

## Supplemental information

### Uptake and impact of carbon dots and their copper complex on tomato health

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Table S1. pH of 0.2 mg/mL CDs before and after exposure to aqueous 68 mg/mL Cu(NO<sub>3</sub>)<sub>2</sub>

	Before Cu(NO <sub>3</sub> ) <sub>2</sub> addition	After Cu(NO <sub>3</sub> ) <sub>2</sub> addition
pH	6.51 ± 0.04	5.11 ± 0.06

Notes: pH of 68 µg/mL Cu(NO<sub>3</sub>)<sub>2</sub> is 6.02 ± 0.03; final concentration after Cu(NO<sub>3</sub>)<sub>2</sub> addition is 30.2 µg/mL

Table S2. Maximum loading capacity of Cu<sup>2+</sup> into CDs, measured using ICP-OES

Mass of Cu <sup>2+</sup> (mg)	Mass of Cu-CD Complex (mg)	Loading Capacity (w/w%)
1.735	5.720	30.5
1.936	6.410	30.2
2.122	6.852	31.0

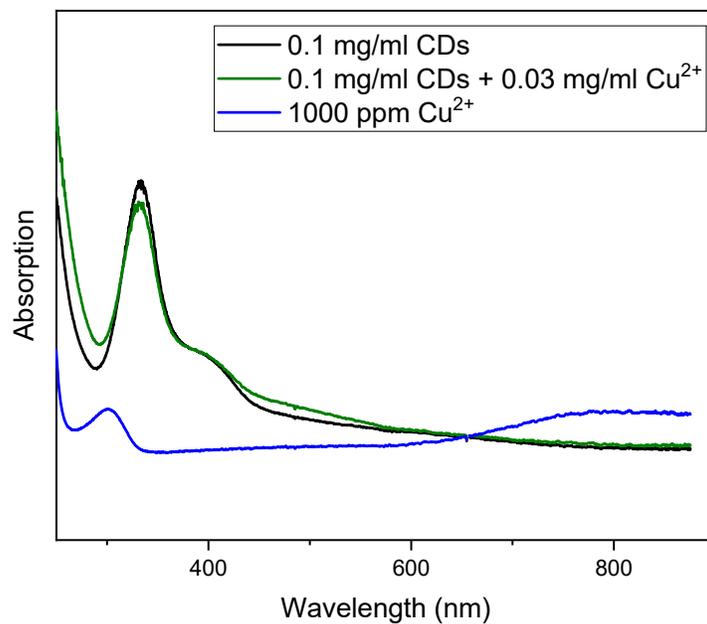


Figure S1. UV-vis absorption spectra of 0.1 mg/mL CDs with and without 0.03 mg/mL  $\text{Cu}^{2+}$  as well as the absorption spectrum of 1000 ppm aqueous  $\text{Cu}^{2+}$  from  $\text{Cu}(\text{NO}_3)_2$ .

Table S3. Fluorescence lifetime of CDs exposed to 0, 20, 50 and 100 ppm aqueous  $\text{Cu}^{2+}$

$\text{Cu}^{2+}$ ppm	Avg $\tau$	error	$\tau_1$	error	$\tau_2$	error
0	4.873	0.400	3.371	0.085	7.989	0.114
20	5.409	0.442	3.339	0.087	8.006	0.080
50	5.612	0.433	3.143	0.134	7.576	0.064
100	5.692	0.417	3.492	0.136	8.949	0.254

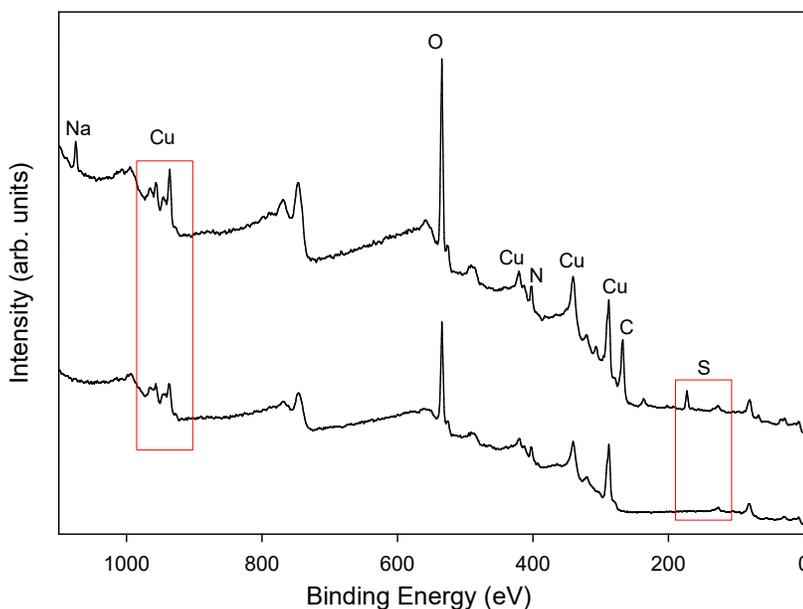


Figure S2. XPS survey spectra of Cu-CD complexes after dialysis for 2 days (top) and 7 days (bottom).

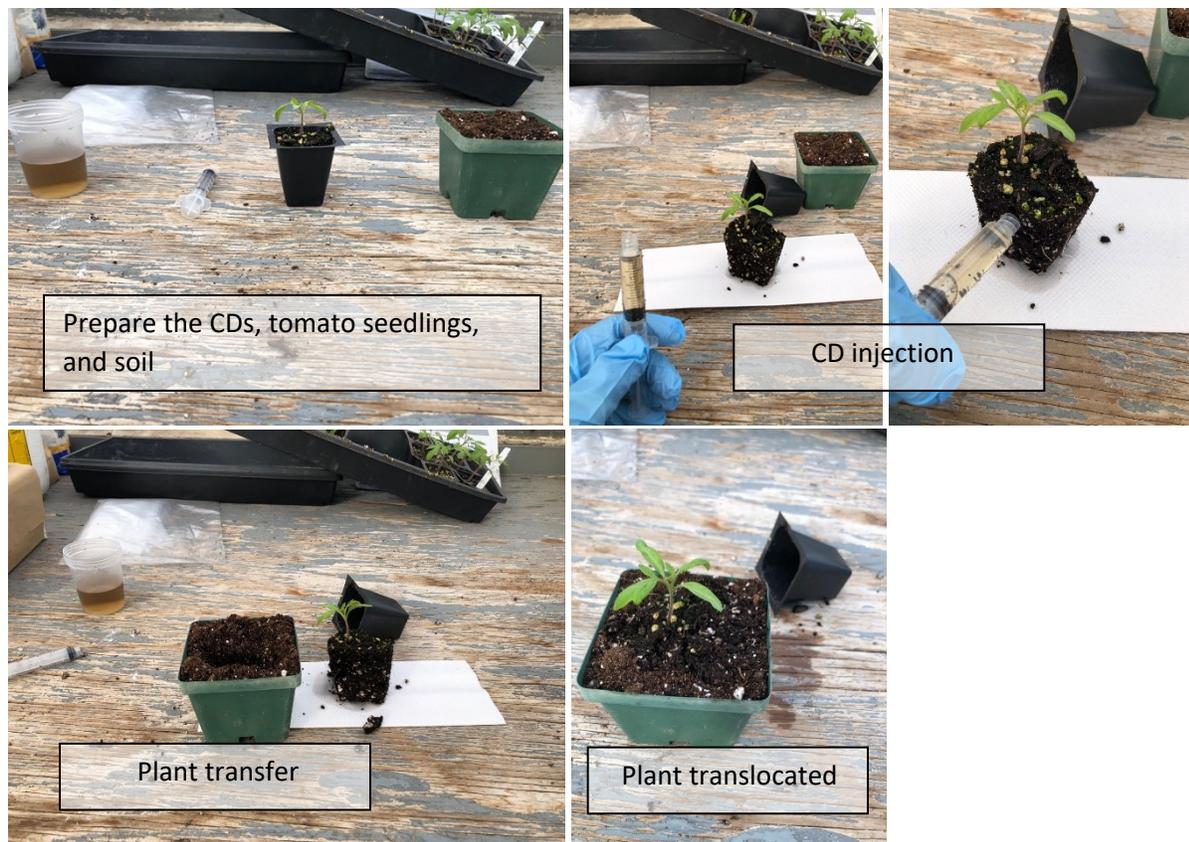


Figure S3. Root ball injection procedure, starting in the top row and proceeding from left to right.

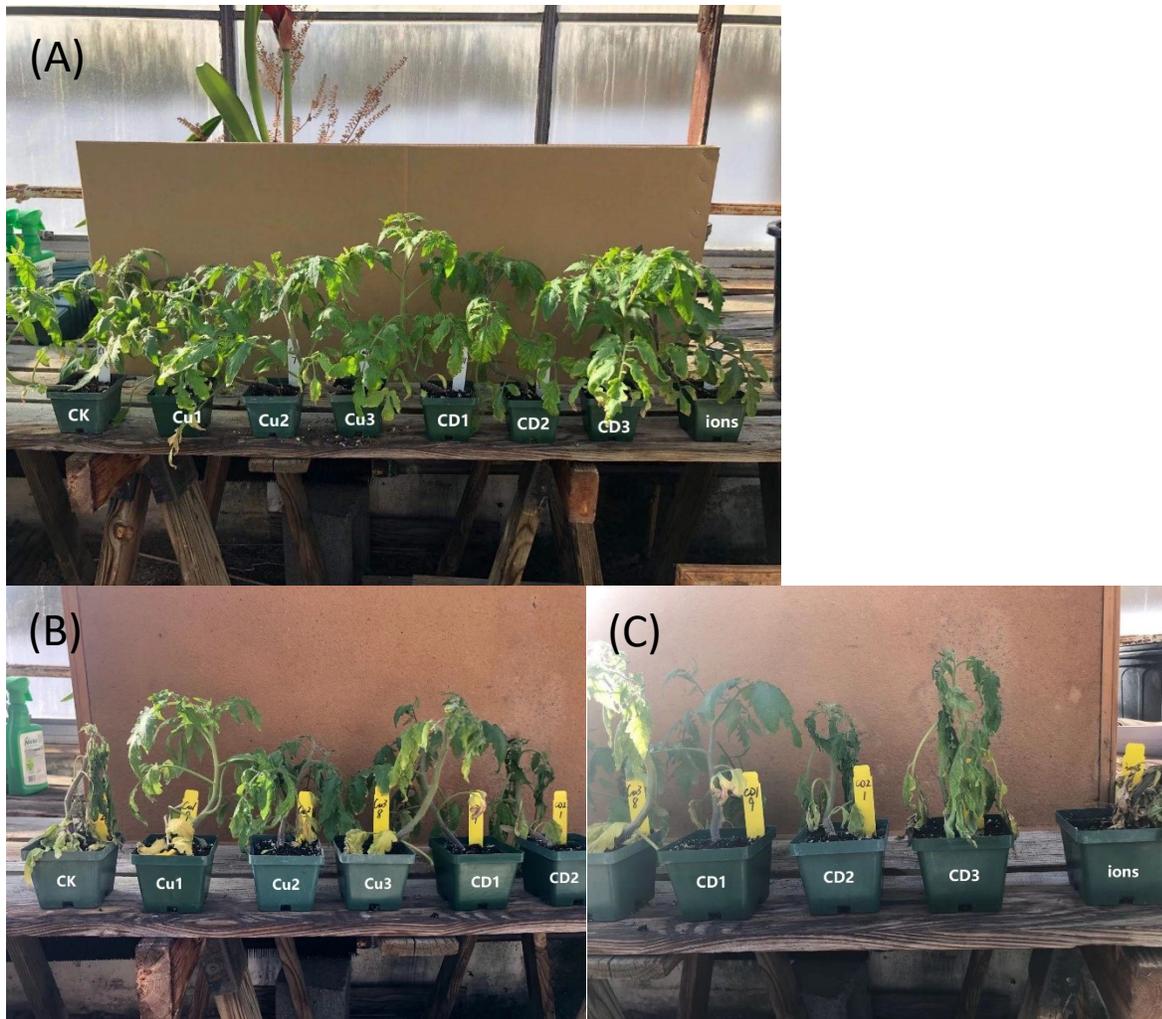


Figure S4. Representative end-of-experiment images of tomatoes that received root ball injections for (A) healthy groups and (B and C) Fusarium-infected disease groups treated with deionized water (CK); 500 ppm CDs with 153 ppm  $\text{Cu}^{2+}$  (Cu1); 250 ppm CDs with 76.5 ppm  $\text{Cu}^{2+}$  (Cu2); 125 ppm CDs with 38 ppm  $\text{Cu}^{2+}$  (Cu3); 500 ppm CDs (CD1); 250 ppm CDs (CD2); 125 ppm CDs (CD3); or 87.5 ppm aqueous  $\text{Cu}^{2+}$  (ions).

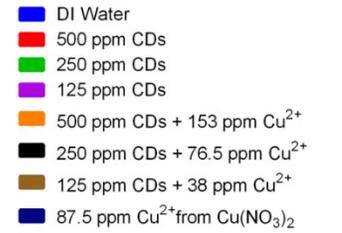
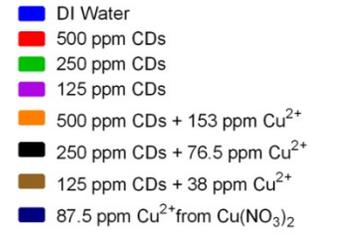
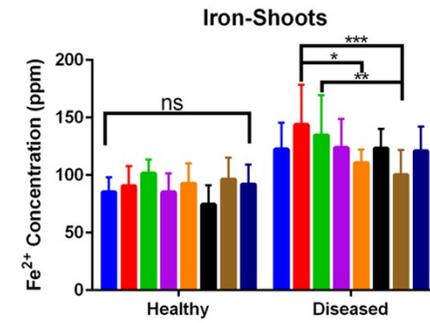
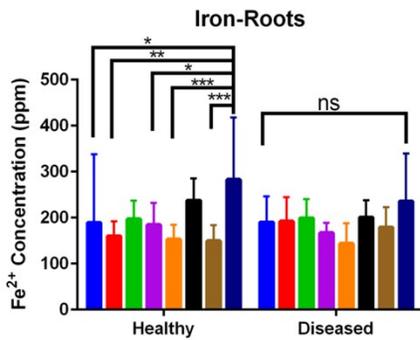
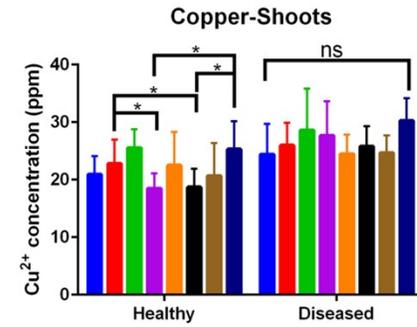
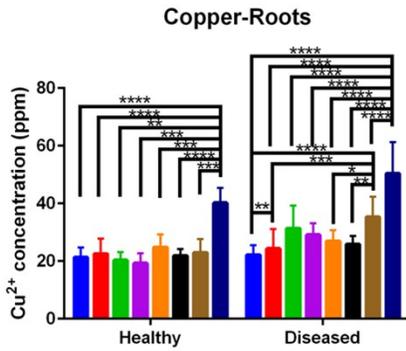
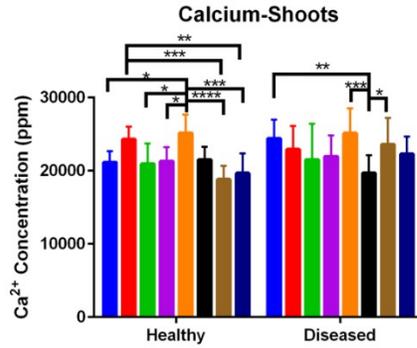
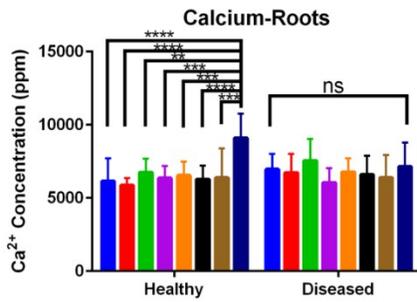
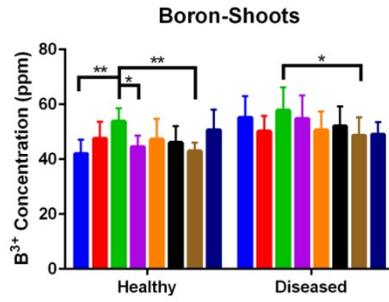
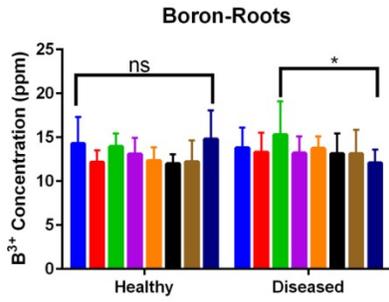
Table S4. Additional statistical differences in Figure 7 from the healthy root biomass data

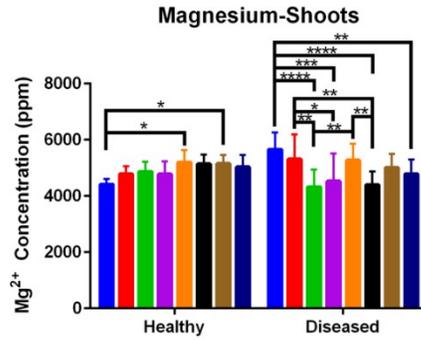
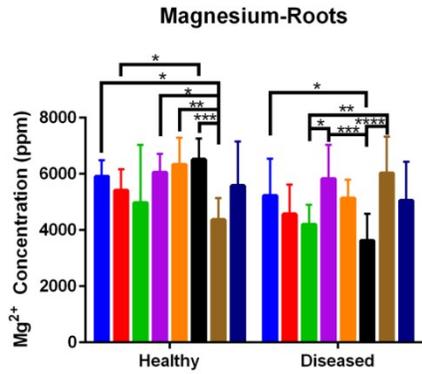
	Significance
DI Water vs 250 ppm CDs + 76.5 ppm $\text{Cu}^{2+}$	*
250 ppm CDs vs 250 ppm CDs + 76.5 ppm $\text{Cu}^{2+}$	*
250 ppm CDs + 76.5 ppm $\text{Cu}^{2+}$ vs 125 ppm CDs + 38 ppm $\text{Cu}^{2+}$	**
250 ppm CDs + 76.5 ppm $\text{Cu}^{2+}$ vs 87.5 ppm $\text{Cu}^{2+}$ from $\text{Cu}(\text{NO}_3)_2$	*

Note: \* denotes  $p \leq 0.05$ ; \*\* denotes  $p \leq 0.01$

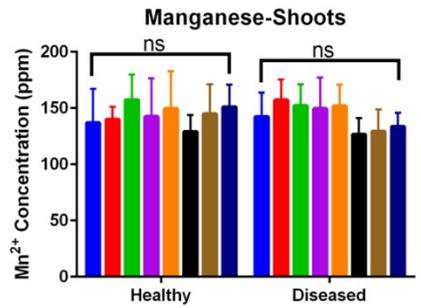
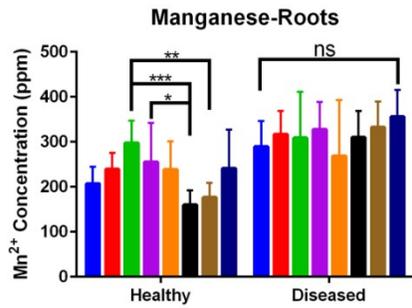
Table S5. Additional nano-enabled micronutrient delivery systems explored in literature and their benefits to the target plant

Nanoparticle Used	Delivered Nutrient	Plant Target	Improvement to Plant
Molybdenum nanofertilizer <sup>1</sup>	molybdenum	Green bean	Increased plant biomass by 36.47% compared to molybdate
Molybdenum disulfide nanoparticles <sup>2</sup>	molybdenum	Soybean	Increased yield by 30% compared to traditional molybdate fertilizer
Hollow silica nanoparticles <sup>3</sup>	silicic acid	Tomato	Increased shoot P by 17.63%, K by 10.00%, and Mg by 11.53%. Increased root P by 51.56%, Mn by 33.13%, and Cu by 151.83%
Porous silica nanoparticles <sup>3</sup>	silicic acid	Tomato	Increased shoot K by 7.95% and Mg by 10.49% Increased root P by 41.41%, Mn by 43.85%, and Cu by 42.00%
ZnO nanoparticles <sup>4</sup>	zinc	Eggplant	Increased fruit yield by 22.6% under drought conditions
CuO nanoparticles <sup>5</sup>	copper	Soybean	Increased shoot biomass by 53% under drought conditions
Chitosan-coated mesoporous silica nanoparticles <sup>6</sup>	silicic acid	Soybean	Reduced <i>Fusarium</i> disease progression by 15% Increased chlorophyll content by 32% Increased Zn, Mn, Mg, K, B content by 23–68%
Nickel nanoparticles <sup>7</sup>	nickel	Lettuce and tomato	Reduced disease severity by 58.4% for lettuce and 57.0% for tomato
Ceria nanoparticles <sup>8</sup>	cerium	rice	Increased N in roots and shoots under low nitrogen stress by 6-12% and 22-30%, respectively
Poly(acrylic acid)-coated CeO <sub>2</sub> nanoparticles <sup>9</sup>	cerium	cotton	Increased seedling root length by 56%, increased fresh weight by 41%, and increased dry weight by 38%

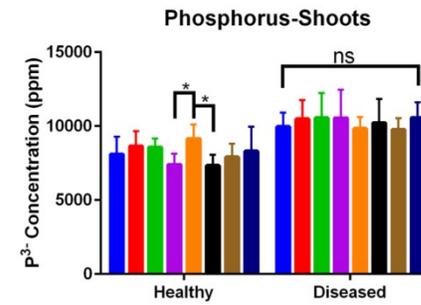
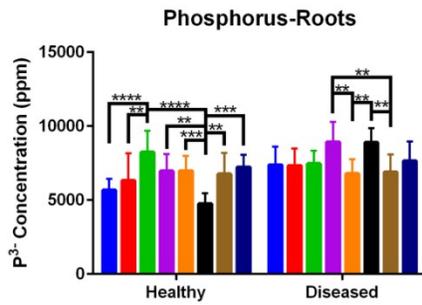




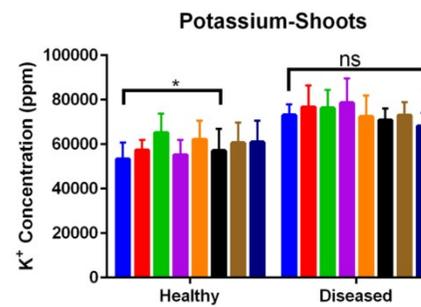
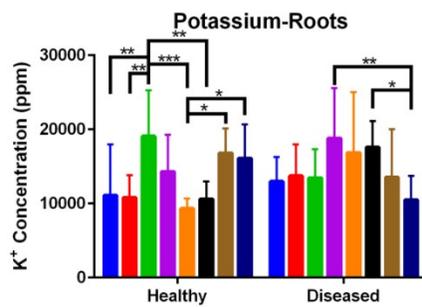
- DI Water
- 500 ppm CDs
- 250 ppm CDs
- 125 ppm CDs
- 500 ppm CDs + 153 ppm Cu<sup>2+</sup>
- 250 ppm CDs + 76.5 ppm Cu<sup>2+</sup>
- 125 ppm CDs + 38 ppm Cu<sup>2+</sup>
- 87.5 ppm Cu<sup>2+</sup> from Cu(NO<sub>3</sub>)<sub>2</sub>



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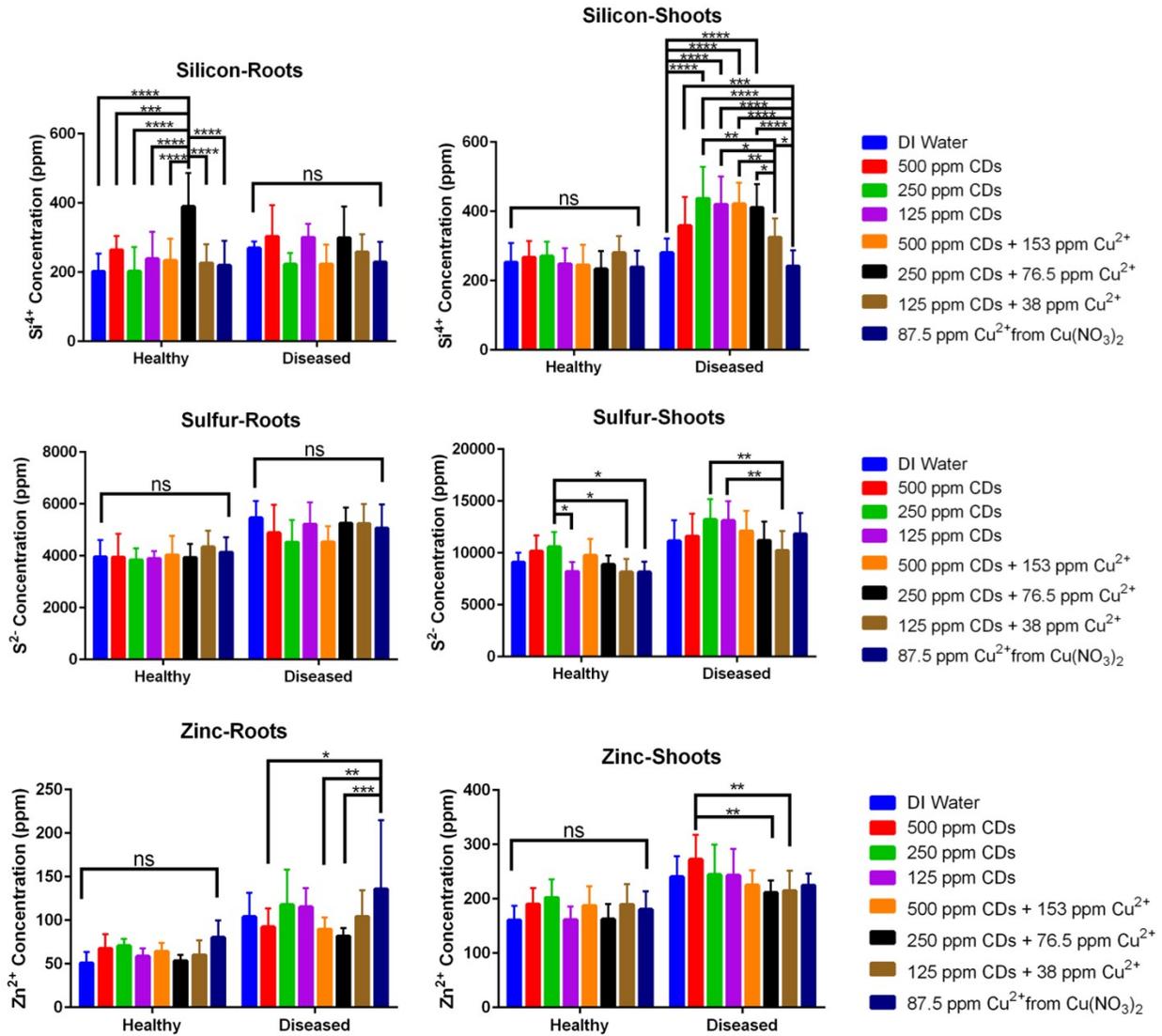


Figure S5. Elemental analysis of different relevant ion concentrations in healthy and diseased plants' roots and shoots. Error bars represent standard deviations across 9-10 plant replicates. Statistical differences were determined through a two-way ANOVA with a Tukey multiple comparisons test.

Table S6. Final survival rates and representative images for tomato seeds in diseased soil treated with varying concentrations of CDs and Cu-CD complexes using seed vacuum infiltration

	Final Survival Rates	Representative Photo
DI Water	22.5%	
500 ppm CDs	12.5%	

250 ppm CDs	22.5%	
125 ppm CDs	10.0%	
500 ppm CDs + 153 ppm Cu <sup>2+</sup>	40.0%	
250 ppm CDs + 76.5 ppm Cu <sup>2+</sup>	35.0%	
125 ppm CDs + 38 ppm Cu <sup>2+</sup>	20.0%	
87.5 ppm Cu <sup>2+</sup> from Cu(NO <sub>3</sub> ) <sub>2</sub>	12.5%	

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Table S7. Final survival rates and representative images for tomato seeds in healthy soil treated with varying concentrations of CDs and Cu-CD complexes using seed vacuum infiltration

	Final Survival Rates	Representative Photo
DI Water	97.5%	
500 ppm CDs	92.5%	
250 ppm CDs	90.0%	
125 ppm CDs	92.5%	
500 ppm CDs + 153 ppm Cu <sup>2+</sup>	95.0%	
250 ppm CDs + 76.5 ppm Cu <sup>2+</sup>	97.5%	
125 ppm CDs + 38 ppm Cu <sup>2+</sup>	95.0%	
87.5 ppm Cu <sup>2+</sup> from Cu(NO <sub>3</sub> ) <sub>2</sub>	87.5%	

## References

- (1) Muñoz-Márquez, E.; Soto-Parra, J. M.; Noperi-Mosqueda, L. C.; Sánchez, E. Application of Molybdenum Nanofertilizer on the Nitrogen Use Efficiency, Growth and Yield in Green Beans. *Agronomy* **2022**, *12* (12), 3163. <https://doi.org/10.3390/agronomy12123163>.
- (2) Li, M.; Zhang, P.; Guo, Z.; Cao, W.; Gao, L.; Li, Y.; Tian, C. F.; Chen, Q.; Shen, Y.; Ren, F.; Rui, Y.; White, J. C.; Lynch, I. Molybdenum Nanofertilizer Boosts Biological Nitrogen Fixation and Yield of Soybean through Delaying Nodule Senescence and Nutrition Enhancement. *ACS Nano* **2023**, *17* (15), 14761–14774. <https://doi.org/10.1021/acsnano.3c02783>.
- (3) Wang, L.; Wang, Y.; Deng, C.; Eggleston, I.; Gao, S.; Li, A.; Alvarez Reyes, W. R.; Cai, K.; Qiu, R.; Haynes, C. L.; White, J. C.; Xing, B. Optimizing SiO<sub>2</sub> Nanoparticle Structures to Enhance Drought Resistance in Tomato (*Solanum Lycopersicum* L.): Insights into Nanoparticle Dissolution and Plant Stress Response. *J. Agric. Food Chem.* **2025**, *73* (16), 9983–9993. <https://doi.org/10.1021/acs.jafc.5c03048>.
- (4) Semida, W. M.; Abdelkhalik, A.; Mohamed, G. F.; Abd El-Mageed, T. A.; Abd El-Mageed, S. A.; Rady, M. M.; Ali, E. F. Foliar Application of Zinc Oxide Nanoparticles Promotes Drought Stress Tolerance in Eggplant (*Solanum Melongena* L.). *Plants (Basel)* **2021**, *10* (2), 421. <https://doi.org/10.3390/plants10020421>.
- (5) Zhou, J.; Wang, Y.; Zuverza-Mena, N.; Dimkpa, C. O.; White, J. C. Copper-Based Materials as an Effective Strategy for Improving Drought Resistance in Soybean (*Glycine Max*) at the Reproductive Stage. *ACS Agric. Sci. Technol.* **2024**, *4* (7), 735–746. <https://doi.org/10.1021/acsagscitech.4c00193>.
- (6) O’Keefe, T. L.; Deng, C.; Wang, Y.; Mohamud, S.; Torres-Gómez, A.; Tuga, B.; Huang, C.-H.; Alvarez Reyes, W. R.; White, J. C.; Haynes, C. L. Chitosan-Coated Mesoporous Silica Nanoparticles for Suppression of *Fusarium Virguliforme* in Soybeans (*Glycine Max*). *ACS Agric. Sci. Technol.* **2024**, *4* (5), 580–592. <https://doi.org/10.1021/acsagscitech.4c00025>.
- (7) Yadav, D. R. Applications of Nickel Nanoparticles for Control of *Fusarium* Wilt on Lettuce and Tomato. *International Journal of Innovative Research in Science, Engineering and Technology* **2016**. <https://doi.org/10.15680/IJIRSET.2016.0505132>.
- (8) Wang, Y.; Zhang, P.; Li, M.; Guo, Z.; Ullah, S.; Rui, Y.; Lynch, I. Alleviation of Nitrogen Stress in Rice (*Oryza Sativa*) by Ceria Nanoparticles. *Environ. Sci.: Nano* **2020**, *7* (10), 2930–2940. <https://doi.org/10.1039/D0EN00757A>.
- (9) An, J.; Hu, P.; Li, F.; Wu, H.; Shen, Y.; White, J. C.; Tian, X.; Li, Z.; Giraldo, J. P. Emerging Investigator Series: Molecular Mechanisms of Plant Salinity Stress Tolerance Improvement by Seed Priming with Cerium Oxide Nanoparticles. *Environ. Sci.: Nano* **2020**, *7* (8), 2214–2228. <https://doi.org/10.1039/D0EN00387E>.