SUPPORTING INFORMATION APPENDIX

Soybean Susceptibility to Manufactured Nanomaterials with Evidence for Food Quality and Soil Fertility Interruption

John H. Priester[1,2,3], Yuan Ge[1,2,3], Randall E. Mielke[1,2,3,4], Allison M. Horst[1,2,3], Shelly Cole Moritz[2], Katherine Espinosa[5], Jeff Gelb[6], Sharon L. Walker[7], Roger M. Nisbet[2,3,8], Youn-Joo An[9], Joshua P. Schimel[2,3,8], Reid G. Palmer[5,10], Jose A. Hernandez-Viezcas[3,11], Lijuan Zhao[3,11], Jorge L. Gardea-Torresdey[3,11], Patricia A. Holden[*1,2,3]

[1] Bren School of Environmental Science and Management, University of California, Santa Barbara, CA, USA
[2] Earth Research Institute, University of California, Santa Barbara, CA, USA
[3] UC Center for the Environmental Implications of Nanotechnology (UC CEIN), University of California, Santa Barbara, CA, USA
[4] Jet Propulsion Laboratory, California Institute of Technology – NASA, Planetary Science, Pasadena, CA, USA
[5] Department of Agronomy, Iowa State University, Ames, IA, USA
[6]Xradia Corporation, Pleasanton, CA, USA
[7]Department of Chemical & Environmental Engineering, University of California, Riverside, CA, USA
[8]Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA, USA
[9]Department of Environmental Science, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143–701, Korea
[10]Corn Insects and Crop Genetics Research, USDA-ARS, Ames, IA, USA
[11] Department of Chemistry, The University of Texas at El Paso, El Paso, TX, USA

* Corresponding author

Table of Contents

Table S1: Soybean Plant Stem Elongation Rate Constants and Maximum Stem Length According to Treatment[*]				
Treatment	**Rate Constant (d^{-1})**	**Time Period (d)**	**R^2 Range**	**Max. Length (cm)**
Control	0.057 ± 0.004[b]	1 – 36	0.98 – 0.99	33.3 ± 1.3[b]
Low nano-CeO_2	0.047 ± 0.002[a,c]	1 – 43	0.93 – 0.94	24.1 ± 4.1[a,c]
Med nano-CeO_2	0.058 ± 0.004[b]	1 – 29	0.90 – 0.99	27.8 ± 3.1[b,c]
High nano-CeO_2	0.055 ± 0.003[b]	1 – 36	0.95 – 0.98	30.9 ± 1.0[b,c]
Low nano-ZnO	0.061 ± 0.004[b]	1 – 22	0.96 – 0.99	33.8 ± 3.0[b,c]
Med nano-ZnO	0.055 ± 0.005[b,c]	8 – 36	0.86 – 0.96	29.2 ± 2.7[b,c]
High nano-ZnO	0.060 ± 0.002[b]	1 – 36	0.96 – 0.98	34.1 ± 2.7[b]

[*]n = 4, except for the low nano-ZnO treatment where n = 3; like letters apply within a column and indicate no significant difference (P > 0.05) within plant part.

Table S2: Rate Constants for Soybean Plant Leaf Cover (% Covering Soil From Aerial Photos) and Maximum Leaf Cover (% Cover), According to Treatment[*]				
Treatment	**Rate Constant (d^{-1})**	**Time Period (d)**	**R^2 Range**	**Max. Area (% cover)[**]**
Control	0.128 ± 0.014[b]	1 – 29	0.88 – 0.94	257.6 ± 26.9[a]
Low nano-CeO_2	0.083 ± 0.013[a,c]	1 – 43	0.58 – 0.99	175.1 ± 40.4[a]
Med nano-CeO_2	0.120 ± 0.007[b]	1 – 29	0.78 – 0.96	236.9 ± 35.3[a]
High nano-CeO_2	0.132 ± 0.030[b,c]	1 – 29	0.77 – 0.99	260.4 ± 30.7[a]
Low nano-ZnO	0.117 ± 0.016[b,c]	1 – 29	0.85 – 0.92	222.7 ± 26.6[a]
Med nano-ZnO	0.124 ± 0.015[b]	1 – 29	0.82 – 0.97	232.9 ± 33.2[a]
High nano-ZnO	0.130 ± 0.023[b,c]	1 – 29	0.93 – 0.94	232.7 ± 20.2[a]

[*]n = 4, except for the low nano-ZnO treatment where n = 3; like letters apply within a column and indicate no significant difference ($P > 0.05$) within plant part.
[**]Maximum value from the time course shown in Fig. S1.

Table S3: Soybean Pods Per Plant, Seeds Per Pod, Pod Dimensions, and Pod Aspect, According to Treatment[*]					
Trmt	**Pods per Plant**	**Seeds per Pod**	**Pod Length (cm)**	**Pod Width (cm)**	**Ratio: Pod Length to Width**
Control	17.0 ± 1.5[a,b]	1.6 ± 0.1[a,b]	2.7 ± 0.2[a,b]	0.8 ± 0.1[a,b]	3.6 ± 0.1[a]
Low nano-CeO_2	10.3 ± 4.1[a]	1.9 ± 0.1[a,b]	3.2 ± 0.3[b]	0.9 ± 0.1[a,b]	3.5 ± 0.1[a]
Med nano-CeO_2	15.3 ± 3.4[a,b]	1.7 ± 0.1[b]	2.9 ± 0.2[a,b]	0.9 ± 0.1[a,b]	3.6 ± 0.1[a]
High nano-CeO_2	16.8 ±2.6[a,b]	1.7 ± 0.1[a]	2.6 ± 0.2[a]	0.8 ± 0.1[a]	3.7 ± 0.1[a]
Low nano-ZnO	13.0 ± 1.5[a]	1.7 ± 0.3[a]	2.8 ± 0.3[a,b]	0.8 ± 0.1[a,b]	3.5 ± 0.1[a]
Med nano-ZnO	12.8 ± 1.9[a]	1.7 ± 0.1[a,b]	3.0 ± 0.2[a,b]	0.9 ± 0.1[b]	3.5 ± 0.1[a]
High nano-ZnO	20.0 ± 2.9[b]	1.7 ± 0.1[a,b]	2.7 ± 0.2[a,b]	0.8 ± 0.1[a,b]	3.6 ± 0.1[a]

[*]n = 4, except for the low nano-ZnO treatment where n = 3; like letters apply within a column and indicate no significant difference (P > 0.05) within plant part.

Table S4: Moisture Content (g H_2O / g dry biomass) of Soybean Plant Parts According to Treatment[*]					
Trmt	**Stem**	**Leaf**	**Pod**	**Root**	**Nodule**
Control	0.68 ± 0.01[a,b]	0.72 ± 0.00[d]	0.80 ± 0.01[b,c,d]	0.87 ± 0.01[d]	0.80 ± 0.01[a]
Low nano-CeO_2	0.74 ± 0.04[a,b,c,d]	0.72 ± 0.01[d]	0.79 ± 0.00[b]	0.85 ± 0.01[c,d]	0.80 ± 0.02[a]
Med nano-CeO_2	0.69 ± 0.01[b]	0.71 ± 0.01[d]	0.82 ± 0.00[c]	0.83 ± 0.01[b,c]	0.81 ± 0.02[a]
High nano-CeO_2	0.68 ± 0.01[a,b,c,d]	0.68 ± 0.01[a,b,c]	0.79 ± 0.00[b]	0.83 ± 0.01[c]	0.80 ± 0.01[a]
Low nano-ZnO	0.66 ± 0.01[a,d]	0.68 ± 0.00[b,c]	0.77 ± 0.00[a]	0.71 ± 0.10[a,b,d]	0.80 ± 0.01[a]
Med nano-ZnO	0.67 ± 0.01[b,c,d]	0.67 ± 0.01[a,c]	0.77 ± 0.00[a]	0.76 ± 0.02[a]	0.77 ± 0.02[a]
High nano-ZnO	0.66 ± 0.00[c,d]	0.67 ± 0.01[a]	0.77 ± 0.00[a]	0.78 ± 0.01[a]	0.77 ± 0.01[a]

[*]n = 4, except for the low nano-ZnO treatment where n = 3; like letters apply within a column and indicate no significant difference (P > 0.05) within plant part.

Table S5: Soybean Plant Part Dry Biomass and Total Dry Biomass (g / plant), According to Treatment[*]						
Trmt	**Stem**	**Leaf**	**Pod**	**Root**	**Nodule**	**Total[**]**
Control	0.90 ± 0.09[a,b]	2.27 ± 0.16[a]	1.82 ± 0.18[b,c]	0.83 ± 0.09[a]	0.19 ± 0.00[a]	6.01 ± 0.32[a,b]
Low nano-CeO_2	0.54 ± 0.21[a]	1.59 ± 0.39[a]	1.44 ± 0.28[a,c]	0.69 ± 0.08[a]	0.14 ± 0.04[a]	4.40 ± 0.96[a]
Med nano-CeO_2	0.80 ± 0.16[a,b]	2.21 ± 0.32[a]	1.52 ± 0.14[a,b]	0.95 ± 0.11[a,b]	0.24 ± 0.05[a,b]	5.72 ± 0.67[a,b]
High nano-CeO_2	0.97 ± 0.13[a,b]	2.43 ± 0.22[a]	1.41 ± 0.09[a]	0.91 ± 0.14[a,b]	0.19 ± 0.03[a,b]	5.90 ± 0.58[a,b]
Low nano-ZnO	0.87 ± 0.10[a,b]	2.06 ± 0.28[a]	1.85 ± 0.17[b,c]	1.23 ± 0.38[a,b]	0.18 ± 0.06[a,b]	6.20 ± 0.76[a,b]
Med nano-ZnO	0.90 ± 0.21[a,b]	2.33 ± 0.44[a]	1.71 ± 0.10[b,c]	1.01 ± 0.14[a,b]	0.26 ± 0.07[a,b]	6.22 ± 0.82[a,b]
High nano-ZnO	1.03 ± 0.06[b]	2.40 ± 0.08[a]	1.95 ± 0.17[c]	1.11 ± 0.05[b]	0.25 ± 0.02[b]	6.74 ± 0.20[b]

[*] n = 4, except for the low nano-ZnO treatment where n = 3; like letters apply within a column and indicate no significant difference ($P > 0.05$) within plant part.
[**] Total plant dry biomass.

Table S6: N_2 Fixation Potential Normalized to Root Nodule Dry Biomass*			
Trmt	**Zero Order Rate (1E8 moles ethylene min^{-1} g^{-1})**	**Points****	**Avg. R^2**
Control	1.97 ± 0.99[b]	5	0.96
Low nano-CeO_2	2.87 ± 0.92[b]	4 to 5	0.96
Med nano-CeO_2	0.39 ± 0.09[a]	5	0.99
High nano-CeO_2	0.28 ± 0.16[a]	4 to 5	0.93
Low nano-ZnO	1.91 ± 1.16[b]	5	0.83[#]
Med nano-ZnO	2.55 ± 1.16[b]	5	0.97
High nano-ZnO	0.99 ± 0.58[b]	5	0.98

*n = 4, except for the low nano-ZnO treatment where n = 3; like letters apply within a column and indicate no significant difference (P > 0.05) within plant part.
**number of points used in regression, including a t=0 point of 0.0 moles ethylene.
#poor fit to the zero-order (linear) model because, as indicated by a superior fit to a first-order model (exponential), either the substrate or enzyme were not saturating.

Table S7: Concentration of Zinc in Various Plant Parts Harvested from the Ceria Treatments (mg Zn kg^{-1} dry tissue)*					
Trmt	**Root**	**Nodule**	**Stem**	**Leaf**	**Pod**
Control	31.61 ± 2.12[a]	19.68 ± 3.59[a]	19.48 ± 1.46[a]	85.59 ± 7.59[a]	32.04 ± 2.83[a]
Low nano-CeO_2	27.72 ± 1.74[a]	25.24 ± 2.57[a]	18.12 ± 2.90[a]	88.51 ± 8.54[a]	34.60 ± 15.38[a]
Med nano-CeO_2	31.45 ± 2.37[a]	25.07 ± 3.47[a]	20.47 ± 1.12[a,b]	98.33 ± 8.94[a]	40.22 ± 9.79[a]
High nano-CeO_2	32.72 ± 3.23[a]	32.59 ± 5.88[a]	27.25 ± 3.14[b]	102.22 ± 16.31[a]	52.12 ± 15.20[a]

*n = 4 individual plants; like letters apply within a column and indicate no significant difference (P > 0.05) within plant part.

Table S8: Characteristics of soil used in this study		
Characteristic	**Mean***	**SE***
Saturation % (SP)	28.00	NA**
pH	6.78	0.01
Sand (%)	66.00	0.00
Silt (%)	22.00	0.00
Clay (%)	12.00	0.00
Estimated Soluble Salts (EC) (dS m^{-1})	0.58	0.00
Cation Exchange Capacity (CEC) (meq per 100g)	8.72	0.09
B, saturated paste extract (meq L^{-1})	0.25	0.00
Ca, saturated paste extract (meq L^{-1})	2.43	0.01
Ca, exchangeable (meq per 100 g)	6.30	0.03
Cl, saturated paste extract (meq L^{-1})	0.50	0.01
Cu, DTPA extraction (ppm)	6.50	0.10
Cu, total (ppm)	32.00	0.00
Fe, DTPA extraction (ppm)	27.10	0.30
Fe, total (ppm)	12700.00	0.00
K, exchangeable (ppm)	290.00	16.00
K, exchangeable (meq per 100 g)	0.74	0.04
Mg, saturated paste extract (meq L^{-1})	1.04	0.02
Mg, exchangeable (meq per 100 g)	1.55	0.01
Mn, DTPA extraction (ppm)	6.75	0.05
Mn, total (ppm)	314.50	1.50
Na, saturated paste extract (meq L^{-1})	1.34	0.01
Na, exchangeable (ppm)	29.00	1.00
Na, exchangeable (meq per 100 g)	0.13	0.01
P, extractable (ppm)	51.30	3.00
Zn, DTPA extraction (ppm)	9.35	0.05
Zn, total (ppm)	84.00	0.00
HCO_3^-, saturated paste extract (meq L^{-1})	1.00	0.00
CO_3^{2-}, saturated paste extract (meq L^{-1})	<0.1	NA
Total C (%)	0.71	0.00
Organic Matter, loss on ignition (LOI) (%)	1.44	0.04
Total N (%)	0.07	0.00
NH_4^+, extractable (ppm)	0.23	0.02
NO_3^-, extractable (ppm)	7.85	0.10

* Each sample (except saturation % and CO_3) was measured in duplicate. The Means and standard errors of the duplicates are shown.

** NA = not available; a single measurement was made.

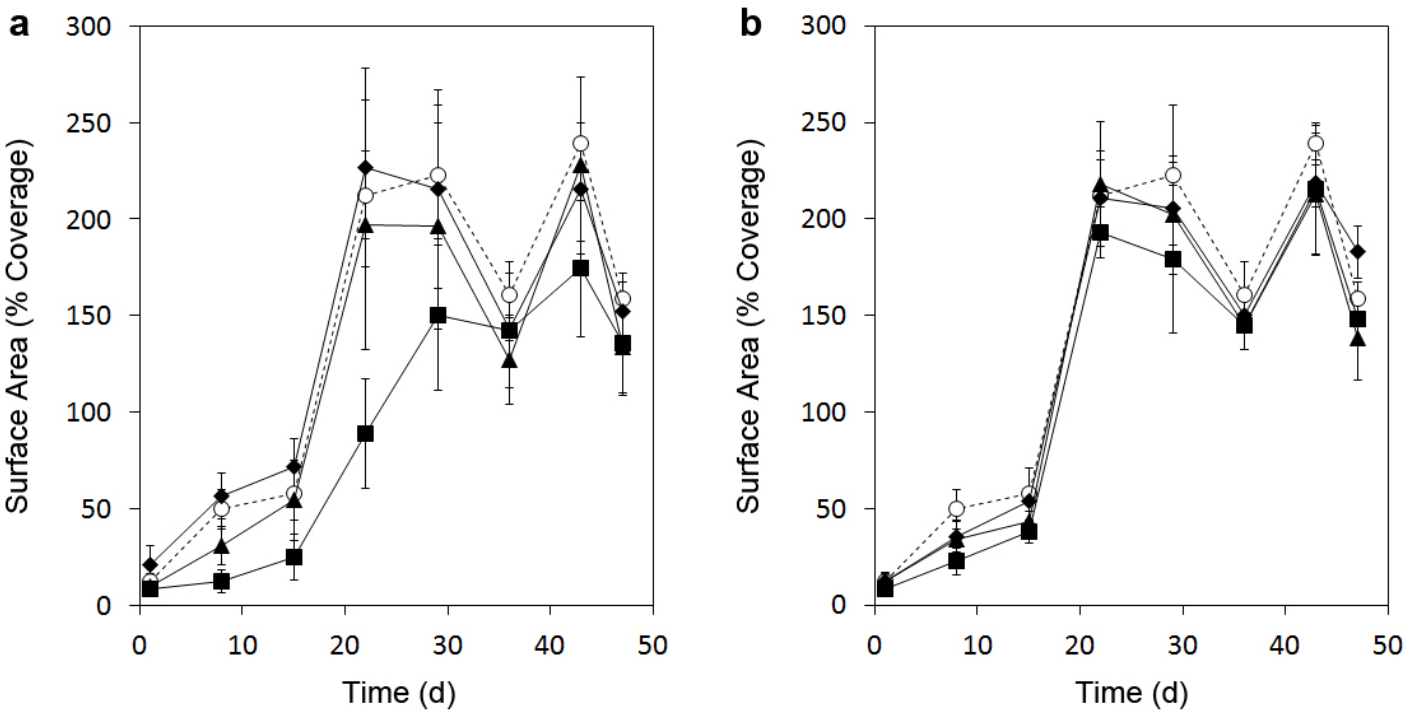

Figure S1. Time course of soybean leaf cover, expressed as a percent coverage projected onto pot soil surface area. a. Leaf cover versus time for control (open symbols), low nano-CeO_2 (squares), medium nano-CeO_2 (triangles) and high nano-CeO_2 (diamonds) treatments. **b**. Leaf cover versus time for control (open symbols), low nano-ZnO (squares), medium nano-ZnO (triangles) and high nano-ZnO (diamonds) treatments. Error bars represent the standard error of the mean (n= 4 plants, except Zn low where n=3 plants).

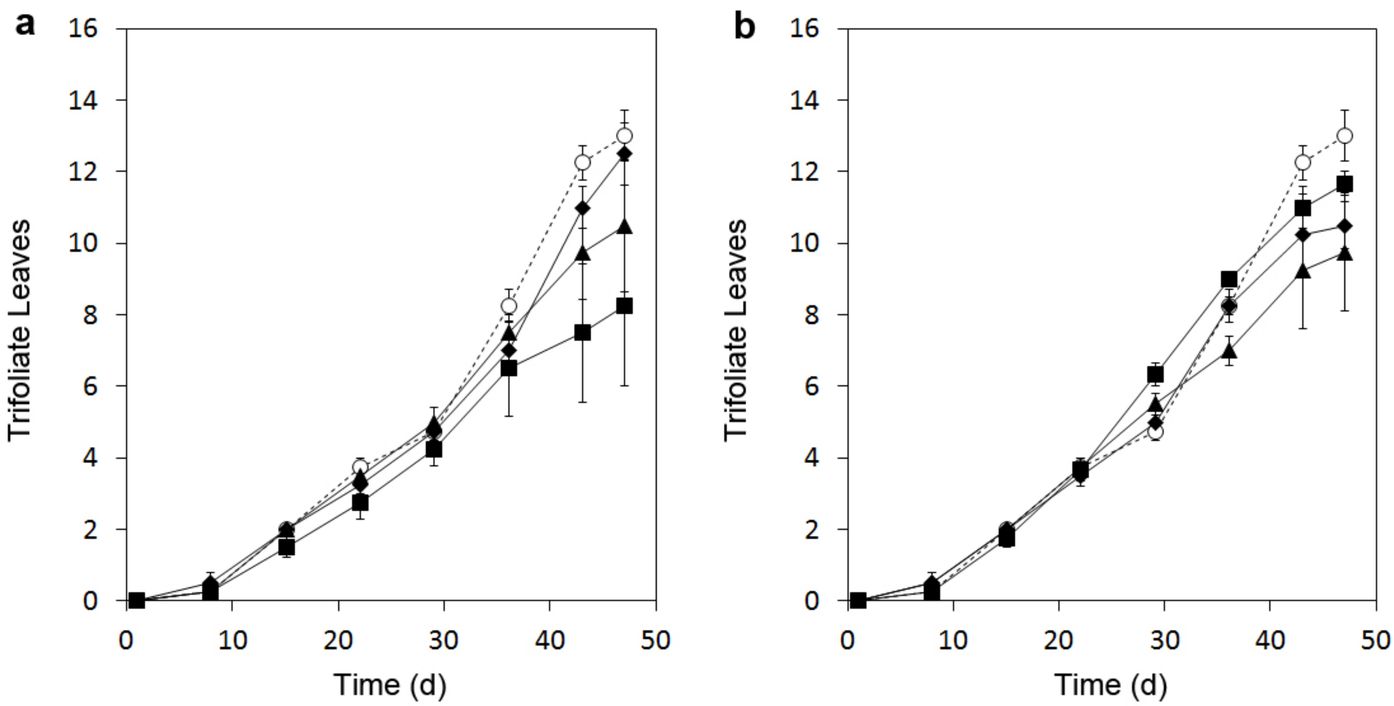

Figure S2. Time course of soybean vegetative developmental stage. a. Vegetative developmental stage (as determined by trifoliate leaf count) versus time for control (open symbols), low nano-CeO_2 (squares), medium nano-CeO_2 (triangles) and high nano-CeO_2 (diamonds) treatments. **b.** Vegetative developmental stage versus time for control (open symbols), low nano-ZnO (squares), medium nano-ZnO (triangles) and high nano-ZnO (diamonds) treatments. Error bars represent the standard error of the mean (n= 4 plants, except Zn low where n=3 plants).

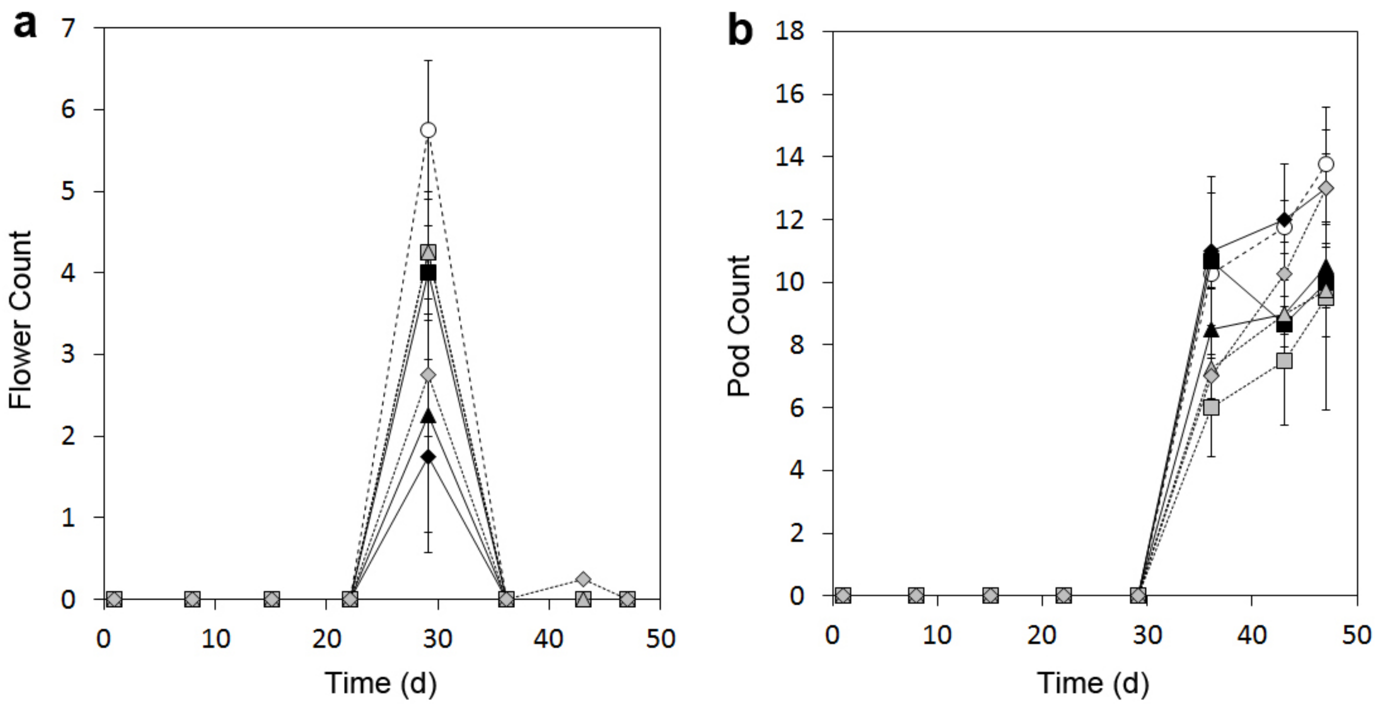

Figure S3. Soybean reproductive developmental stage versus time. a. Reproductive developmental stage (as determined by flower count) versus time for control (open symbols), nano-CeO_2 (grey symbols) and nano-ZnO (black symbols) treatments. Low, medium and high nanoparticle concentrations are represented by squares, triangles and diamonds, respectively. **b.** Reproductive developmental stage (as determined by pod count) versus time for control (open symbols), nano-CeO_2 (grey symbols) and nano-ZnO (black symbols) treatments. Low, medium and high nanoparticle concentrations are represented by squares, triangles and diamonds, respectively. Error bars represent the standard error of the mean (n= 4 plants, except low nano-ZnO where n=3 plants).

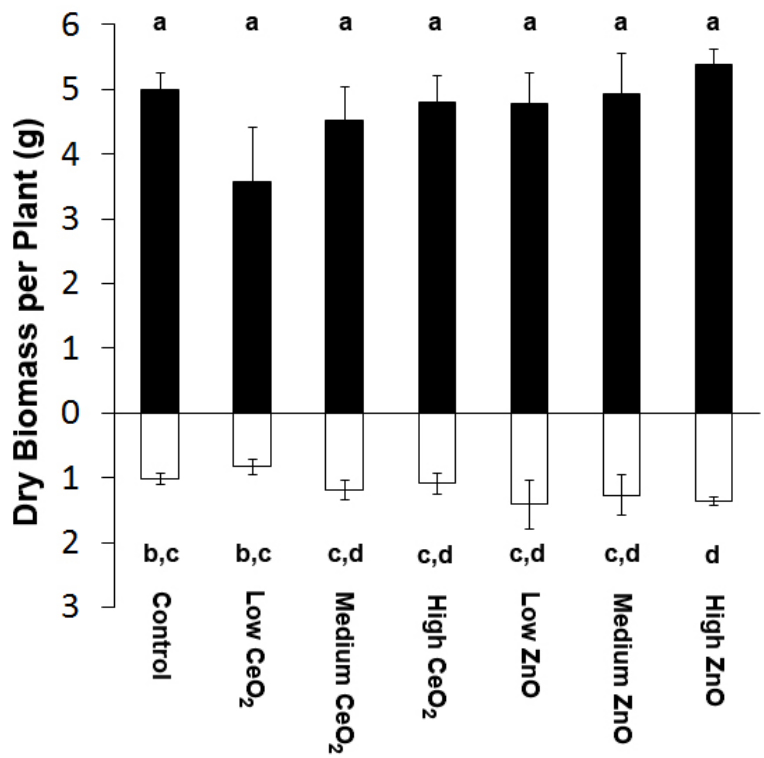

Figure S4. Soybean plant dry biomass. Total above- (black bars) and below- (white bars) ground dry biomass. Like letters indicate differences that are *not* significant (T-test; $P > 0.05$).

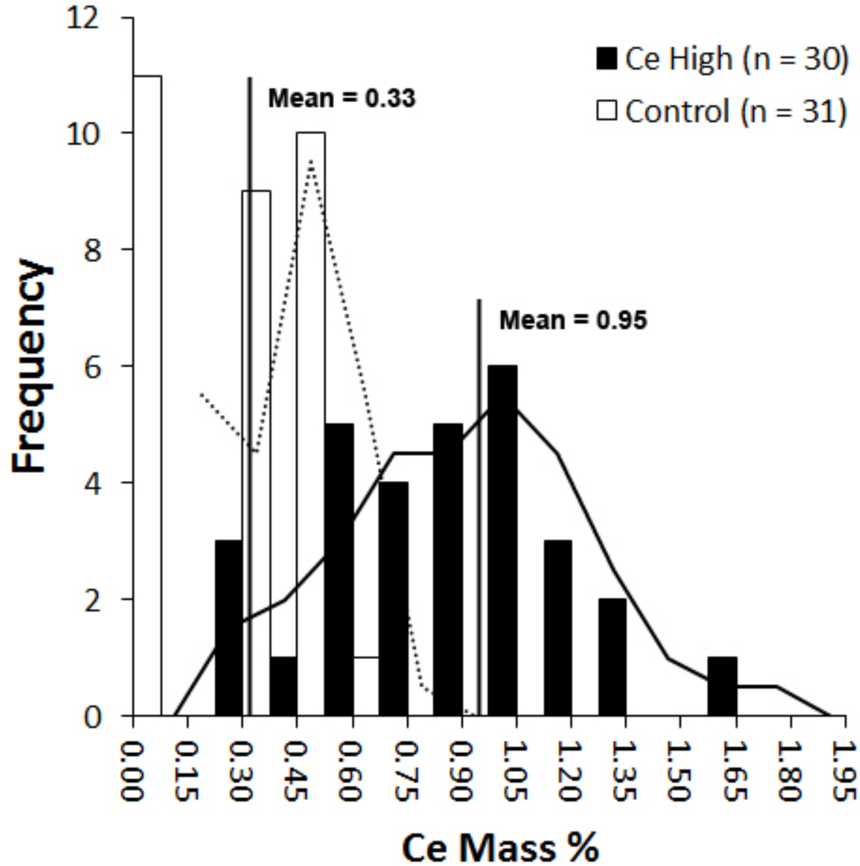

Figure S5. Frequency distribution of Ce atomic mass % measurements for soybean root nodules. Ce mass percent was measured by energy dispersive spectrometry (EDS) for soybean root nodules (Figure 2). The displayed means were calculated using a histogram with a bin width of 0.15. The arithmetic means (*not* calculated from the above graph) for the high nano-CeO_2, and Control treatments were 0.81 ± 0.06 and 0.21 ± 0.03 Ce mass %, respectively. The arithmetic means were significantly different (T – Test; $P < 0.05$) for all treatments, including between the control and high nano-CeO_2 shown here. Measurement numbers in parentheses in the legend are for individual EDS scans for several (3 or more) individual thin sections prepared from a single specimen.

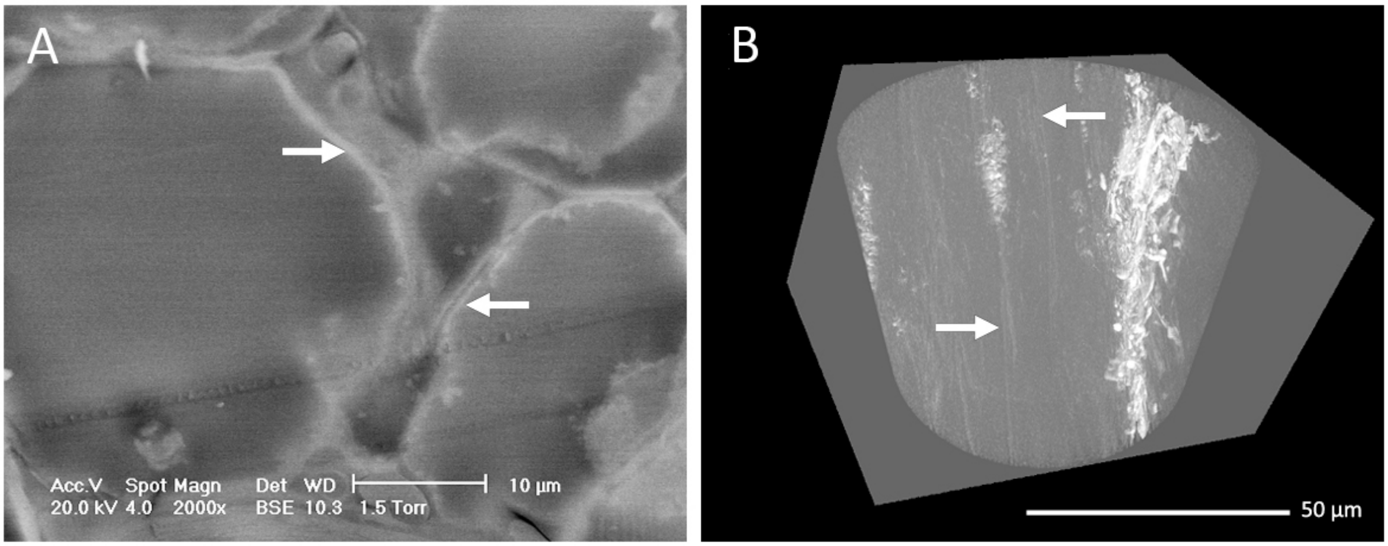

Figure S6. Micrographs of Ce accumulations in soybean root nodules. a. Backscatter ESEM micrograph of a root nodule from the high nano-CeO_2 treatment. Arrows show regions of increased electron density as indicated by brightness. **b.** X-ray microscopy (XRM) image of a root nodule from the high nano-CeO_2 treatment. Arrows indicate regions of increased electron density, which correspond to those shown in (a).

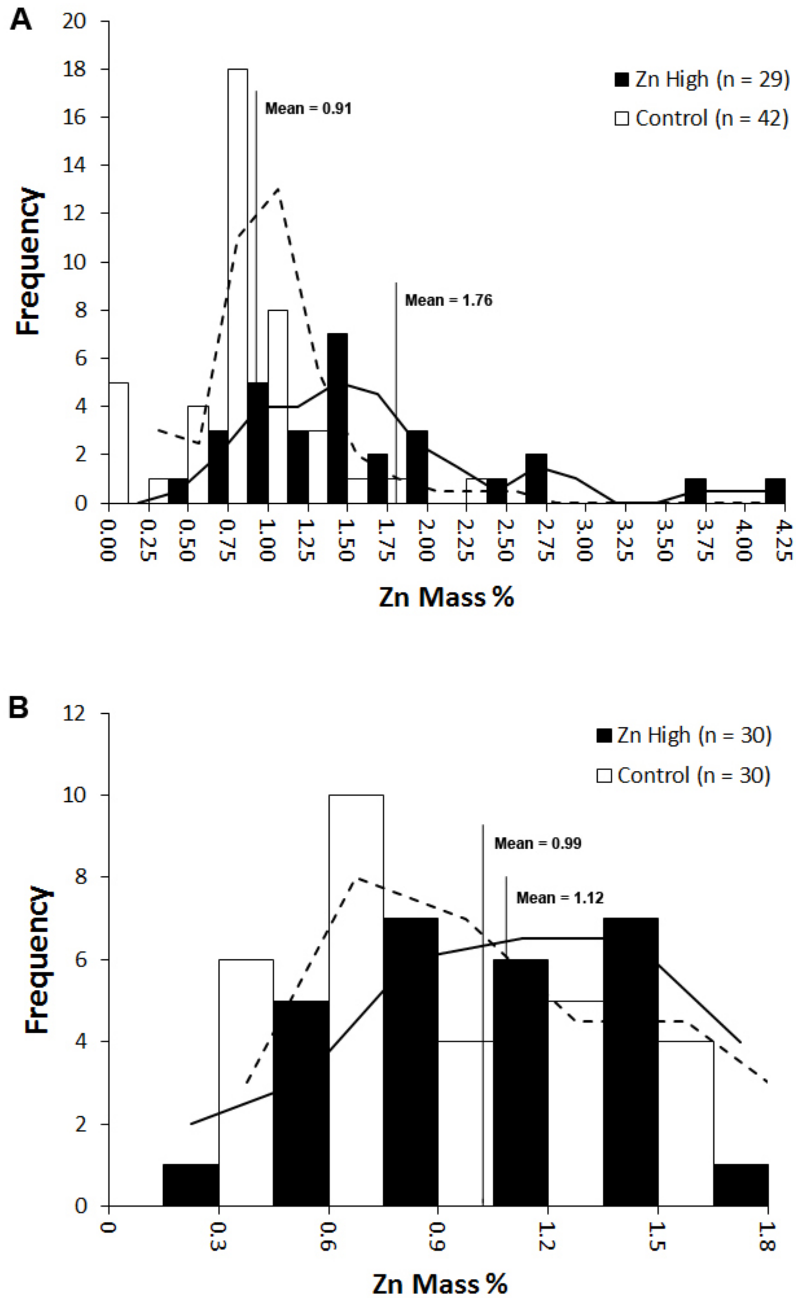

Figure S7. Frequency distribution of Zn atomic mass % in soybean leaves and pods. a. Zn mass % as measured by energy dispersive spectrometry (EDS) for soybean leaves. **b**, Zn mass % as measured by EDS for soybean pods. The displayed means were calculated using histograms with bin widths of 0.25 (leaves) and 0.3 (pods). The arithmetic means (*not* calculated from the above graph) for the high nano-ZnO and control leaves were 1.53 ± 0.16 and 0.69 ± 0.07 mass %, respectively. The arithmetic means for the high nano-ZnO and control pods were 0.83 ± 0.08 and 0.68 ± 0.08 mass %, respectively. The arithmetic means were significantly different for the leaf treatments (T – Test; $P < 0.05$), but not for the pod treatments. Measurement numbers in parentheses in the legend are for individual EDS scans for several (3 or more) individual thin sections prepared from a single specimen.

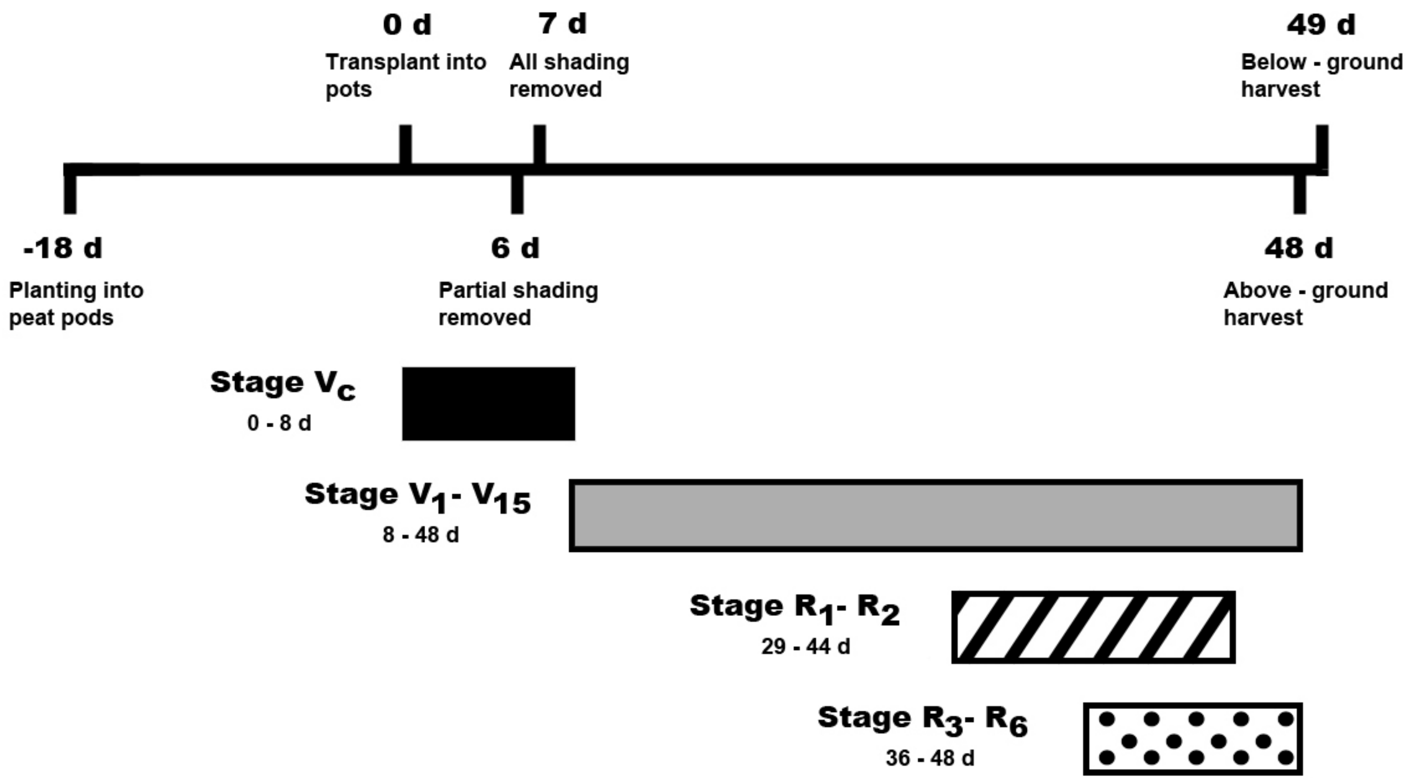

Figure S8. Planting and plant cultivation timeline. Stages denoted with a V represent vegetative growth. Stages denoted with an R represent reproductive growth (R_1 – R_2 = flowering, R_3 – R_6 = pod development).

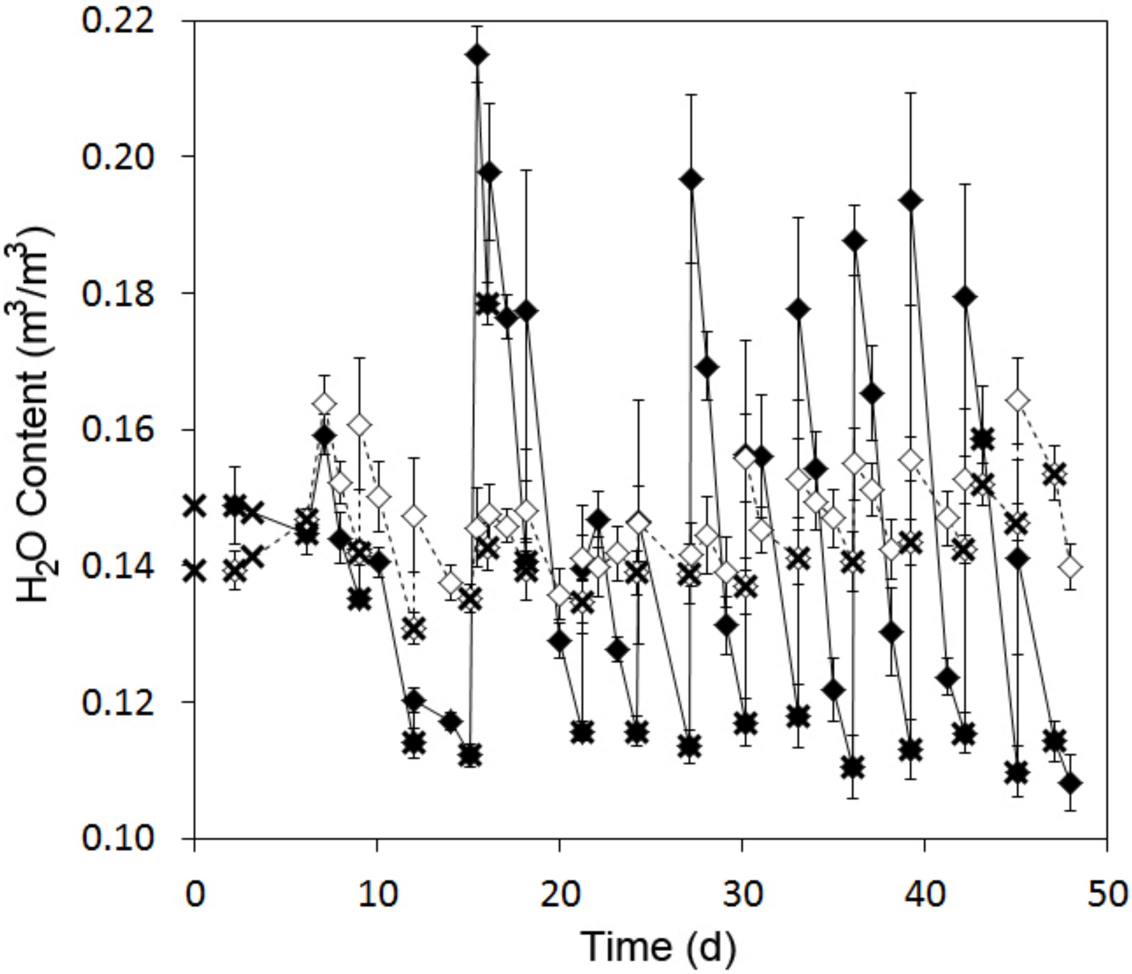

Figure S9. Volumetric soil water content versus time. Soil water content versus time for unplanted (open symbols) and planted (closed symbols) pots. (×) symbols represent irrigation time points. Error bars represent the standard error of the mean across all pots that were instrumented for semi - continuous measurement (n= 5 unplanted; n = 5 planted).

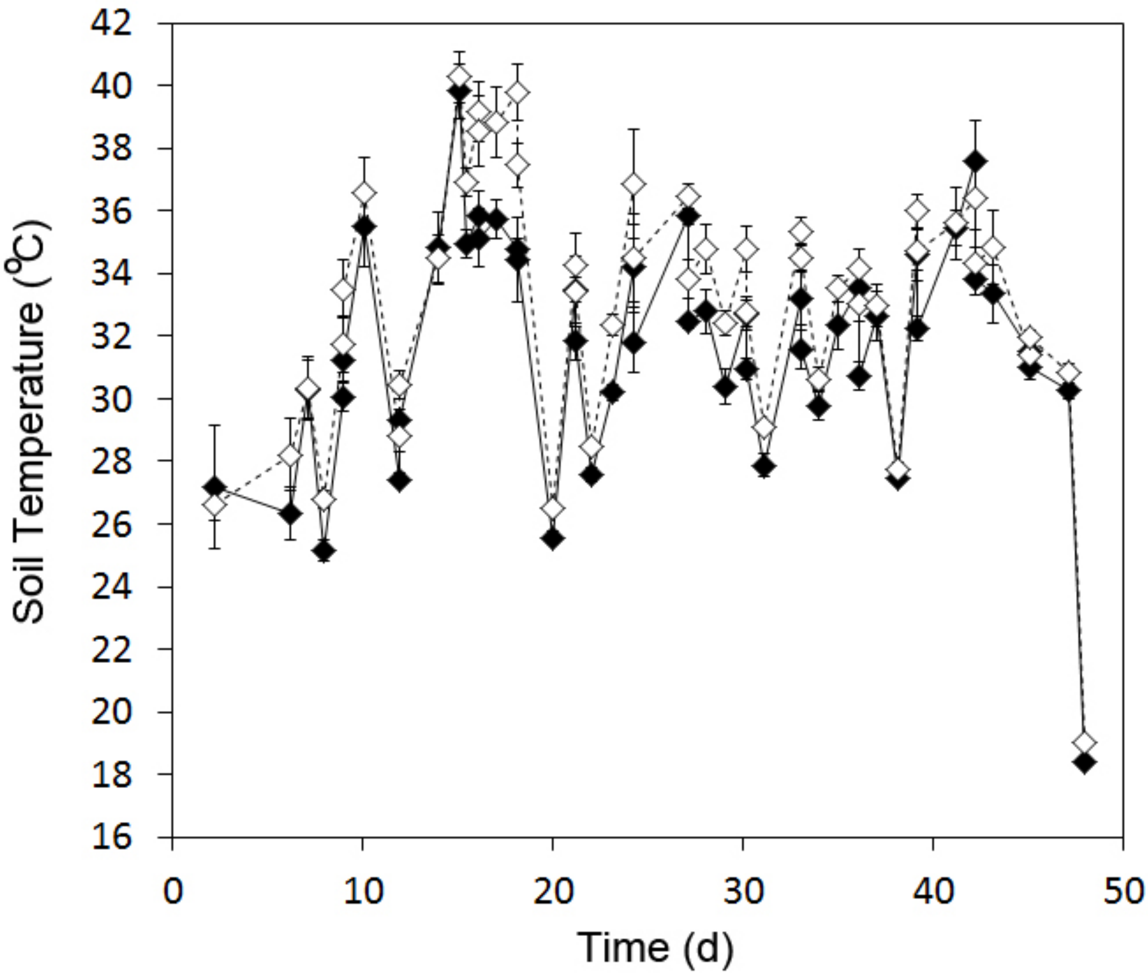

Figure S10. Soil temperature versus time. Soil temperature versus time for unplanted (open symbols) and planted (closed symbols) pots. Error bars represent the standard error of the mean across all pots that were instrumented for semi - continuous measurement (n= 5 unplanted; n = 5 planted).

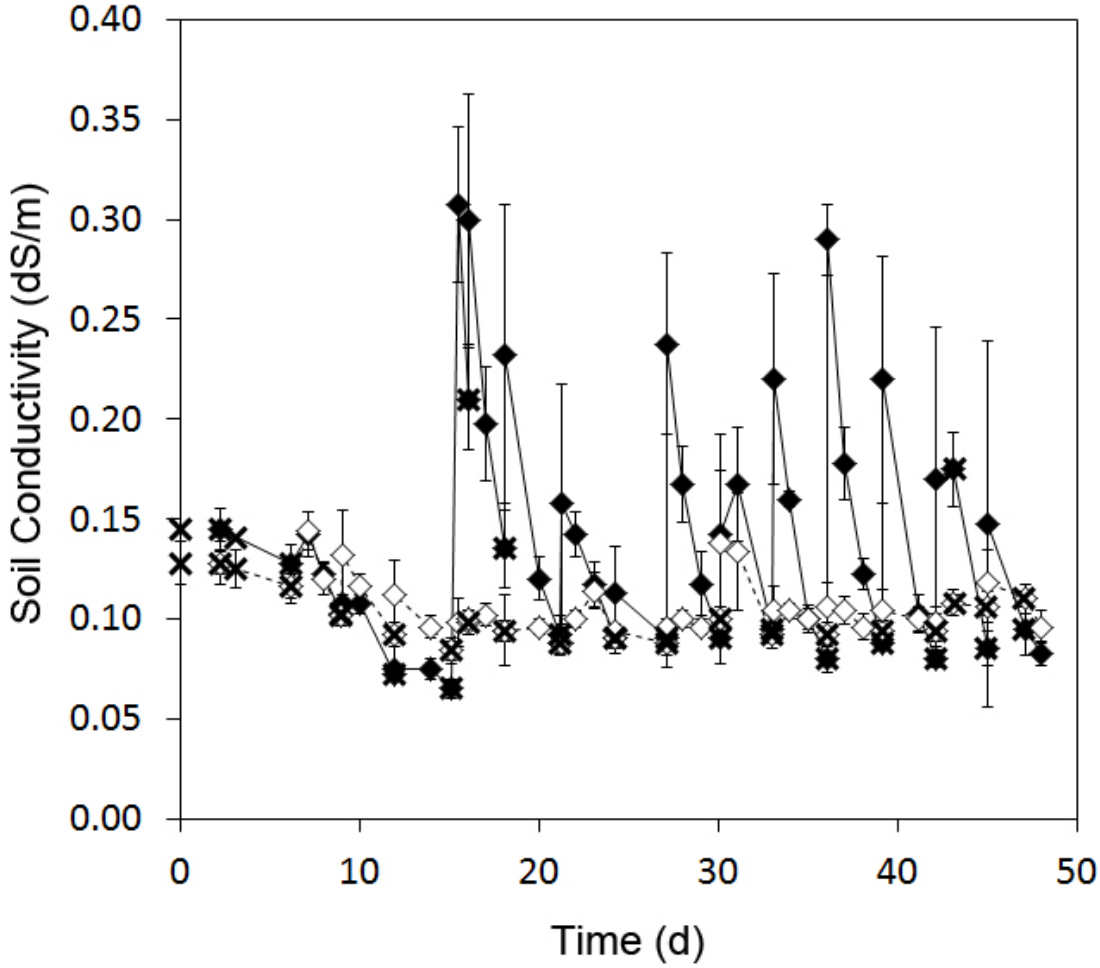

Figure S11. Soil conductivity versus time. Soil conductivity versus time for unplanted (open symbols) and planted (closed symbols) pots. (×) symbols represent irrigation time points. Error bars represent the standard error of the mean across all pots that were instrumented for semi - continuous measurement (n= 5 unplanted; n = 5 planted).

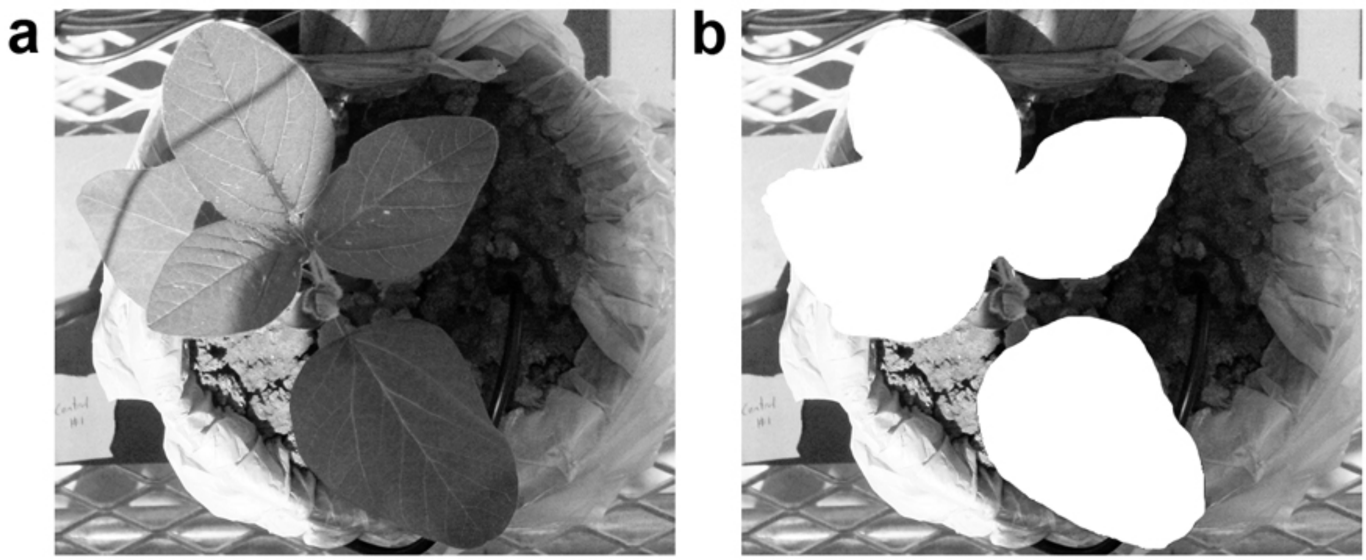

Figure S12. Aerial photograph of control soybean plant. a. Photograph of control soybean plant (8 days after planting into pot) showing one trifoliate leaf and two unifoliate leaves. **b**. The region measured for total leaf cover is shown in white.

Discussion of Potential Plant and Human Toxicity Implications from Soybean Zn Accumulation

The significant translocation of Zn from soil into soybean biomass raises questions regarding implications to plant health, and to the health of humans consuming the plant tissues. Herein, we: a) first discuss effects of very high total Zn concentrations on soybean plants, and on metal accumulation in edible tissue (bean pod), and b) then discuss possible implications for human health.

Total Zn Effects to Soybean Plants

High Zn can negatively impact soybean plants. For example, in the study of Shute and Macfie (2006), approximately 2000 mg kg^{-1} Zn in soil resulted in decreased soybean biomass (1); endpoint leaf Zn concentrations were 400 mg kg^{-1}, and bean pod concentrations were 100 mg kg^{-1} (1). In the current study, the highest nano-ZnO concentration applied to soil was 500 mg kg^{-1} (Methods). Accounting for the difference in molecular weights of ZnO (81.39 g $mole^{-1}$) versus Zn (65.39 g $mole^{-1}$), the highest soil treatment concentration in the current study was approximately 400 mg Zn kg^{-1} soil. At this highest Zn application, the final bean pod and leaf Zn concentrations were 82 and 344 mg Zn kg^{-1} plant tissue, respectively (Table 2, main manuscript). According to Borkert *et* al. (1998), soil characteristics substantially influence Zn toxicity to soybean; also, the "plant critical toxicity level" (CTL, i.e. the lowest metal concentration in leaf tissue that corresponds to diminished plant growth measured as final dry mass), varies with soybean cultivar (2). Here, the final leaf Zn concentration for the high nano-ZnO treatment (Table 2, main manuscript) was similar to the concentrations at which soybean growth was shown to be impaired in prior studies (1, 2). Yet, here, there was little indication that soybean was impaired by the tissue Zn burden, as conveyed in the main manuscript. Still, while the high nano-ZnO soil application level concentration was not particularly toxic to soybean in our study, considering that agricultural fields undergo biosolids application for decades, and that biosolids Zn concentrations can be much higher (3) compared to our treated soils, it is conceivable that chronic nano-ZnO loading to soils with ongoing biosolids application could cause total soil Zn to build up to levels that are toxic to soybean. Thus, our study is still useful in forewarning the potential plant uptake response. Additionally, intact nano-ZnO, which is cytotoxic (4), could confer impacts related specifically to this form of Zn.

Total Zn Effects to Humans Consuming Soybean

The second issue relates to the potential for hazards to humans ingesting Zn-enriched soybean. We provide the following analyses, focusing on bean pods because they are directly ingested and thus provide a direct means of exposure to food borne Zn. In our study, the highest concentration of Zn bioaccumulating in beans was approximately 8.2 mg Zn per 100 g bean; the control beans contained approximately 3 mg Zn per 100 g bean. According to the U.S.

Department of Agriculture, nutritional edamame contains approximately 1.3 mg Zn per 100 g of bean (http://ndb.nal.usda.gov/ndb/foods/show/3032). However, this is based on the "unfrozen" bean weight which is a wet mass basis for Zn concentration. If edamame contains 80% water by mass (according to Table S4 herein), then the dry mass basis for nutritional Zn in edamame is 6.5 mg Zn per 100 g soybean dry mass. Thus, the concentration of Zn in the beans in our study was similar to that considered as typical for nutritional edamame.

Next, it is relevant to consider if chronic ingestion of edamame with such Zn levels could be harmful to human health. According to the National Institutes of Health (NIH), tolerable upper intake levels (UILs) for Zn range from 4 - 40 mg (http://ods.od.nih.gov/factsheets/zinc-HealthProfessional/), depending on age, with lower tolerated levels for infants, and higher tolerated levels with increasing age. Assuming that 100 g (moist mass) of edamame providing approximately 10 grams of protein (http://ndb.nal.usda.gov/ndb/foods/show/3032) which is approximately 1/5th of the daily protein intake recommended for adults, and assuming an adult that derives all protein from soybean, then 500 g moist mass per day (or 100 g dry mass per day) of edamame would be required to meet a soy-only dietary protein requirement. As above, ingesting 100 g dry soybean mass from our study would deliver approximately 8 mg Zn, which is well below the tolerable UIL for adults. Since children would consume less, as their protein requirements are less than adults, they should also not be at risk. Bioaccumulation levels would need to be approximately five times more total Zn than we report to reach bean Zn concentrations that are harmful to human health. Whether such bioaccumulation levels would occur, e.g. as a consequence of long term biosolids application to soils with Zn soil concentrations greatly exceeding those studied here, is not directly predictable from our study. Further, we caution that the toxicity to humans may also depend on speciation of Zn in plant tissues. The critical unknown in the above analysis are the impacts to humans, through ingestion of soybeans, from exposure to nano-ZnO. Nano-ZnO is known to be cytotoxic through its dissolution process that generates cell-damaging and inflammatory free radicals (4). If nanoparticulate ZnO is accumulated in edible plant tissue, a question that we intend to address in later research, then the above analysis based on total Zn would need revision.

Summary

In summary, based on our results and the analyses above, soybean plants are likely to be impacted by high total Zn in soils, but humans may not be impacted by consuming soybeans with total Zn bioaccumulated to the levels reported herein. Critical unknowns regard the state of Zn in tissues, including if nano-ZnO is present and especially toxic with ingested soybean.

Literature Cited

1. Shute T & Macfie SM (2006) Cadmium and zinc accumulation in soybean: A threat to food safety? *Sci. Total Environ.* 371(1-3):63-73.
2. Borkert CM, Cox FR, & Tucker MR (1998) Zinc and copper toxicity in peanut, soybean, rice, and corn in soil mixtures. (Translated from English) *Commun. Soil Sci. Plant Anal.* 29(19-20):2991-3005 (in English).
3. Donner E*, et al.* (2011) X-ray Absorption and Micro X-ray Fluorescence Spectroscopy Investigation of Copper and Zinc Speciation in Biosolids. (Translated from English) *Environ. Sci. Technol.* 45(17):7249-7257 (in English).
4. George S*, et al.* (2010) Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping. *ACS Nano* 4(1):15-29.