Supplementary Information (SI) for Chemical Science.
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Supporting Information

Vacuum Infiltration for Priming of Soybean Seeds: Optimization and Particle Tracking Using Fluorescent Silica Nanoparticles

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Total Protein Content

Total protein content of vacuum infiltration and seed priming suspensions was quantified using the Micro BCA Protein Assay Kit (Thermo Fisher Scientific) to determine the likelihood of nanoparticle-corona formation after seed infiltration. Varied numbers of seeds (6, 12, 15 and 20) were both vacuum infiltrated and seed primed with 30 mL of water and post-treatment solutions were collected. For RITC-UMN and APTES-modified RITC-UMN treatments (500 mg/L), 3 seeds were infiltrated or primed with 5 mL nanoparticle treatment and post-infiltration solutions were kept for analysis. The left-over infiltration and seed priming suspensions were mixed thoroughly with a Micro BCA working reagent in a 96-well plate. The plate was then incubated at 37 ºC for 2 hours. The absorbance was measured at 562 nm on a BioTek Synergy H1 microplate reader (Winooski, Vermont) and absorbance values were converted into concentration by using a standard curve.

Salt Infiltration Solution Germination

A rolled paper towel germination test was also used to evaluate the impact of salt solutions used for the ionic strength optimization on the seeds for both vacuum infiltration and seed priming. Ten seeds were vacuum infiltrated or primed with 200 mM K_2SO_4, KNO_3, $MgSO_4$, $Mg(NO_3)_2$ or 50 mM KNO_3 and germinated for a 9-day period; percent germination and biomass were measured every 3 days and radicle length was measured on day 9. The vacuum application appeared to benefit the germination rate as the 200 mM $Mg(NO_3)_2$ and 50 mM KNO_3 had higher germination percentages (40 % and 50%, respectively) compared to their non-vacuum counterparts (0% and 10% respectively), on day 2; although, they eventually all reached the same percentage on day 9. There were some trends for reductions in biomass and radicle length with vacuum treated samples on day 9; however, none were statistically significant impacts.

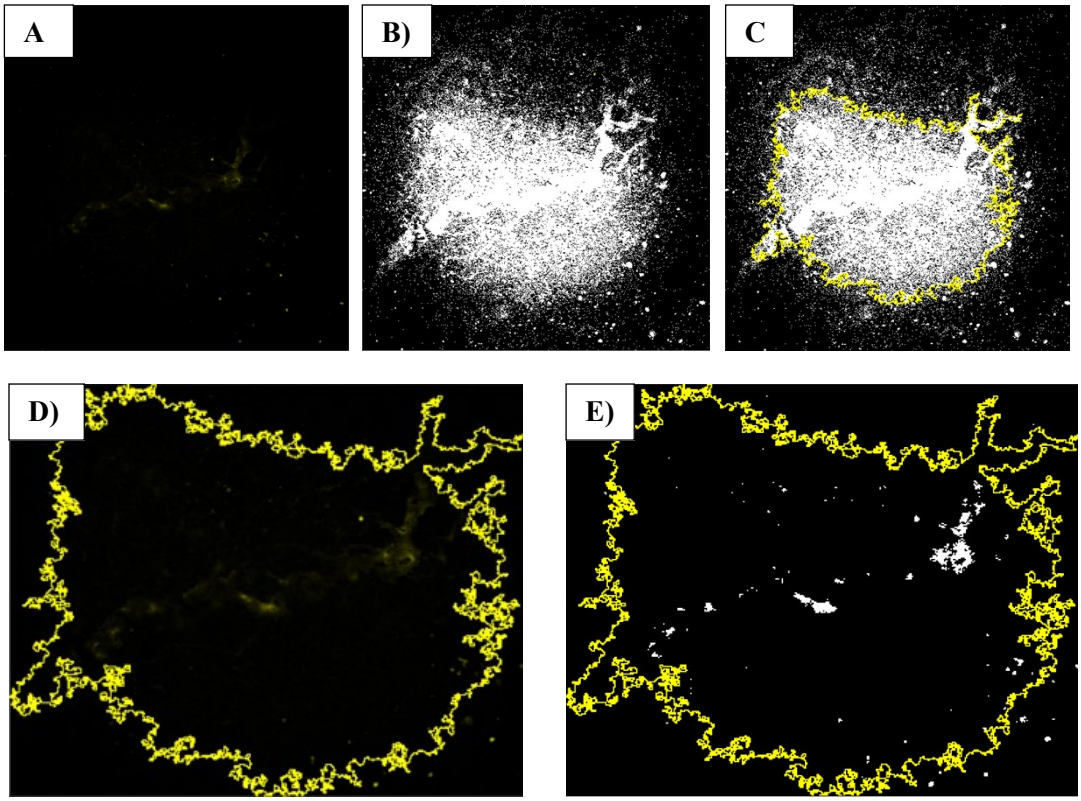


Figure S1: Image processing for analysis after confocal imaging. **A)** An unmixed image of a soybean seed infiltrated with 500 mg/L RITC-UMNs for 10 minutes. **B)** The image is thresholded with percentile thresholding. **C)** A region of interest (ROI) on the outside edge of the seed is located and outlined in yellow. **D)** The image is duplicated to focus on the ROI. **E)** The duplicated image is thresholded using moments thresholding, and a measurement of the average intensity within the ROI is recorded. Measurements for three seed replicates for each treatment were recorded.

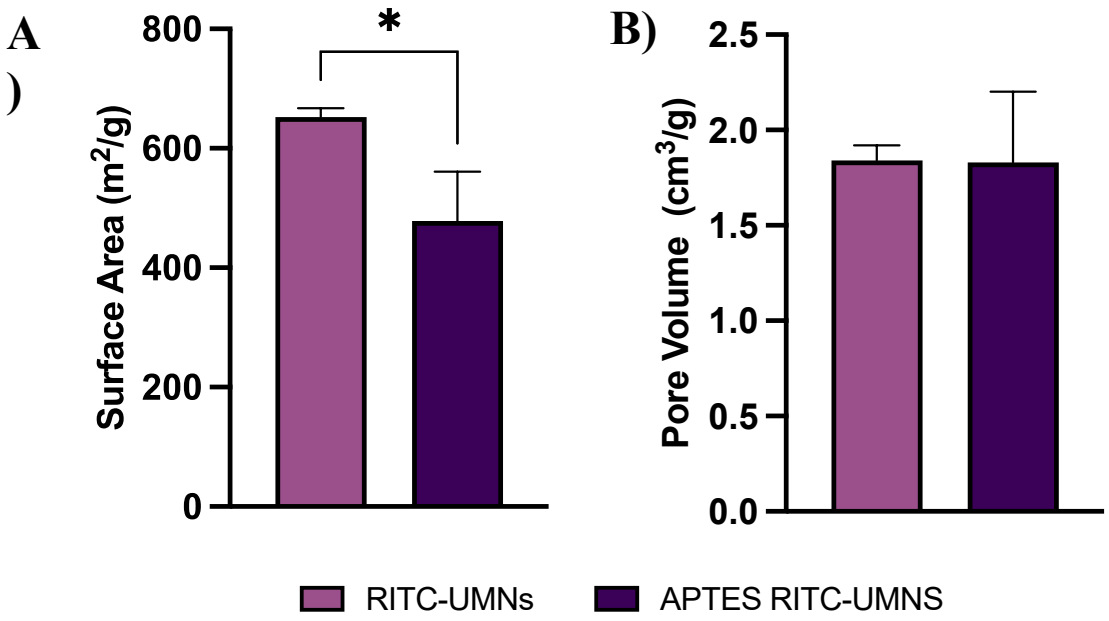


Figure S2: Nitrogen physisorption analysis of RITC-UMNs and APTES RITC-UMNs. Nitrogen physisorption characteristics with **A)** a decrease in the BJH surface area upon APTES modification, and **B)** no change in the BJH pore volume. Error bars represent the standard deviation of three material replicates. Unpaired *t*-tests were used to evaluate statistical significance (*$p < 0.05$).

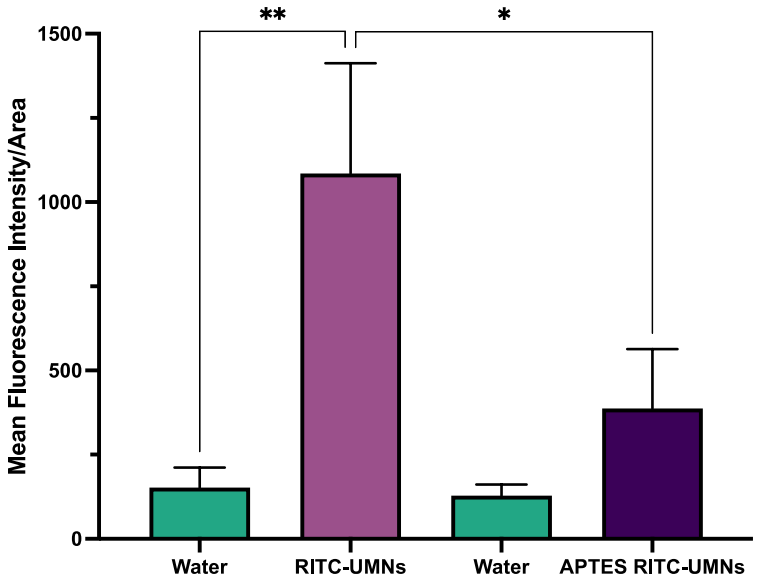


Figure S3: Fluorescence for the seed interior of RITC-UMN and APTES-modified RITC-UMN treatments. 500 mg/L and 30-minute seed samples were used to understand the correlation between the fluorescence observed on the exterior and interior of soybean seeds upon vacuum infiltration. Error bars represent the standard error of three seed replicates. A one-way ANOVA with Tukey's multiple comparisons test was used to evaluate statistical significance (**$p < 0.01$, *$p < 0.05$).

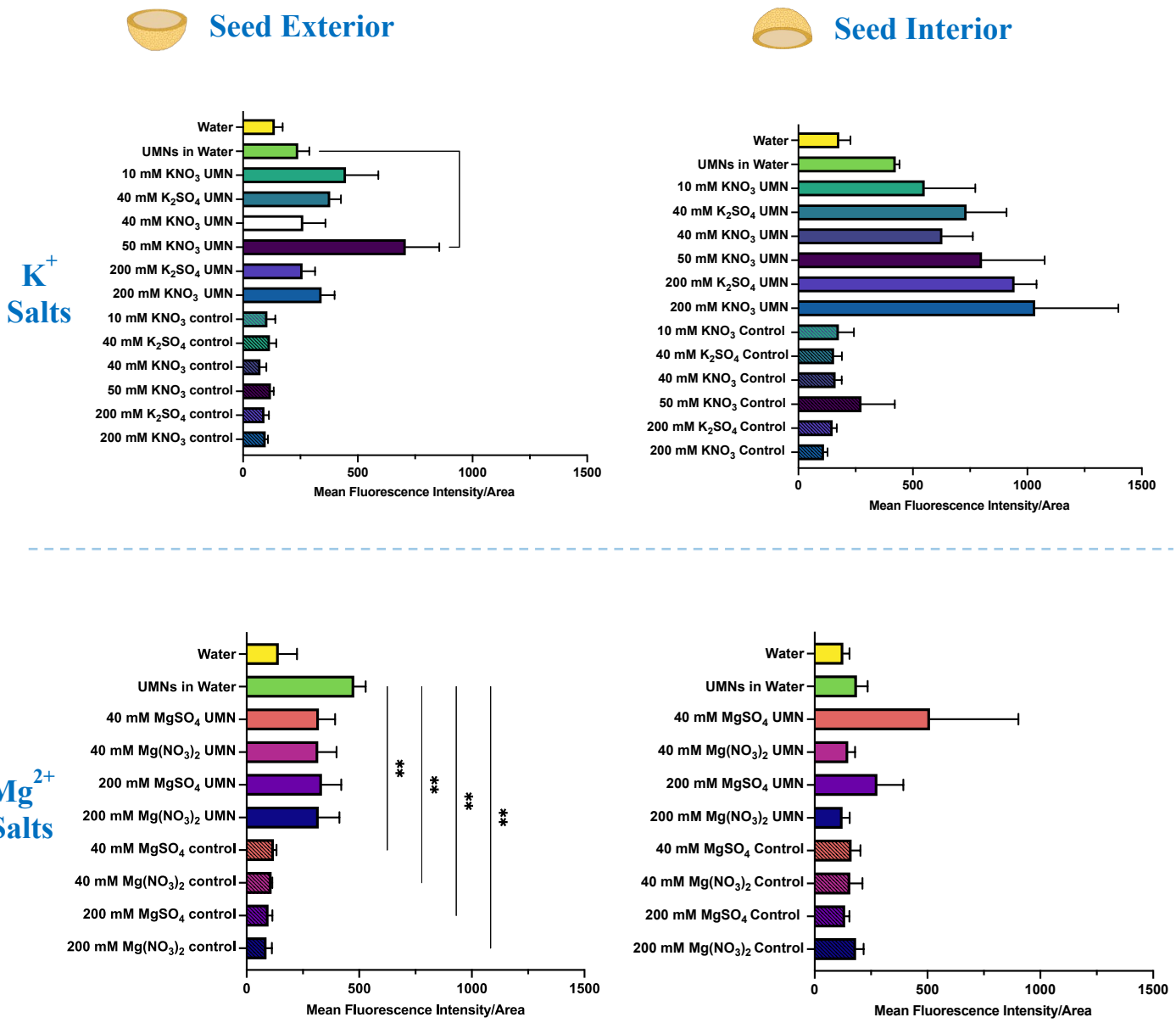


Figure S4: Fluorescent intensities of negatively-charged RITC-UMNs in K– and Mg–based salts as well as salt controls with no UMN treatment for both the seed exterior (left panel) and seed interior (right panel). 10 mM and 50 mM solutions were included for KNO_3 as concentration comparison. Error bars represent the standard error of three seed replicates. A one-way ANOVA with Dunnett's multiple comparisons test was used to evaluate statistical significance (***$p<0.001$, **$p < 0.01$).

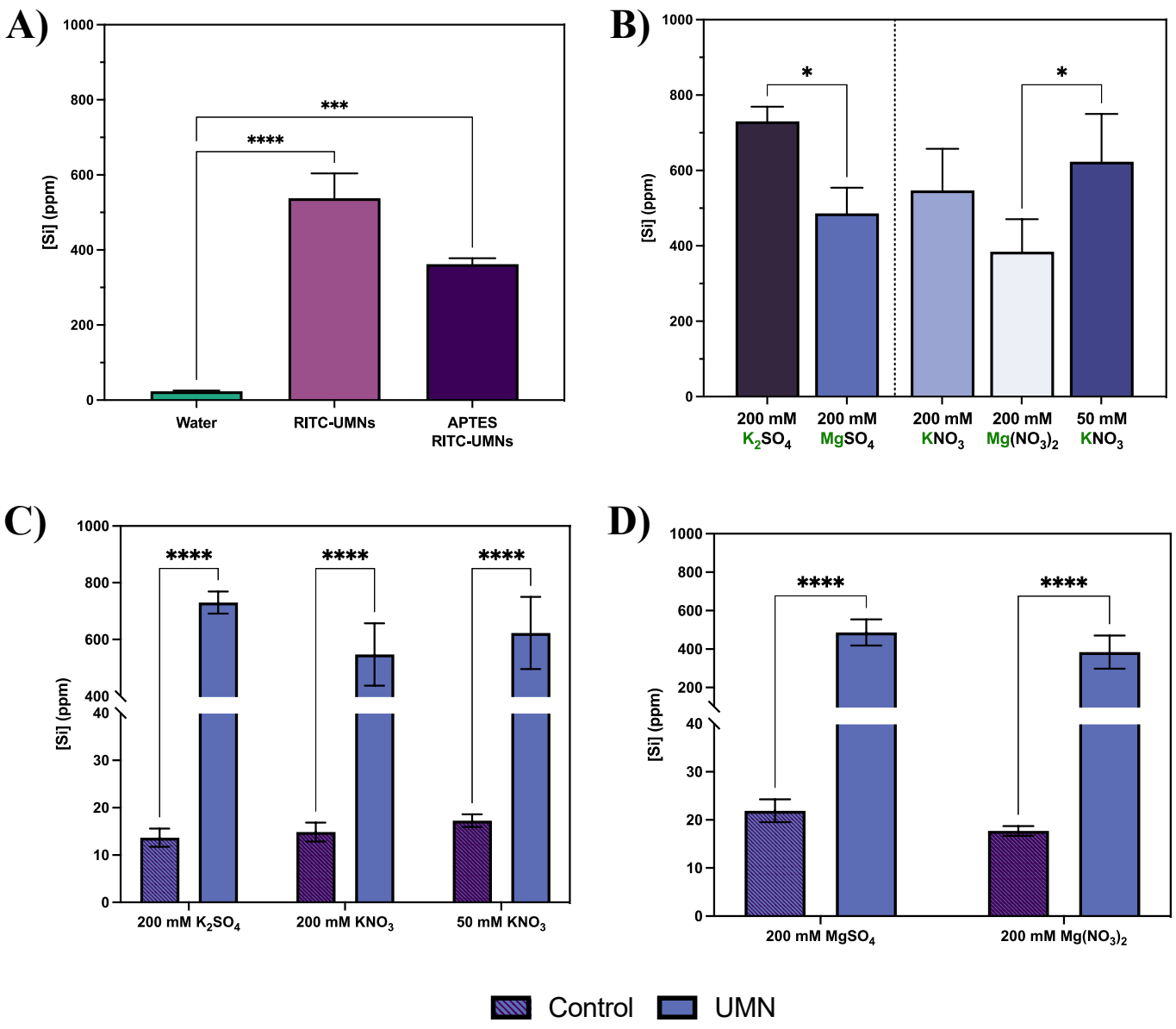


Figure S5: A) Silicon content in soybean seed coat for water, negatively-charged RITC-UMNs and positively-charged APTES RITC-UMNs. **B)** Silicon content in soybean seed coat for UMNs infiltrated with K– and Mg–based salt solutions at 200 mM and 50 mM ionic strength. Silicon content in soybean seed coats for control solutions with no UMN treatment and UMN treatments for **C)** K-based ionic salts and **D)** Mg-based ionic salts. Error bars represent the standard deviation of three replicates. A one-way ANOVA with Tukey's multiple comparisons test was used to evaluate statistical significance (*$p<0.05$, ***$p<0.001$, ****$p<0.0001$). Note: Figure S6c, d have segmented y axes to accurately represent differences in Si concentration between control and UMN treatments.

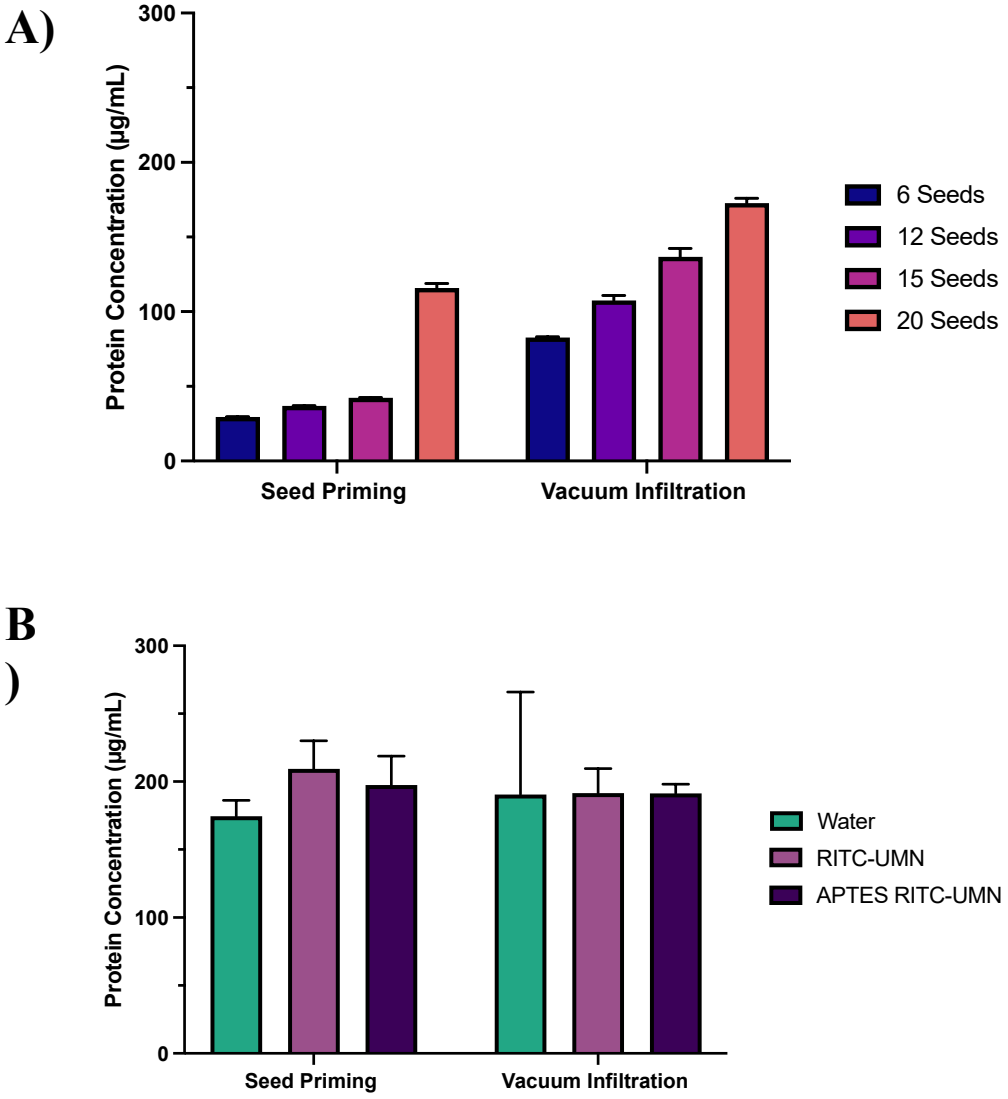


Figure S6: A) Total protein content of vacuum infiltration and seed priming solutions following 10-minute infiltrations with different amounts of seeds and no nanoparticles. **B)** Total protein content of vacuum infiltration and seed priming solutions after 10-minute infiltrations at 500 mg/L RITC-UMN concentration. Error bars represent standard deviation of three technical replicates for A) and three infiltration/seed priming replicates for B).

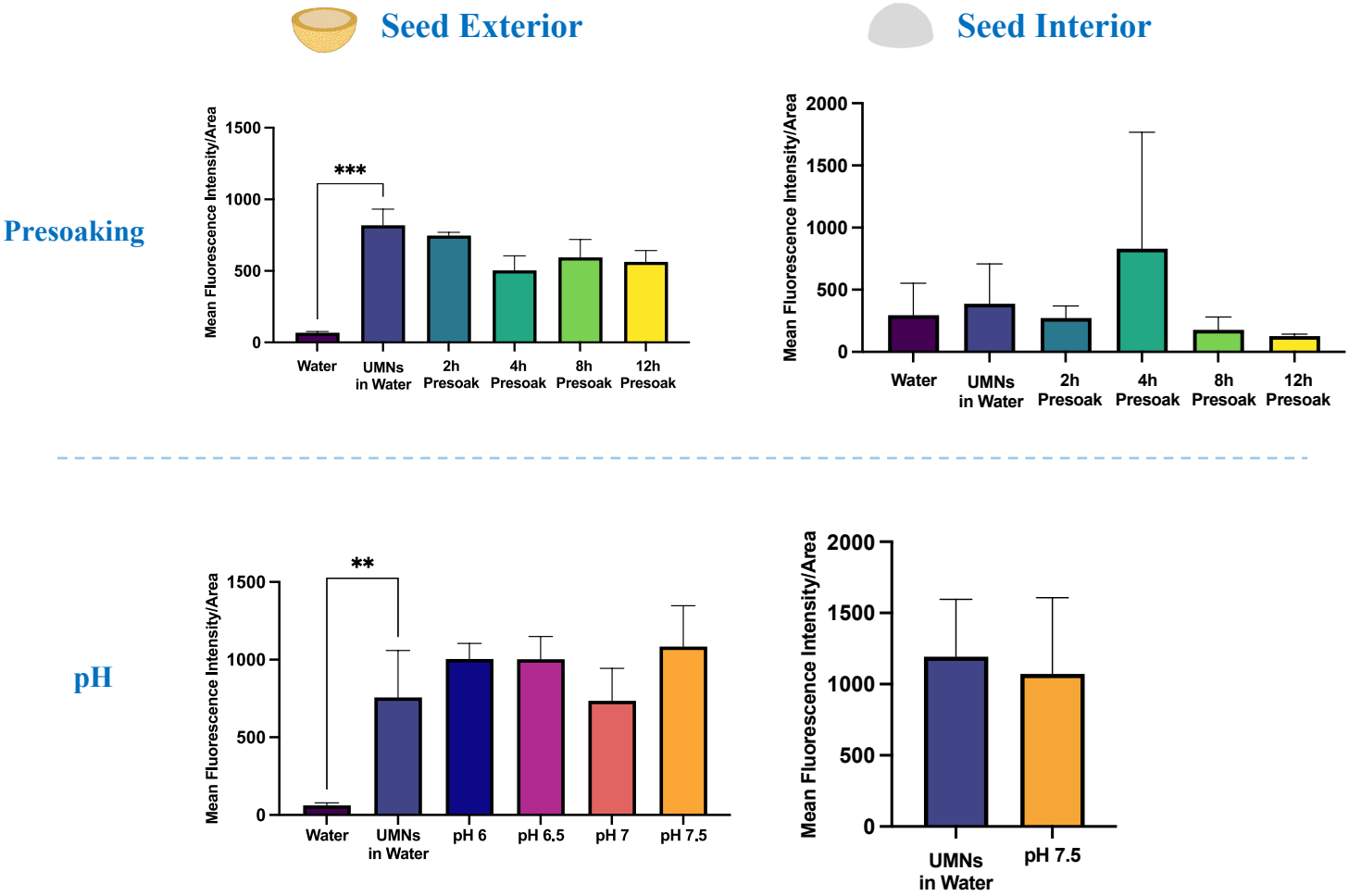
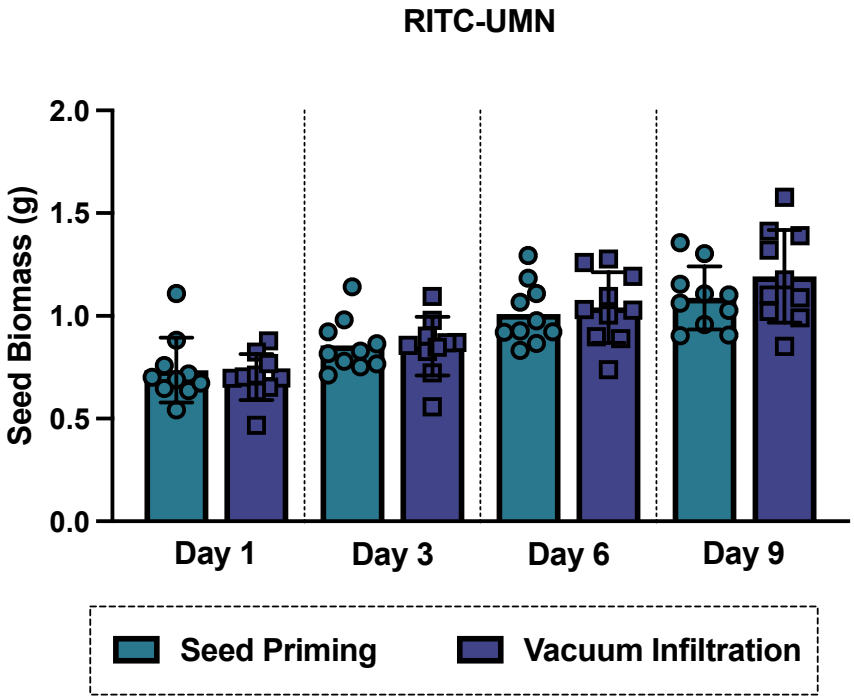


Figure S7: Fluorescent intensities of negatively-charged RITC-UMNs after presoaking seeds prior to infiltration and at different solution pHs for both seed exterior (left panel) and seed interior (right panel). Error bars represent standard error of three seed replicates. A one-way ANOVA with Dunnett's multiple comparisons test was used to evaluate statistical significance (***$p<0.001$, **$p < 0.01$).

A)



B
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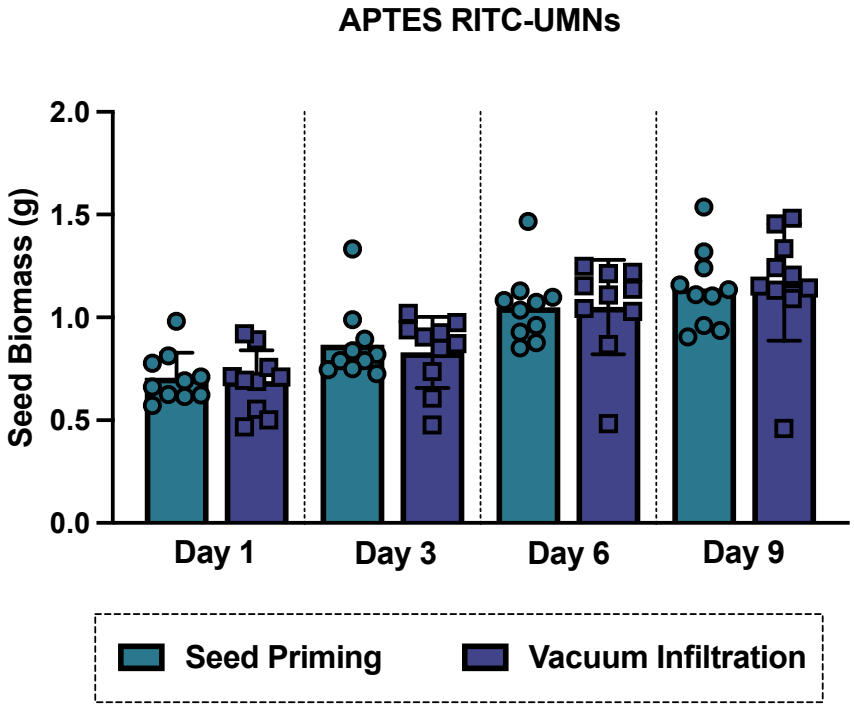

Figure S8: Soybean seed biomass monitored throughout germination test for **A)** RITC-UMNs and **B)** APTES RITC-UMNs following seed priming and vacuum infiltration. Error bars represent standard deviation of 10 seed replicates.

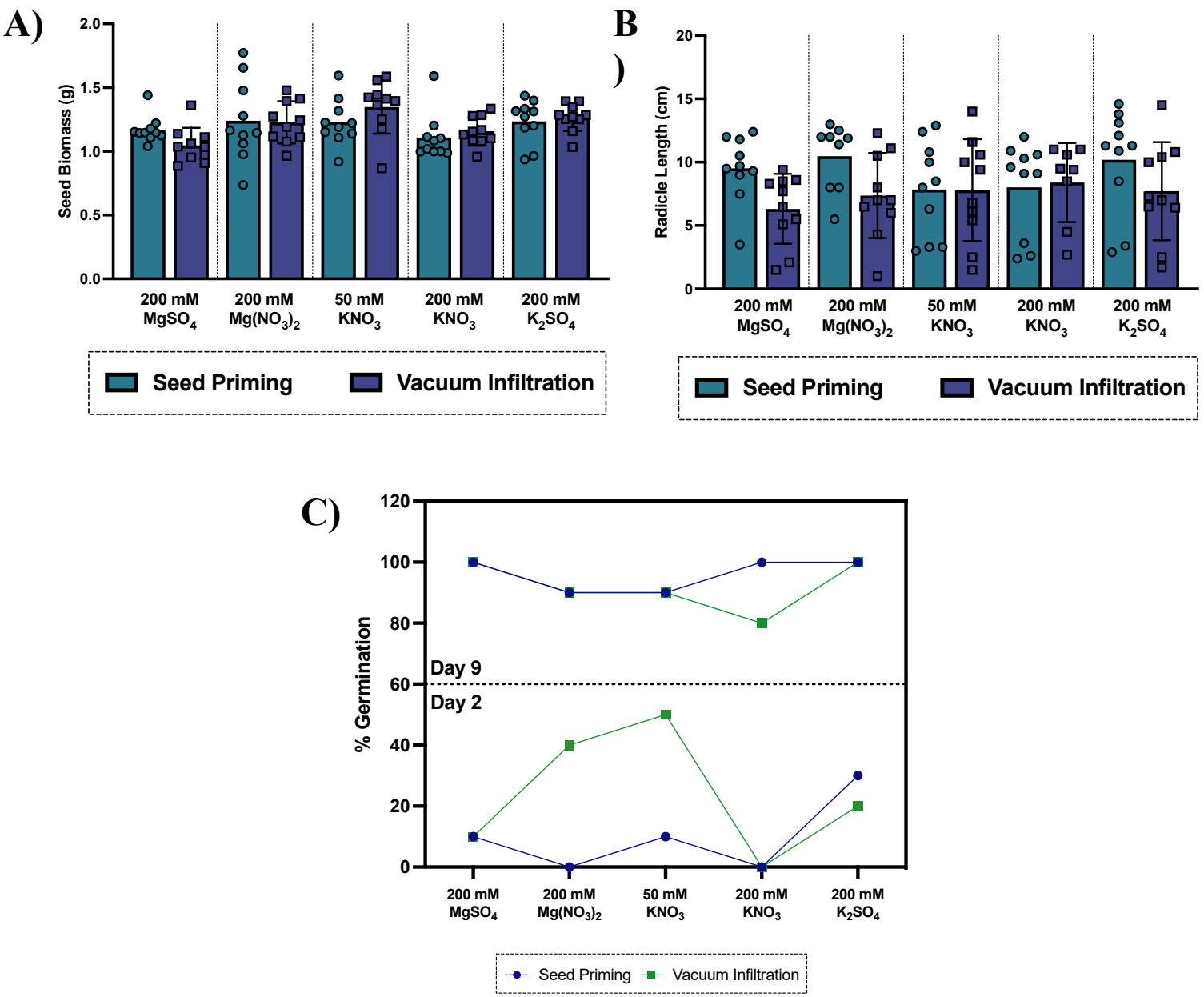

Figure S9: Comparison of **A)** seed biomass and **B)** radicle length for soybean seeds germinated with K^+ and Mg^{2+} salts for both seed priming and vacuum infiltration. **C)** Percent germination for day 2 (bottom) and day 9 (top graph) soybean seeds treated via seed priming and vacuum infiltration. A two-way ANOVA with Sidak's multiple comparisons test was used to evaluate statistical significance.