Supporting Information

The Effect of Nanoparticle Surface Charge on Freshwater Algae Growth, Morphology, and Lipid Production

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Instruction for replicating ImageXpress analysis are below. These setting were used at 60x magnification and may need to be adjusted for other objectives. These modules are located under the "Segment" tab.

Step 1: Set image names and select Cy5, DAPI, and GFP channels.

Step 2: Filter DAPI image using Top Hat function. Set pixel size to 12, filter shape to circle, and name filter output image.

Step 3: Find nuclei using "Find Round Objects" function. Choose Top Hat filtered DAPI image as source. Set approximate minimum width to 0.8 μm and approximate maximum width to 1.77 μm. Set intensity above local background to 1562, and name result "nuclei."

Step 4: Using result "Nuclei" from step 3, mark the center of each nucleus as a single pixel using "Mark Object Centers" function. Name the result. This will allow for nuclei per cell counts, as number of pixels per cell will correspond to the nuclei count per cell.

Step 5: Create an image from the Mark Object Centers mask created in step 4 using the "Mask to Image" function. The mask to image here was named "Nuclei center Mask to Image."

Step 6: Use "Top Hat" function to filter Cy5 images. Choose Cy5 as source, pixel size 22, and filter shape circle. Name result (named "TopHat Cell" here).

Step 7: Use the "Gaussian Filter" function to blur "TopHat Cell," which aids in recognition of the sickle-shaped *R. subcapitata* cell. Rename ("blurred cell" in this example).

Step 8: Use function "Adaptive Threshold" to identify cells. Set source as "blurred cell," approximate minimum width as 2.3 μm and maximum width as 4 μm. Use 1000 as intensity above local background and name result ("Cell" used as name here).

Step 9: Create a filter mask from "Cell" result using the "Filter Mask" function. Choose Integrated Intensity as as "Measurement," "Range Filter" for "Filter Type" and set both minimum and maximum values to 1. Name "Filter Mask 1." This identifies all cells with 1 nuclei.

Step 10: Use the "Filter Mask" function again. Choose "Nuclei center Mask to Image" as the image source and "Cell" as the mask source. Use "Integrated Intensity as measurement, "RangeFilter" as filter type, and set both minimum and maximum values to 2. Name this output "Filter Mask 2." This identifies all cells with 2 nuclei.

Step 11: Use the "Filter Mask" function again with same settings as steps 9 and 10. This time, change the minimum and maximum values to 4. This identifies all cells with 4 nuclei. Name "Filter Mask 4."

Step 12: Create a final "Filter Mask" as above, but change minimum value to 5, and do not include a maximum value. This creates a filter for any cells which have more than 4 nuclei.

Step 13: Use "Top Hat" function to filter GFP images. Set source as "GFP," size to 10 pixels, and filter shape to "Circle." Name "Top hat gfp."

Step 14: Use "Find Round Objects" function to identify lipid droplets. Set source as "Top hat gfp," approximate minimum width to 0.8 μm, approximate maximum width to 6 μm, and intensity above local background to 2278. Name result "lipid droplets."

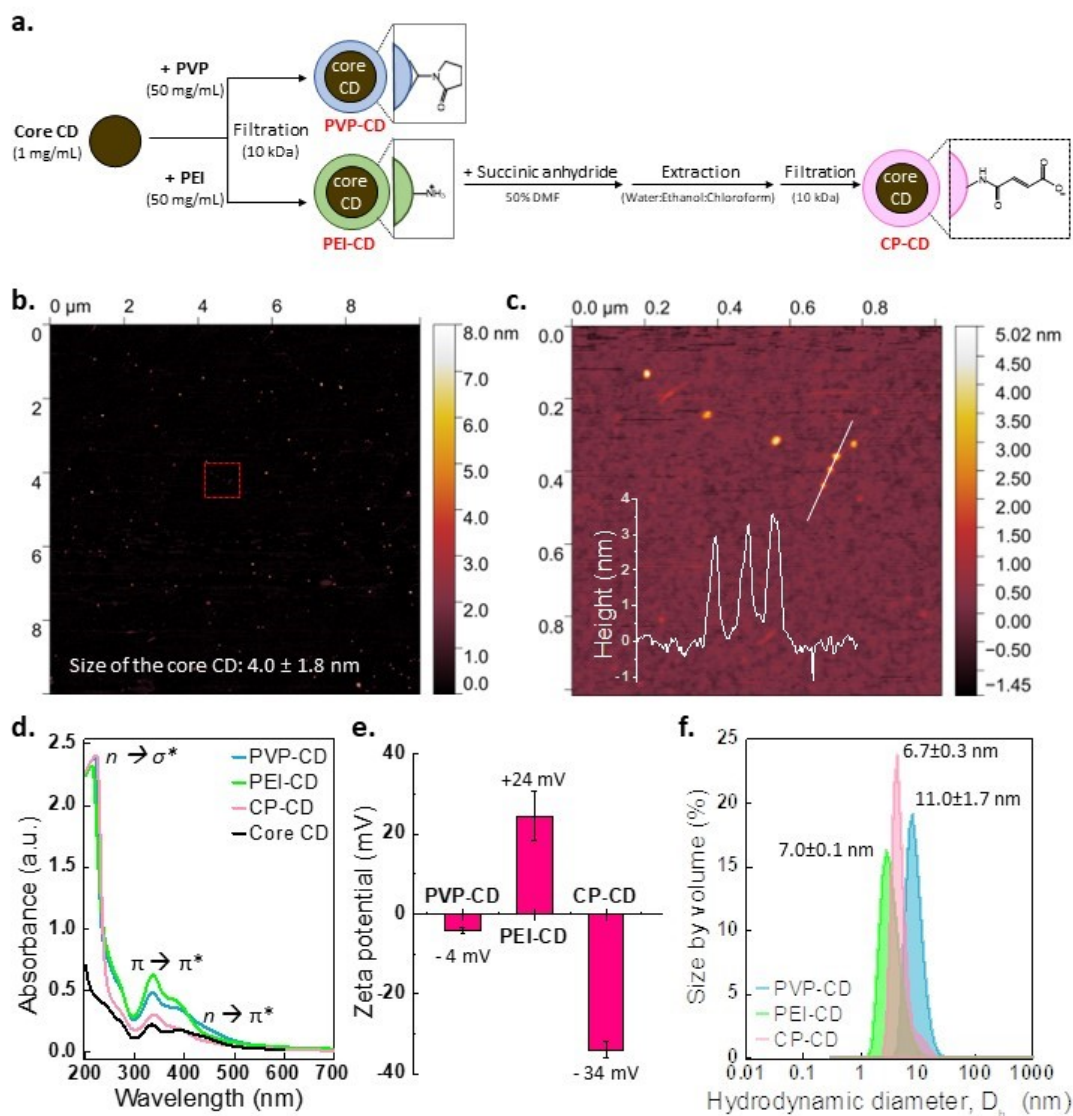After analysis, choose which aspects of the analyzed images to measure in the "Measure" tab.

Figure S1. Carbon Dot (CD) Synthesis and Characterization. a. An illustrated procedure outlining the synthesis process of the charge-varied carbon dots. **b.** Atomic Force Microscopy (AFM) images alongside the height profiles of the synthesized core CDs, revealing an average height of 4.0 ± 1.8 nm for the core carbon dot. **c.** A magnified view of the section demarcated by the red dashed box in figure b, complemented by the height profile of core CDs. **d.** UV spectrums characterizing the carbon dots. **e.** Evaluations of the carbon dot's surface charge and **f.** the hydrodynamic diameter, as measured with a Zetasizer.
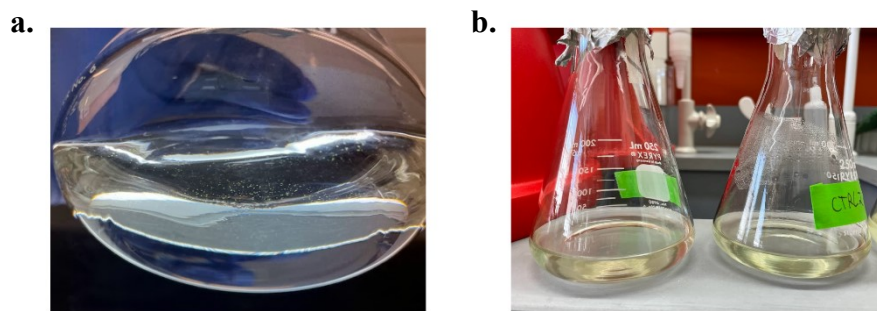
a.

b.

Figure S2. (a) Example of visible algal clumping in an exposure flask after PEI-CD treatment, compared to **(b)** control flasks with cells in suspension.
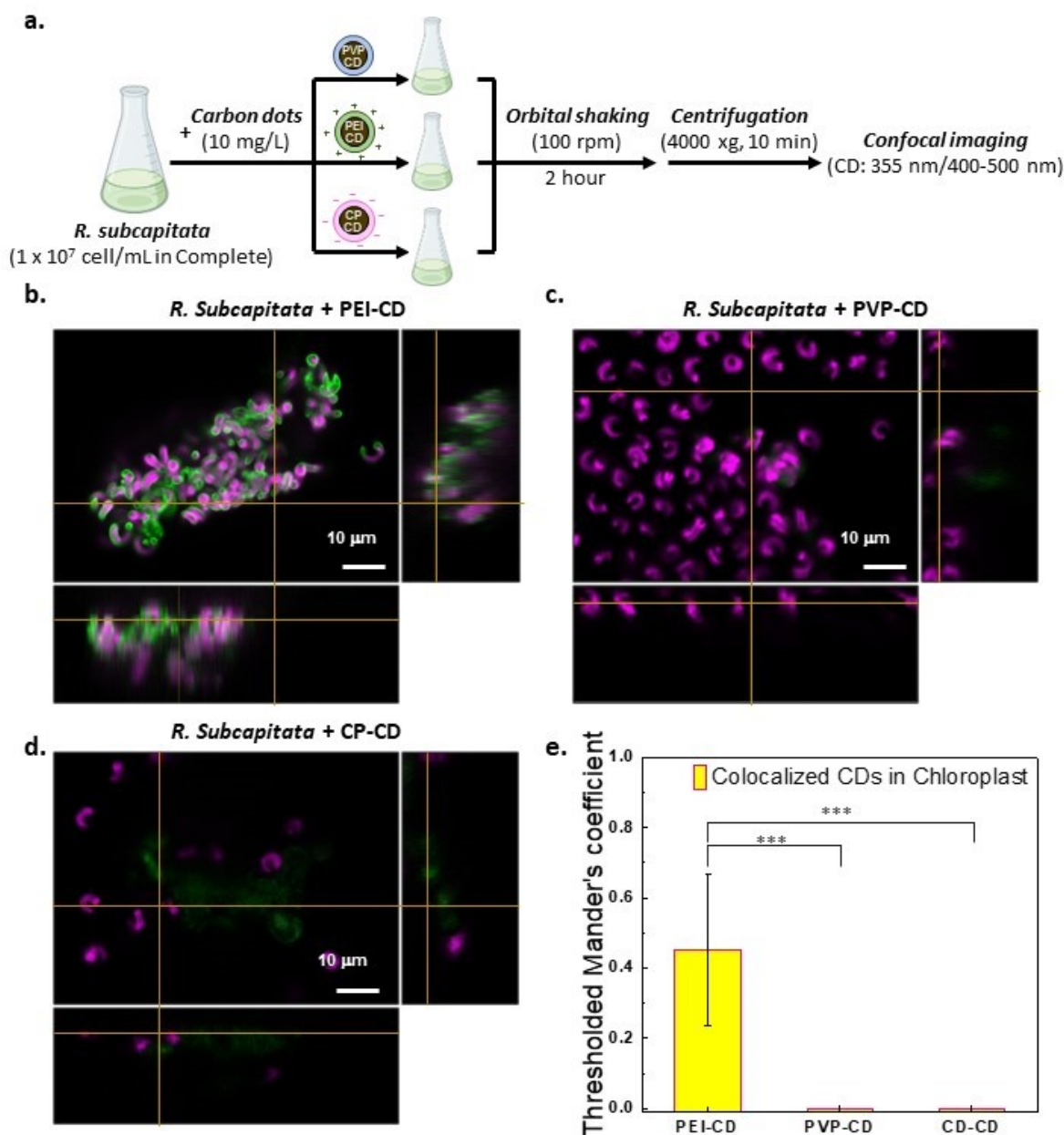
Figure S3. Carbon Dots and Algae Interaction Visualized and Quantified Using Confocal Microscopy. a. The process diagram for preparing *R. subcapitata* and carbon dots (CD) for confocal microscopy. **b.** Confocal microscopy images and orthogonal projections for R. subcapitata combined with PEI-CD, **c.** PVP-CD, and **d.** CP-CD. Microalgae samples were imaged by laser scanning confocal microscopy (X-880 , Carl Zeiss Microscopy, Germany) using a ×40 water objective (C-Apochromat 40x/1.2 W, Carl Zeiss Microscopy, Germany). Confocal imaging of CDs and chloroplast autofluorescence was conducted under 355 nm laser excitation for CDs and 514 nm for chloroplast (20% power). The emission detection ranges were configured as 400–500 nm and 650–760 nm, respectively.

The z-stack focal plane depth was established with a 2 μm interval. **e.** Analysis of the colocalized CDs within the chloroplast, determined through z-stack imaging calculations. The Thresholded Mander's coefficient (tM1) represents the ratio of overlapping pixels in the carbon dot channel (channel 1) with the chlorophyll channel (channel 2). The tM1 value is presented along with its p-value, established via one-way ANOVA (Analysis Of VAriance) with the Tukey test *** ($p<0.001$). *n=3-7*
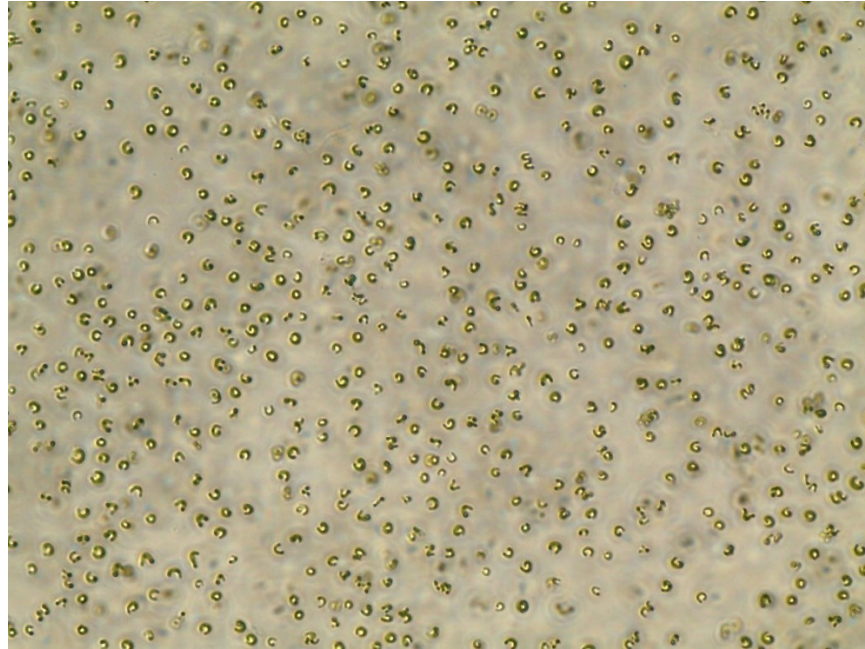

Figure S4. An example brightfield image of a dense, *R. subcapitata* culture not exposed to carbon dots. Image obtained using an EVOS XL Core Imaging System using 40X objective.
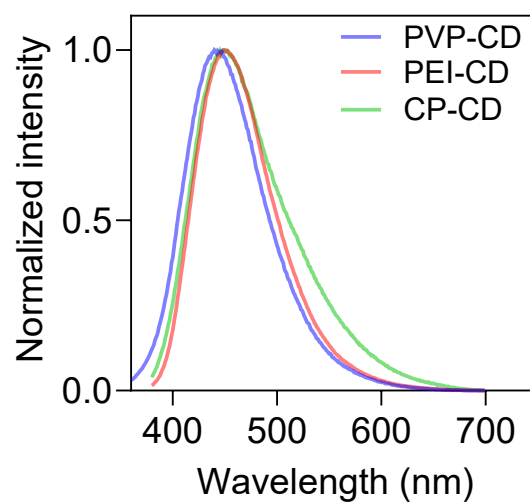
Figure S5. Fluorescence spectra of PVP-, PEI-, and CP-CD at the maximum emission wavelength of 355 nm, illustrating comparable fluorescence emission properties.