution one. Then, the mixture was crushed by a probe ultrasonicator (S-250D, Branson, USA) for 1 min in an ice bath at 200 W with a working/pausing duration cycle of 3 s/7 s. The mixture was then centrifuged at 10000 rpm and 4 °C for 10 min to separate the insoluble

residue. The supernatant (50 μL) was mixed with 50 μL reagent four and 900 μL working solution. Absorbance of the mixture at 340 nm at 20 s and 2 min 20 s was recorded, respectively.

Soluble protein content. Soluble protein content was analyzed by a staining method with Coomassie brilliant blue with the assistance of a total protein quantitative assay kit (Nanjing Jiancheng Technology Co., Ltd. China).[2] Specifically, 0.05 mL supernatant was mixed with 3 mL Coomassie Brilliant Blue solution and allowed to stand for 5 min, after then its absorbance at 595 nm was measured.

Soluble sugar content. Soluble sugar content in plant leaves was measured by the method of sulfuric acid anthrone using a plant soluble sugar content test kit (Nanjing Jiancheng Technology Co., Ltd. China).[3] In brief, 0.3 g leaves of lettuce were cut and ground with 10 mL phosphate buffer (PBS, pH 7.8). The mixture was centrifuged at 4000 rpm for 10 min, and the supernatant in each treatment was stored at 4 °C for further use. 0.2 mL supernatant, 0.1 mL substrate solution and 1 mL concentrated sulphuric acid were mixed and heated in a boiling water bath for 10 min. After cooling down by flowing water, the absorbance at 620 nm was measured.

Chloroplasts isolation

Chloroplasts were isolated from lettuce leaves using differential centrifugation, following the instruction of a chloroplast isolation kit (Beijing Leagene Biotechnology Co., Ltd. China). The lettuce leaves were homogenized in a pre-chilled mortar and pestle in ice-cold isolation buffer. The slurry was filtered through 3-layer cheese cloth followed by centrifugation at 300 g for 2 min at 4 ^{o}C. The supernatant was centrifuged again at 1000 g for another 2 min at 4 ^{o}C. The supernatant was carefully discarded and the pellet was re-suspended in an extraction buffer and used for spectroscopic analysis.

Chlorophyll was extracted in chilled 80% acetone and estimated spectrophotometrically at 652 nm,[4] to correct for the suspension concentration difference of chloroplasts used for spectrometric determination.

Plant tissue digestion

Approximately 50 mg of shoot or root tissues were transferred to a digestion tube containing 3 mL concentrated HNO_3. The mixture was heated at 120 ^oC for 1 h. After cooling down to ambient temperature, 0.5 mL H_2O_2 was added for further digestion at 120 ^oC for an additional 30 min.

Sorption experiments

To test sorption of nutrient elements to CDs in the systems amended with CDs and the associated influence on their uptake by plant, sorption experiments were conducted in 50 mL solution that contained different amounts of CDs (0, 10, 20, 30, and 40 mg/L) dispersed in 50% Hoagland's nutrient solution. These tubes were shaken on a rotary shaker for one week, to keep the exposure duration of the nutrient elements to CDs identical to that in the hydroponic system. After that, the mixture was filtered with a 3 kDa Amicon Ultra-4 Centrifugal Filter Unit. It was confirmed that the nutrients were not sorbed to the filter. Concentrations of the nutrient elements including K, Ca, Mg, P, Fe, Mn and Zn in the filtrate were measured using ICP-OES as mentioned in the main text.

Figures

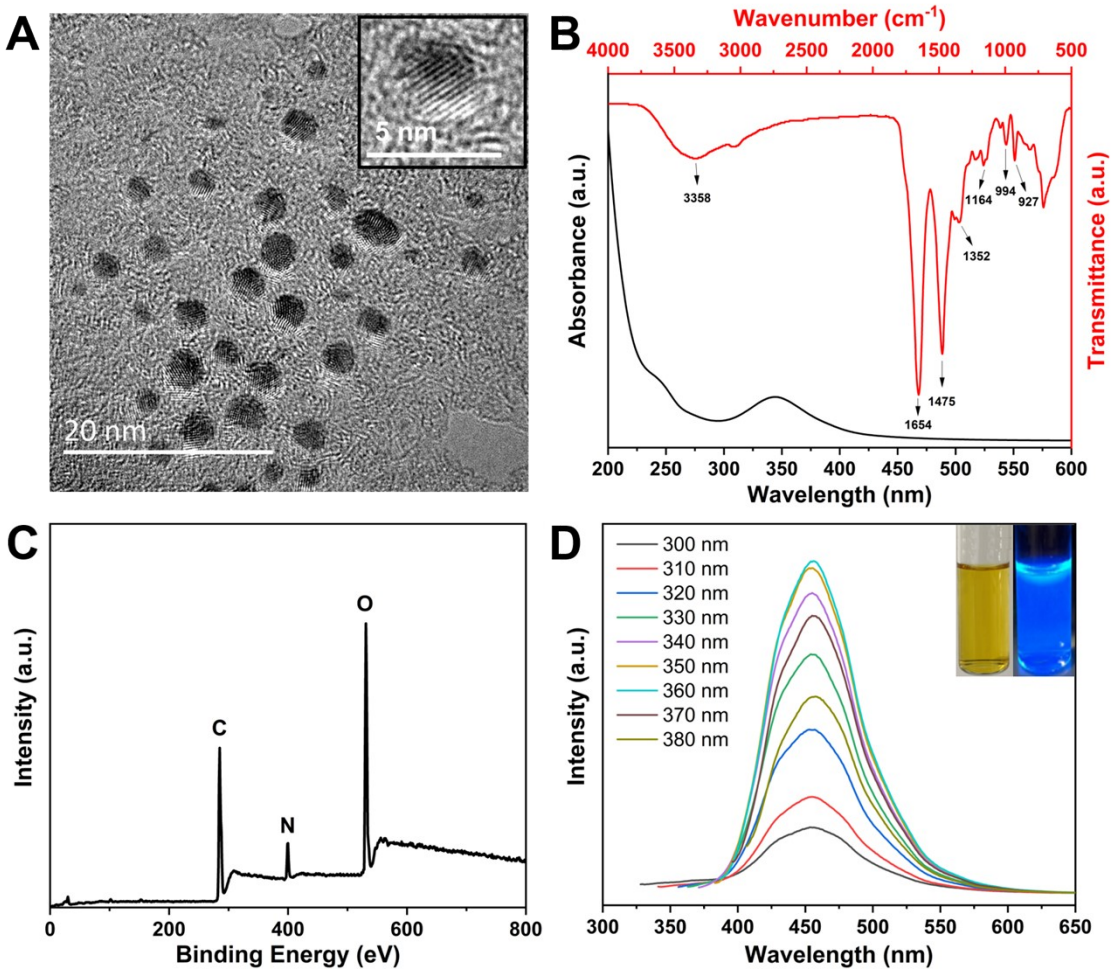

Fig. S1 Characterization of CDs. (A) TEM image (inset: a high-resolution TEM image of single CDs particles). (B) FTIR and UV-Vis spectra of CDs. (C) Full XPS spectrum of CDs. (D) Emission spectra of CD recorded for progressively longer excitation wavelength of 10 nm increments from 300 to 380 nm (inset: the photograph of CDs under visible light and UV light at 370 nm, respectively).

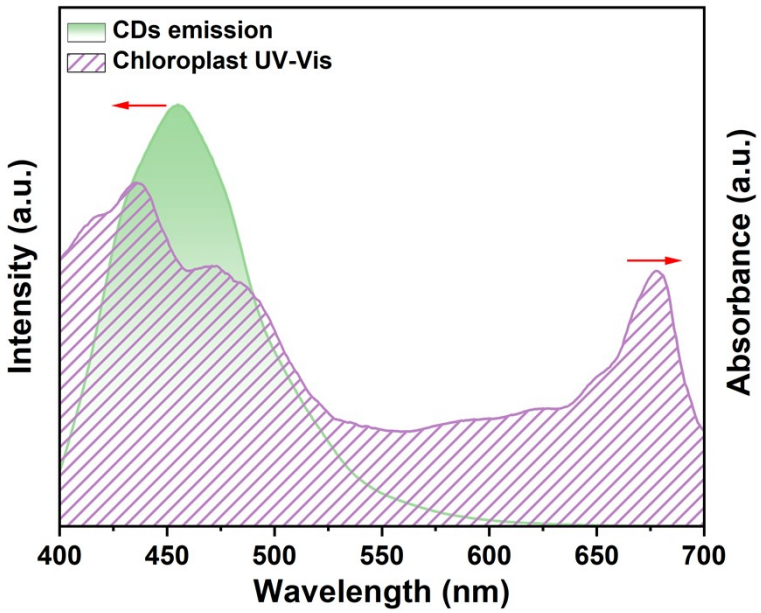

Fig. S2 Emission spectrum of CDs excited at 360 nm and UV-Vis spectrum of chloroplasts isolated from the untreated lettuce leaves.

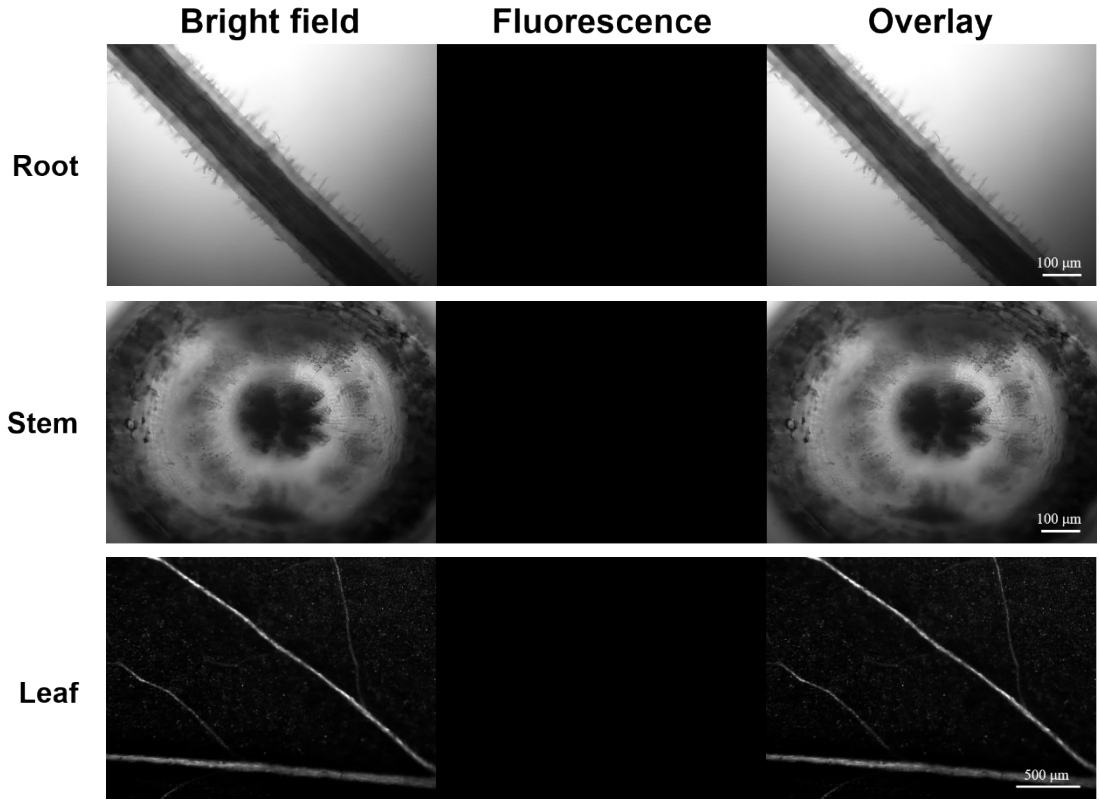

Fig. S3 Fluorescence microscopy images of the pellet of the root hair zone, cross section of stem and leaf in the control plant.

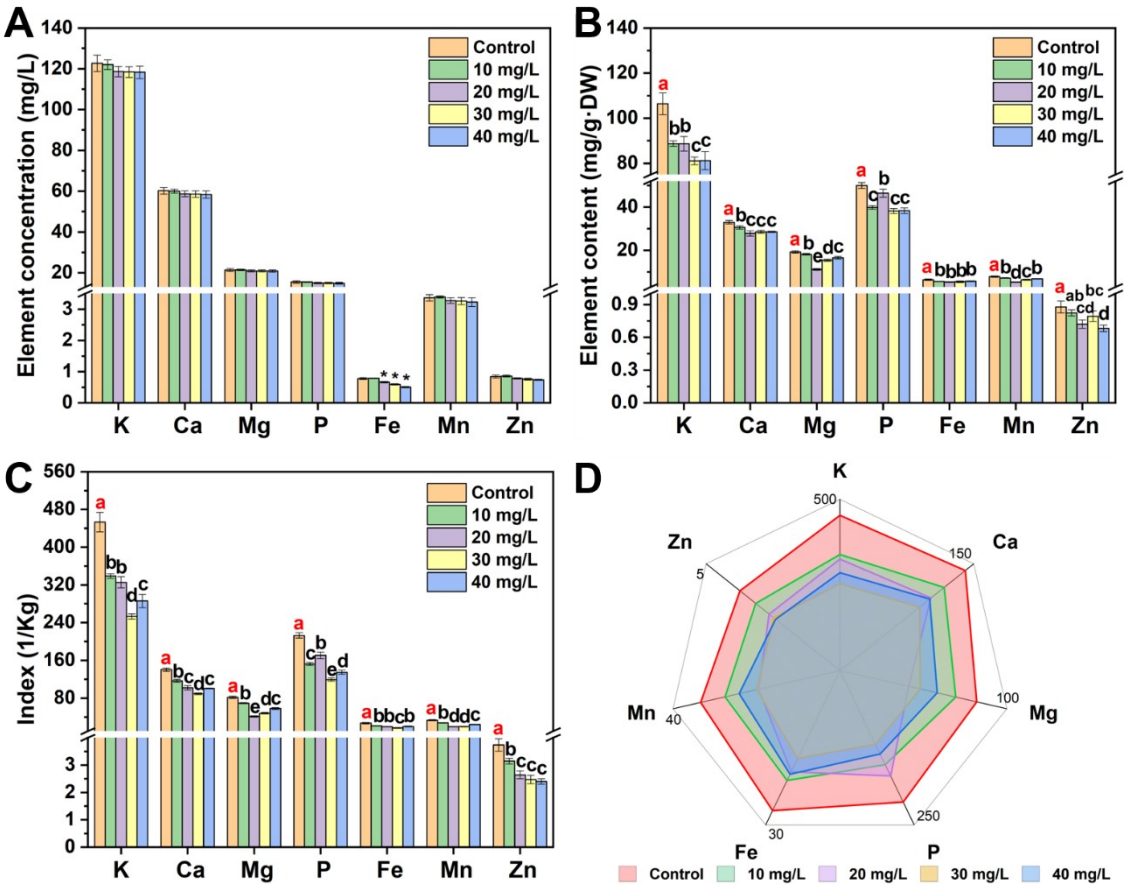

Fig. S4 Effect of CDs at varying concentrations on mineral elements. (A) K, Ca, Mg, P, Fe, Mn and Zn concentration in 50% Hoagland's nutrient solution after exposure to CDs for one week; (B) The nutrient elements in lettuce plant treated with different concentrations of CDs; (C) and (D) The bar and radar plot of an index describing the biomass dilution effect of nutrients in lettuce plant, respectively. Data are means of three replicates ± SD. Those marked with * and different lowercase letters for each element are significantly different at $p \leq 0.05$.

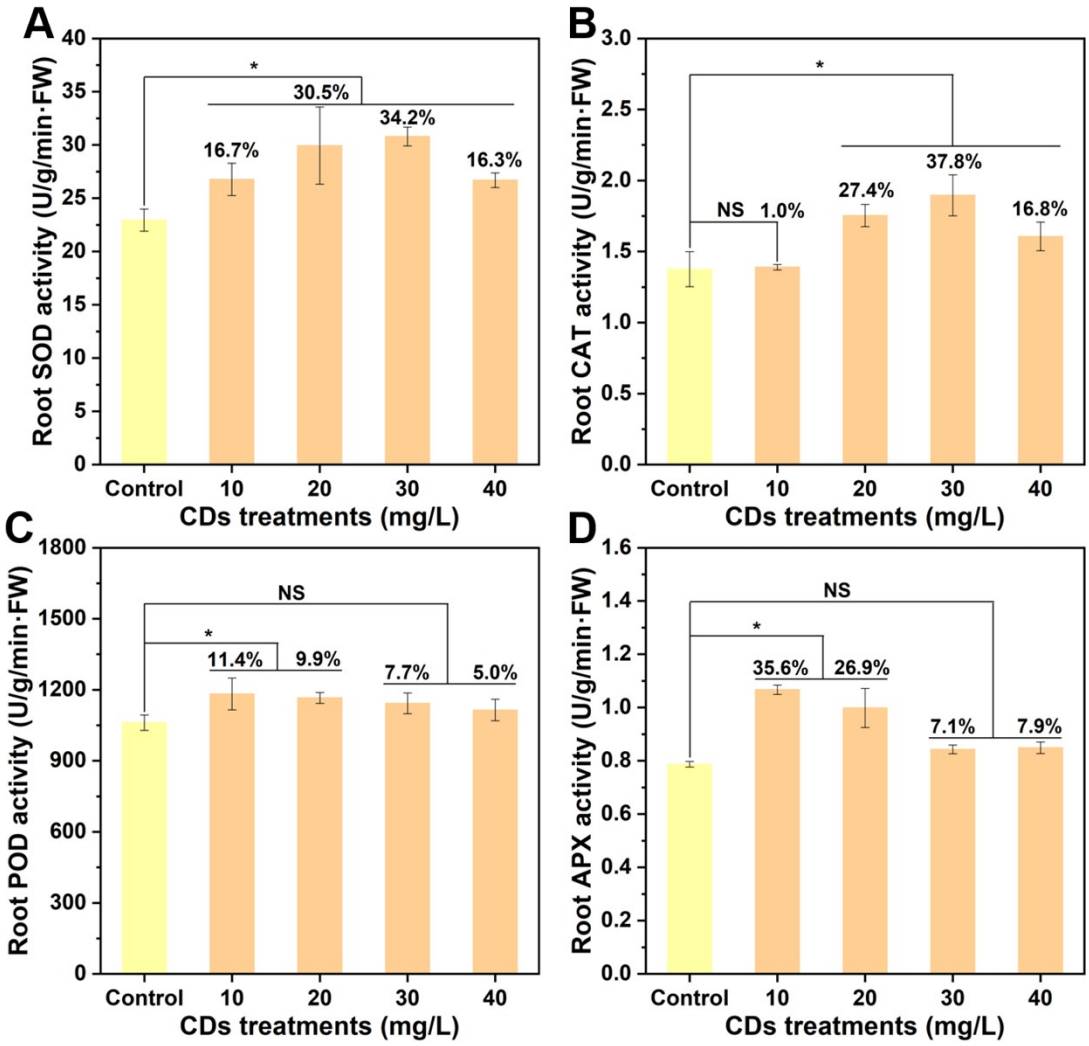

Fig. S5 Effects of CDs on activities of SOD (A), CAT (B), POD (C) and APX (D) in the lettuce root. Data are means of three replicates ± SD. The data marked with NS indicate not significant and those marked with * indicate significant difference from the control ($p \leq 0.05$).

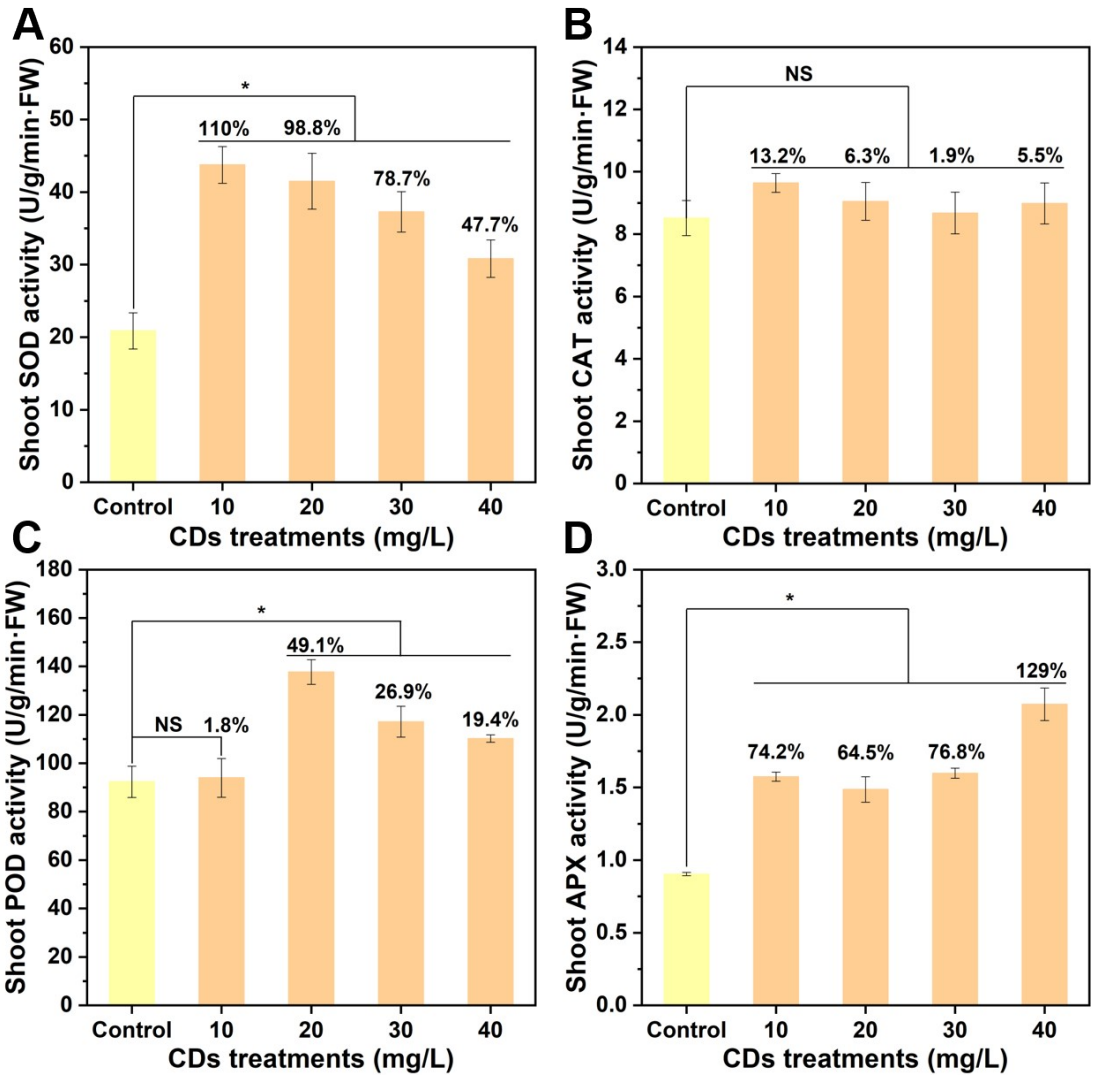

Fig. S6 Effects of CDs on the activities of SOD (A), CAT (B), POD (C) and APX (D) in the lettuce shoot. Data are means of three replicates ± SD. The data marked with NS indicate not significant and those marked with * indicate significant difference from the control ($p \leq 0.05$).

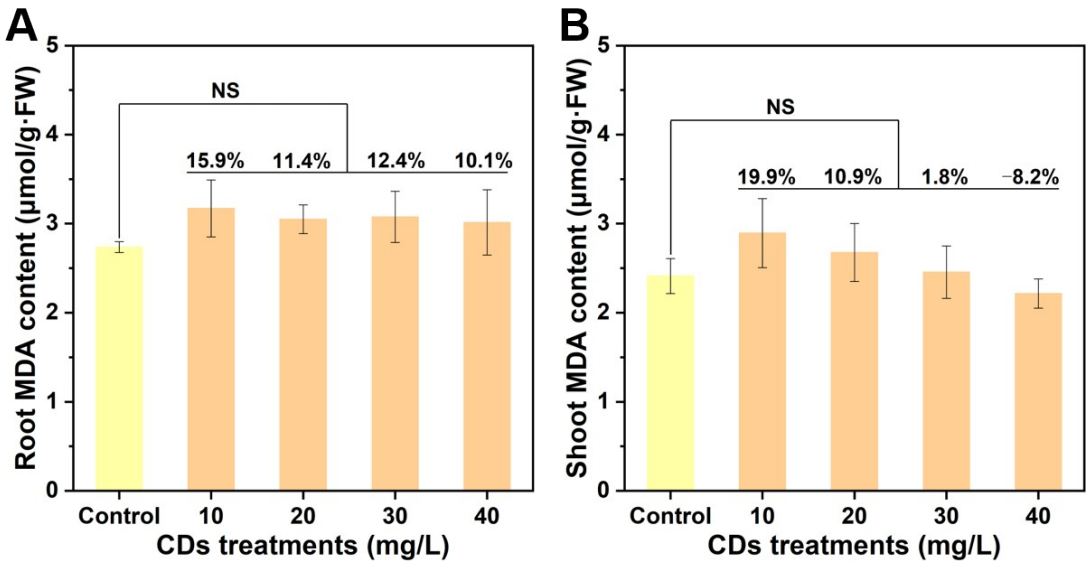

Fig. S7 Effects of CDs on MDA content in roots (A) and shoots (B) of lettuce plant. Data are means of three replicates ± SD. Those marked with NS indicate not significant ($p \leq 0.05$).

References

1. H. K. Lichtenthaler, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Methods Enzymol., 1987, 148, 350-382.
2. H. X. Ren, L. Liu, C. Liu, S. Y. He, J. Huang, J. L. Li, Y. Zhang, X. Huang and N. Gu, Physiological investigation of magnetic iron oxide nanoparticles towards Chinese mung bean, J. Biomed. Nanotechnol., 2011, 7, 677-684.
3. J. Chen, Y. Tian, X. Zhang, C. Zheng, Z. Song, A. Deng and W. Zhang, Nighttime warming will increase winter wheat yield through improving plant development and grain growth in north China, J. Plant Growth Regul., 2014, 33, 397-407.
4. H. Yuan, L. Liu, F. Lv and S. Wang, Bioluminescence as a light source for photosynthesis, Chem. Commun., 2013, 49, 10685-10687.