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

Supporting Information

Carbon dots embedded hydrogels promote maize germination and growth under drought stress

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Number of pages: 17

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Number of Figures: 12

Supplementary texts

Text S1: Gel content

Dry Gelatin and GTACDs (5, 10, 20 and 50 ppm) were prepared in PBS solution (pH=7.4, 10 mL, 4 h) at 25℃, which were weighed (Wi). They were dried to constant weight in the oven at 60ºC, which were weighted (Wd). Gel content percentage using a formula, (1) Gel Content (%) = Wd/Wi *100. [1]

Text S2: Swelling ratio

The swelling behavior was evaluated by measuring the weight difference of Gelatin and GTACDs (5, 10, 20 and 50 ppm) before and after incubation in PBS solution (pH=7.4). The dry hydrogels were cut into a square sample with a side length of 1cm. Due to a certain error in thickness, the dried hydrogel was weighed (Wd) and soaked in PBS (25 mL) at 25℃. At a certain time, the reswelling hydrogels were weighted (Ws) by absorbing excess water on the surface using filter paper. The percentage of swelling is then calculated using a formula,

(2) Swelling (%) =((Ws-Wd)/Wd) *100. [1]

Text S3: Water retention ratio

The reswelling hydrogels from Text S2. were dried in glass dishes at 25ºC. At a certain time, they were weighted (Mt) after absorbing excess water on the surface using filter paper. Finally, they were dried to constant weight

in the oven at 60ºC, which were weighted (Mo).

Water retention ratio (%) = ((Mt-Mo)/Mo) *100. [1]

Text S4: Porosity

Ethanol replacement method was used to cut the freeze-dried GTACDs into small pieces and immerse them into a measuring cylinder containing anhydrous ethanol. The volume of anhydrous ethanol was V1, and the air in the pores of the material was discharged after vacuum-pumping for 5min. At this time, the total volume of anhydrous ethanol and the material was V2. Finally, the material was removed and the remaining volume of anhydrous ethanol was V3.[1]

Porosity = ((V1-V3)/(V2-V3)) *100%

Text S5: TACDs release rate (%)

Dry GTACDs (5, 10, 20 and 50 ppm) were placed in PBS buffer solution (15 mL) in shaking table (50 rpm). 3 mL solution was taken every 12 h, then adding 3mL PBS buffer solution, this process was repeated for five times. TACDs standard curve: a series of concentrations (0, 10, 20, 40, 50 and 100 ppm) of TACDs. y = 0.013x +0.0628 (R^2=0.9997), x is the absorbance at 265 nm, y is the TACDs concentration.[2]

Text S6: Seed coating process:

Firstly, excess water was absorbed on the seeds surface using filter paper. Secondly, Maize seeds were immersed in Gelatin and GTACDs (5, 10, 20 and 50 ppm) solution. Coated maize seeds were placed in the tray above

the ice cube (30 s) for rapid gelation and air-dry (48 h) for using. The Gelatin and GTACDs formed a film on the surface of maize seeds (Figure. 4b). As shown in Figure. S8, the coated seeds can achieve rapid reswelling within 30 min.

Text S7: Element determination

The contents of N elements in maize tissues and soil were analyzed with an elemental analyzer (Elementar, Germany). The content of phosphorus was determined by inductively coupled plasma mass spectrometry (ICP-MS, iCAP-TQ, Thermo Fisher, Germany), dried plant tissue (25 mg) was weighed, 3 mL of deionized water and 3 mL of HNO_3 were added, then microwave digestion was performed at 1400 W and 190℃, and samples were measured after dilution.

Text S8: Gene Expression Analysis

The expression of genes associated with Aquaporins in maize tissues (radicles) with Non-CK, CK, Gelatin, GTACDs (10 ppm) groups were measured by quantitative real time PCR (qPCR). The primers were designed and synthesized by the Sangon Biotech Co., Ltd. (Shanghai, China) (Table S1). Actin was selected as the housekeeping gene. Total RNA was isolated and then used as a template to synthesize first-strand cDNA. The qPCR was performed on a CFX 96 touch Real Time PCR System platform (BioRad, USA). Using the UltraSYBR Mixture (CWBIO, China) following the manufacturer's instructions and qPCR protocol as

described in Yang et al.[3] The qPCR amplification program was 30 s at 95°C, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. The relative expression of each targeted gene was calculated using the 2−ΔΔCT method. All reactions were performed in triplicate, and the data were analyzed using the Bio-Rad CFX Manager software.

Text S9: 16s rRNA gene sequencing of rhizobacteria community

Total microbial genomic DNA was extracted using the DNeasy PowerSoil Kit (QIAGEN, Inc., Netherlands). Analysis of maize rhizosphere soil microbiome, the rhizosphere soil DNA extraction, PCR amplification and sequencing was supported by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The primers used for sequencing were 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWT CTAAT3'). After sequencing the V3 – V4 and ITS regions, the original readings (original PE) were obtained and low-quality fragments were filtered to recover effective tags.

Table S1

The sequences of specific primers used for the qRT-PCR analysis.[3]

Gene	Sequence (5' to 3')
M-actin-F	CCATGAGGCCACGTACAACT
M-actin-R	GGTAAAACCCCACTGAGGA
ZmPIP1,1-F	CCCTACTATGTTACGTGGAGTTC
ZmPIP1,1-R	GCGGCATATTACACAATTGGTA
ZmPIP1,2-F	GCTCAAACAGACAAGGACTAC
ZmPIP1,2-R	CAAGATGATGATGATGATGACTCGAAAG

Gene	Sequence (5’ to 3’)
ZmPIP2,4-F	TACCGGAGCAACGCCTAAG
ZmPIP2,4-R	GAAAACAGCAGCGAGCGA
ZmPIP2,5-F	TGTCGTCGTTGGTTGCCT
ZmPIP2,5-R	CACAACAATCACACTAGCTTGGAA
ZmTIP1,1-F	GCTCGCCGCCGTTTAGTTTCT
ZmTIP1,1-R	GACGACTGCTGGTCCAAGGAAG

Table S2

Physical and chemical properties of soil in different treatment groups.

Indicators	Before	Non-CK	CK	Gelatin	GTACDs
Soil moisture (%)	75.17±0.81^{a}	75.06±0.67^{a}	41.03±0.72^{b}	40.50±0.20^{b}	40.63±0.75^{b}
pH	7.40^{a}	7.41±0.01^{a}	7.38±0.01^{a}	7.38±0.01^{a}	7.37±0.01^{a}
EC (μS cm^{-1})	0.214±0.001^{b}	0.213±0.002^{a}	0.215±0.003^{a}	0.216±0.004^{a}	0.205±0.002^{a}
Eh (mV)	200.67±0.06^{a}	202.86±0.06^{a}	208.97±0.06^{b}	210.93±0.06^{b}	209.93±0.12^{b}
Total C (g/kg)	19.47±0.64^{b}	19.40±0.60^{b}	20.47±2.25^{b}	22.43±2.05^{ab}	23.13±0.15^{a}
Total N (g/kg)	0.90±0.10^{b}	0.93±0.15^{b}	0.76±0.12^{c}	1.03±0.21^{b}	1.27±0.15^{a}
C/N ratio	21.54±2.46^{a}	21.56±2.84^{a}	23.57±3.82^{a}	20.88±3.95^{ab}	20.47±3.32^{b}
TIC (mg/kg)	118.97±2.86^{d}	150.27±1.79^{b}	135.63±3.19^{c}	142.83±4.76^{bc}	180.20±6.99^{a}
TOC (mg/kg)	125.87±1.1^{b}	134.17±3.04^{a}	105.19±1.92^{c}	114.24±5.04^{b}	124.20±1.34^{b}

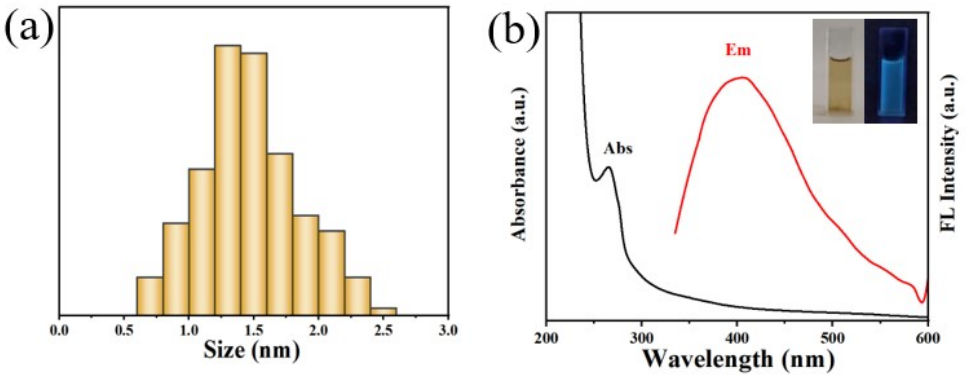

Figure. S1 (a) Particle size distribution of TACDs, (b) Absorption and fluorescence emission spectra of TACDs.

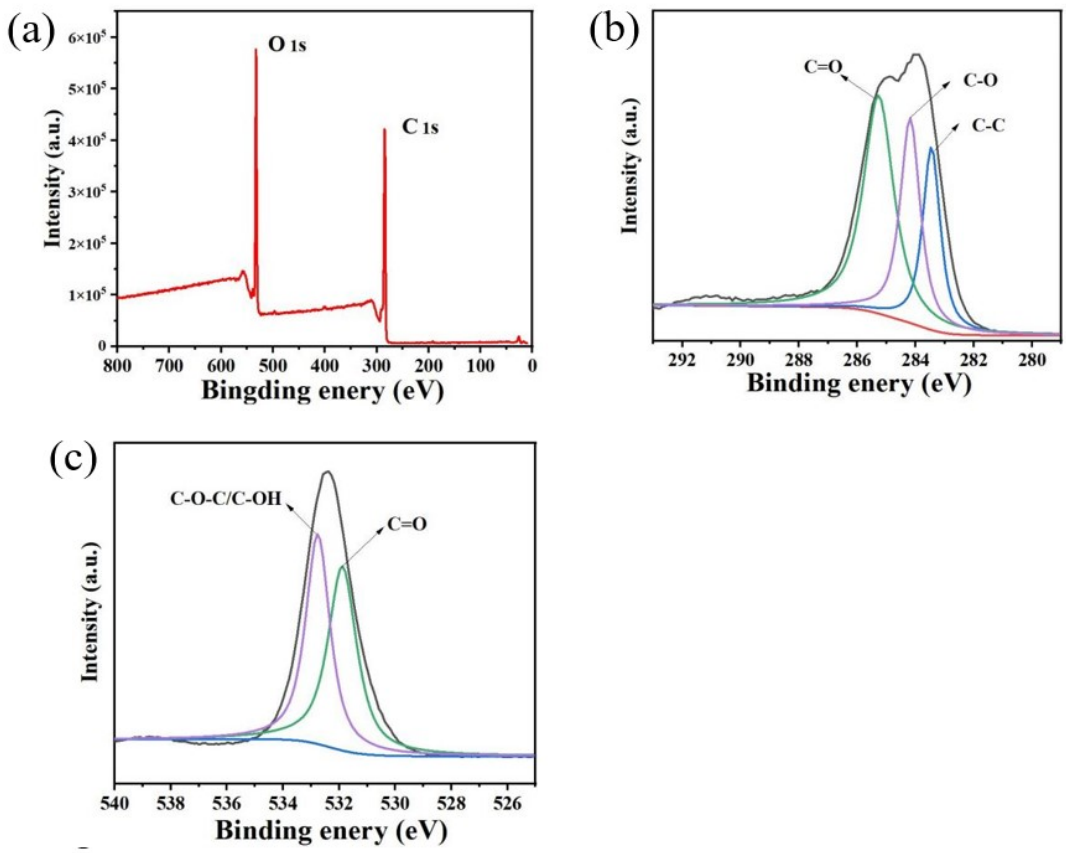

Figure. S2 (a) Full XPS patterns, (b) The XPS spectra of C1s, (c) N1s peaks of TACDs.

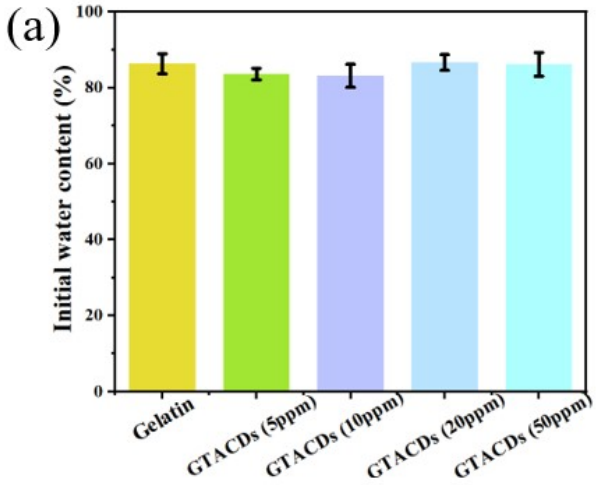

Figure. S3 The initial water content (%) of Gelatin, GTACDs hydrogels (5, 10, 20, 50 ppm) (n=3).

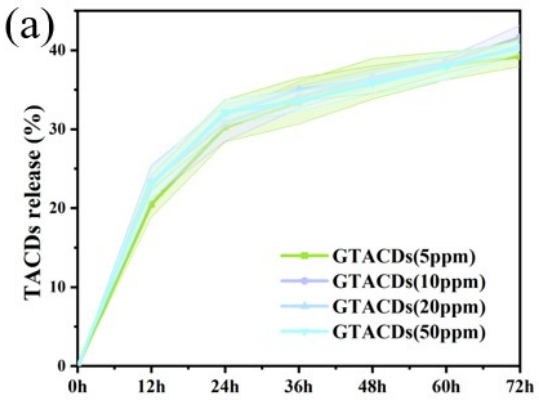

Figure. S4 TACDs release ratio (%) in GTACDs hydrogels (5, 10, 20, 50 ppm) (n=3).

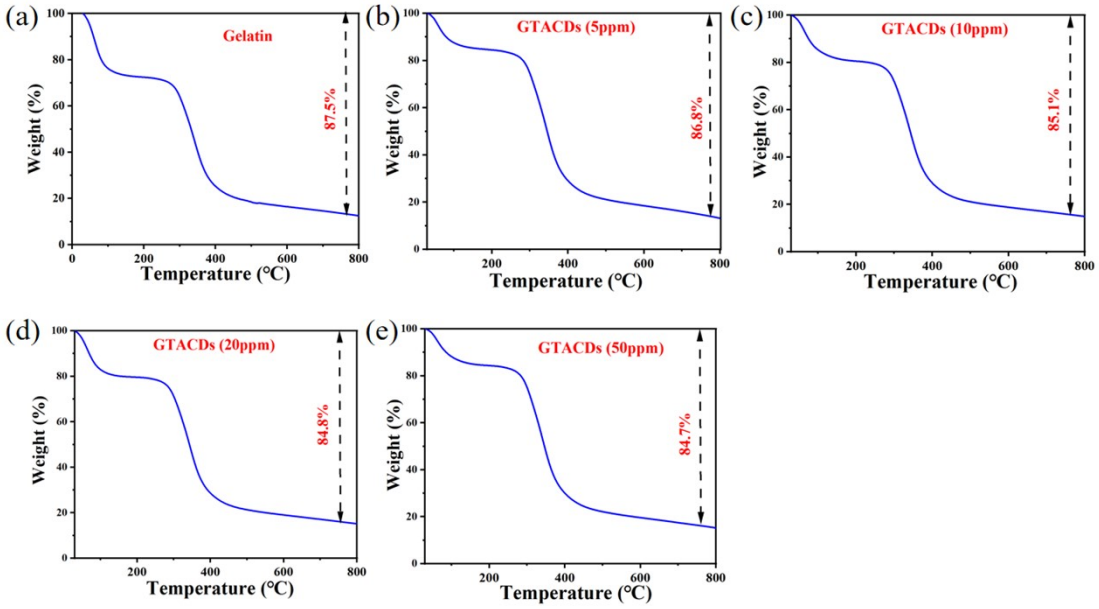

Figure. S5 Thermal gravimetric analysis (TGA) curves of GTACDs hydrogels (5, 10, 20, 50 ppm).

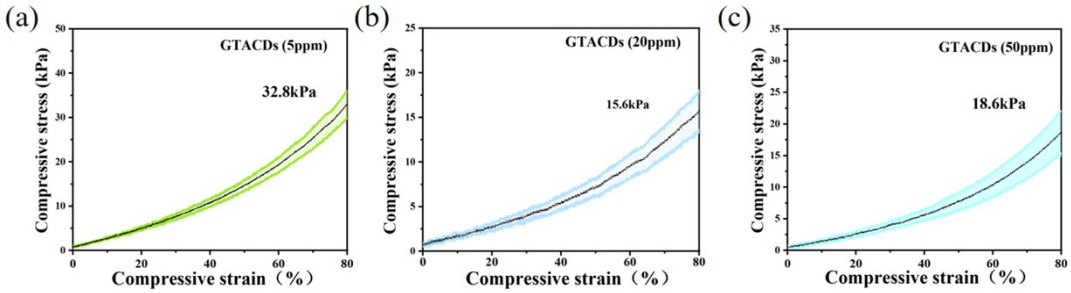

Figure. S6 The compression-strain curves of GTACDs hydrogels (5, 20, 50 ppm) (n=3).

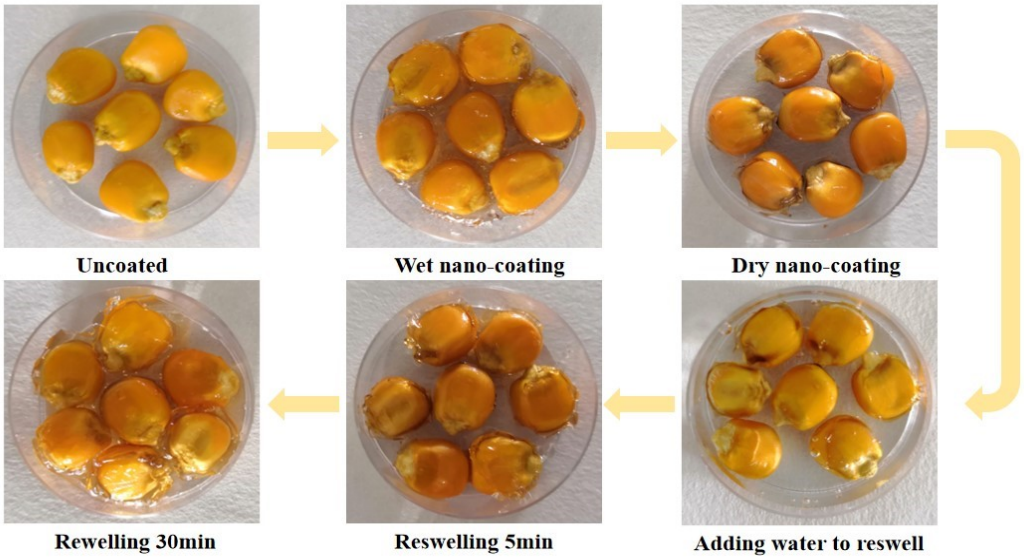

Figure. S7 Reswelling pictures of GTACDs hydrogels as seed coating.

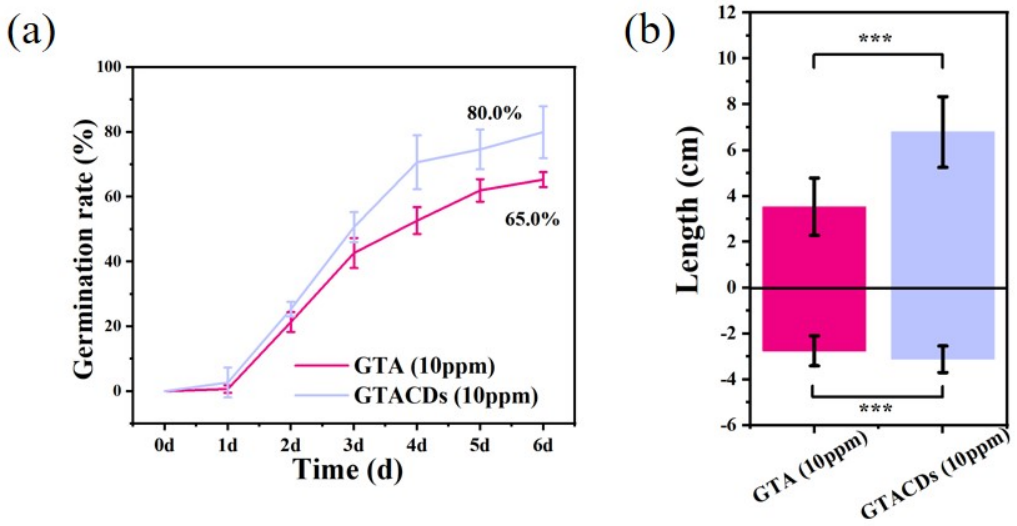

Figure. S8 (a) The germination rate of GTA (10 ppm) hydrogels and GTACDs (10 ppm) hydrogels, (b) The length of shoots and radicles from GTA (10 ppm) hydrogels and GTACDs (10 ppm) hydrogels after 6 days of germination (n=3). $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ as determined by a two-tailed Student's *t* test.

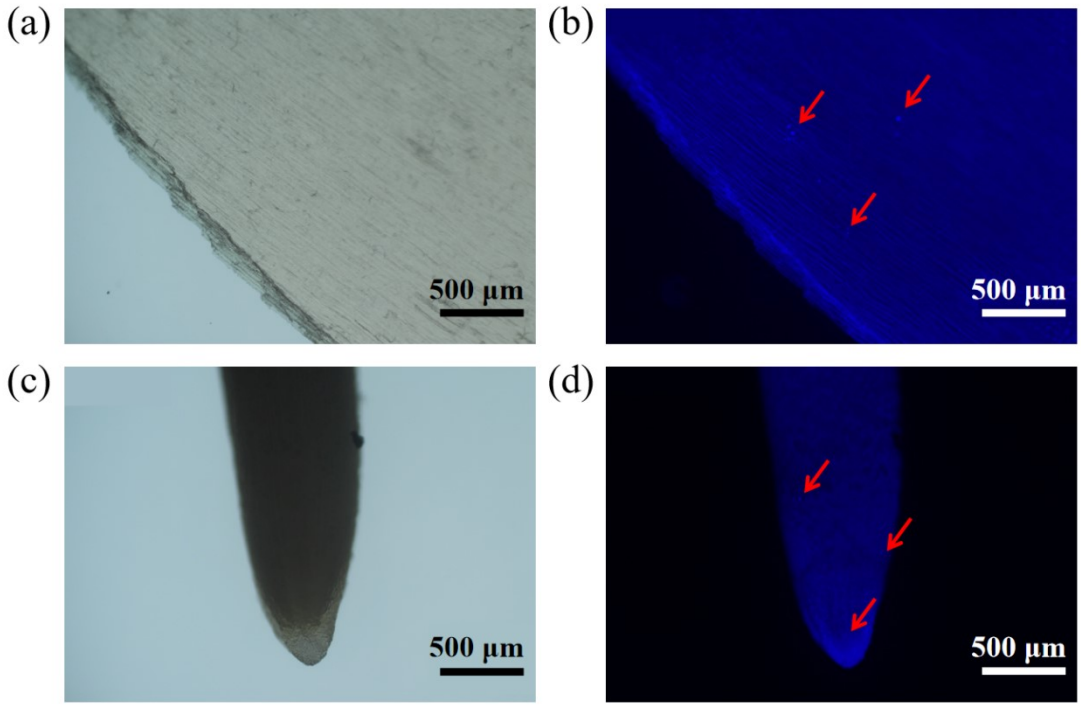

Figure. S9 Fluorescence microscope images of TACDs (λ_{Ex} =361-389 nm) were observed in episperms (a-b) and radicles (c-d) . (The samples were be from two days after germinating.)

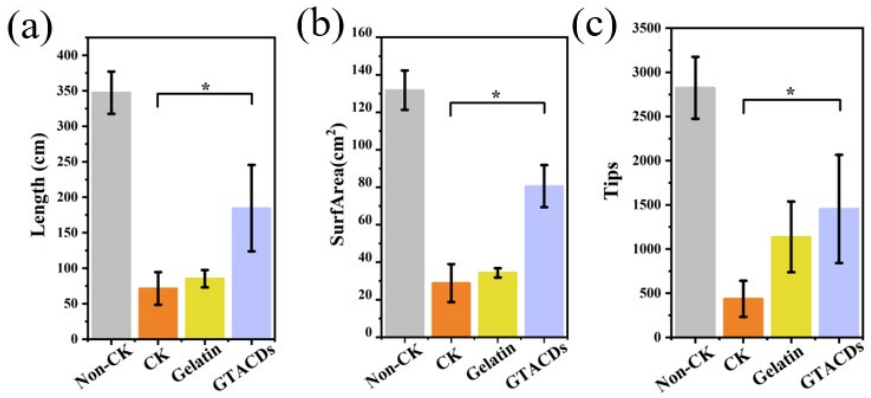

Figure. S10 (a-c) The root length, root surf area, root tips of Non-CK, CK, Gelatin, GTACDs (10 ppm) groups (n=3). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ as determined by a two-tailed Student's *t* test.

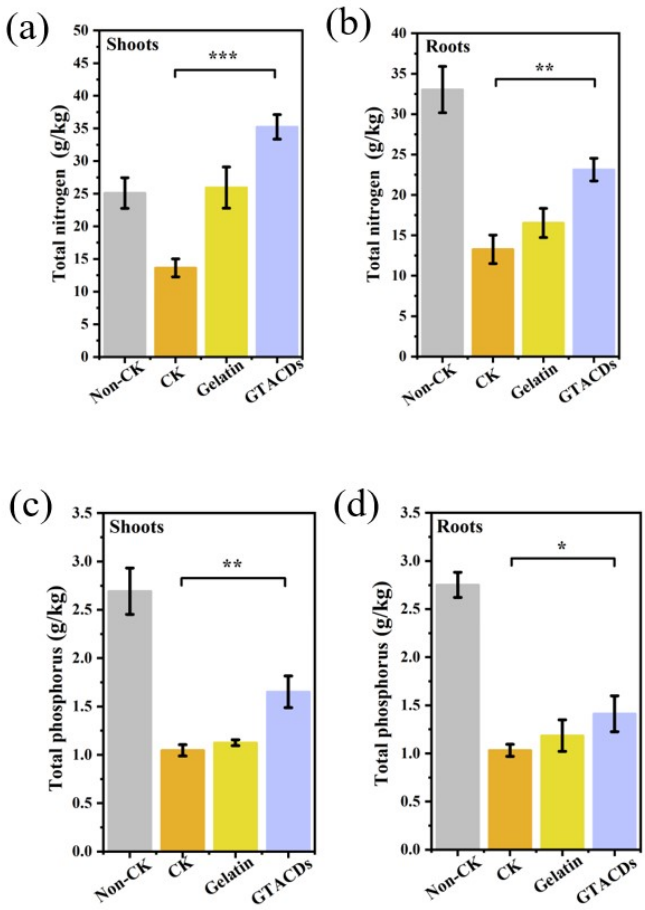

Figure. S11 The total nitrogen, total phosphorus of maize in shoots and roots (n=3). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ as determined by a two-tailed Student's *t* test.

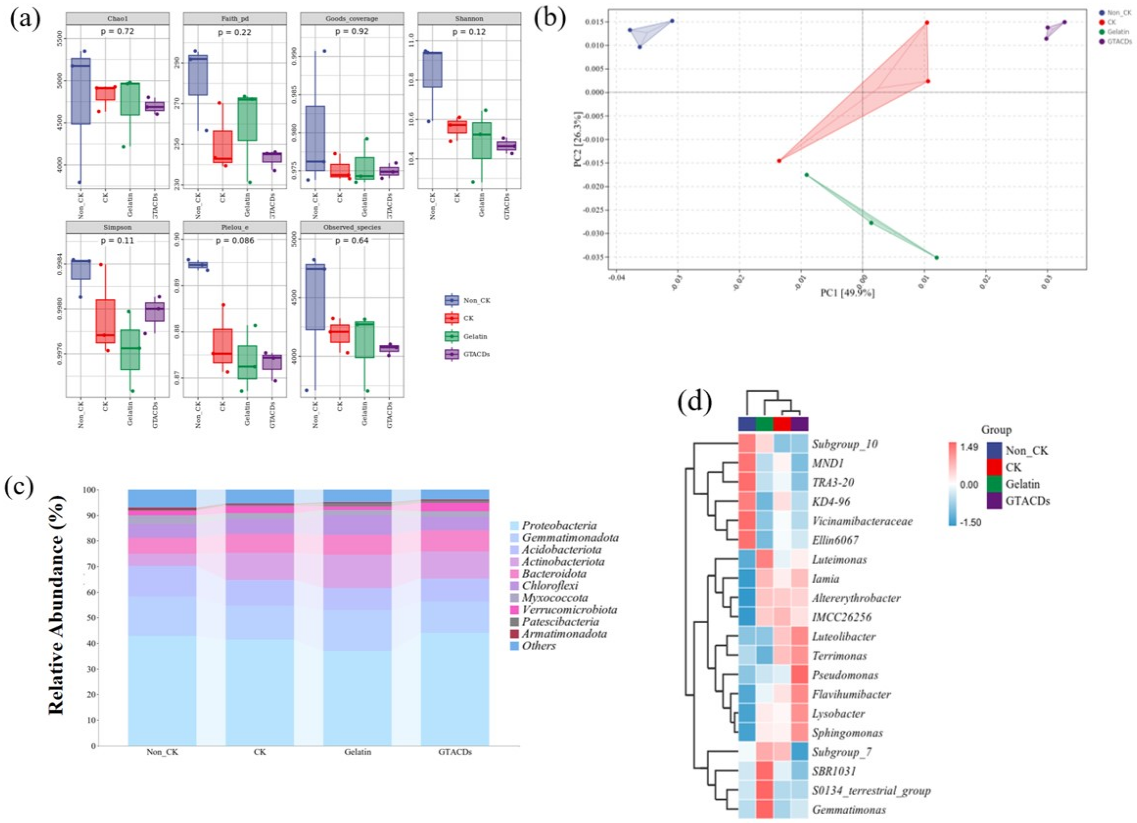

Figure. S12 (a) The alpha diversity, (b) Principal component analysis (PCA), (c) Relative abundance of bacterial at phylum-level, (d) Relative abundance of bacterial at genus-level in rhizobacteria communities of Non-CK, CK, Gelatin, GTACDs group. (n=3)

Reference

(1) Z. Ahmadian, A. Correia, M. Hasany, P. Figueiredo, F. Dobakhti, M. R. Eskandari, S. H. Hosseini, A. Ramin, S. Khorshid, J. Hirvonen, H. A. Santos, M. Shahbazi. A Hydrogen-Bonded Extracellular Matrix-Mimicking Bactericidal Hydrogel with Radical Scavenging and Hemostatic Function for pH-Responsive Wound Healing Acceleration. *Adv. Healthcare Mater.*, 2021, **10**, 2001122.

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(3) H. Yang, C. Wang, F. Chen, L. Yue, X. Cao, J. Li, X. Zhao, F. Wu, Z. Wang, B. Xing. Foliar carbon dot amendment modulates carbohydrate metabolism, rhizospheric properties and drought tolerance in maize seedling. *Sci Total Environ.*, 2022, **809**, 151105.