Supporting Information for

Agglomeration Determines Effects of Carbonaceous Nanomaterials on Soybean Nodulation, Nitrogen Fixation Potential, and Growth in Soil

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ADDITIONAL MATERIALS AND METHODS

Calculation of CNM concentrations in soil solution for designing CNM stability studies

To study the potential agglomeration states of CNMs in moist soil under conditions similar to the main experiment, suspensions of CNMs were prepared in an aqueous soil extract at CNM concentrations expected to occur in the soil solution of the main experiment. In the main experiment, the soil water content was 17.3% (soil dry mass basis) and was either un-amended (control) or amended with 0.1, 100 or 1000 mg kg^{-1} (dry soil basis) of CB, MWCNTs or GNPs. Assuming that all CNMs were within the water phase of the soil and assuming a specific gravity of the soil solution equal to that of water (1 kg L^{-1}), the CNM concentrations in the soil solution of the amended soils were calculated to be 0.58, 578 and 5780 mg L^{-1} (for CNM soil amendments of 0.1, 100 or 1000 mg kg^{-1} soil, respectively).

Based on the above calculations and the limits of the two instruments used to characterize CNM agglomeration and sedimentation (Zetasizer Nano-ZS90 and UV-1800 spectrophotometer), putative CNM colloidal stabilities in the soil solution were studied at CNM concentrations of 10 and 300 mg L^{-1} in the soil extract. A 1:5 w/v (g moist soil per mL water) ratio was used to create the soil extract from unamended soils, following a published method for characterizing soil nitrogen and carbon contents.[1] The dry density of the soil was assumed to be 1.3 g cm^{-3}.[2] Taking into account the dry soil volumetric displacement when suspending soil into 5 parts water, and taking into account the water content in the moist soil, the soil solution was diluted by approximately 39 times in the extract solution. If soil extracts had been prepared similarly using CNM-amended soils, then a similar dilution factor would apply and the CNM concentrations in the soil extracts would have been 0.015, 15 and 147 mg L^{-1} (for CNM soil amendments of 0.1, 100 or 1000 mg kg^{-1} soil, respectively). Thus, amending soil extract to 10 or

300 mg L^{-1} CNM met two criteria for representativeness: similarity to experimental CNM concentrations in the soil solution, and reasonable proportionality of the original soil solution constituents to added CNMs.

Table S1. Physicochemical characterization of CNMs. CB = carbon black. MWCNTs = multi-walled carbon nanotubes. GNPs = graphene nanoplatelets.

	Specific Surface Area (m^2 g^{-1})[a]	**Size (nm)[a]**	**Diameter (nm)[b]**	**Primary Oxidation Temperature (°C)[c]**	**Non-carbon Impurity (wt %)[c]**	**Purity (wt %)[a]**
CB	72[d]	N/A	36.6 ± 8.3[d]	619.9 ± 3.4[e]	1.34 ± 0.34[e]	> 99
MWCNTs	110	Diameter: 20–30 Length: 10000–30000	18.8 ± 4.1	584.9 ± 3.8	2.17 ± 0.25	> 95
GNPs	600–750	Diameter: 2000 Thickness: 8–12	350 ± 320 (80–1600)	623.0 ± 0.2	1.03 ± 0.12	> 97

[a] Reported by the manufacturer. [b] Measured from transmission electron microscopy (TEM) images following method reported in Ge et al.[3] [c] Measured by thermogravimetric analysis (TGA) following methods reported in Ge et al[3] and Mortimer et al[4]. [d] Previously reported in Ge et al.[3] [e] Within the range of previously reported values in Ge et al.[3] N/A not available.

Table S2. Metal content of CNMs (CB = carbon black, MWCNTs = multi-walled carbon nanotubes, GNPs = graphene nanoplatelets; wt %)[a]

Metal[b]	CB	MWCNTs	GNPs
Al	0.011 ± 0.000	0.003 ± 0.002	0.009 ± 0.002
As	-[c]	0.003± 0.000	-
B	-	0.001 ± 0.000	-
Ca	0.011 ± 0.003	0.055 ± 0.008	0.010 ± 0.002
Co	-	0.002 ± 0.000	-
Cr	-	0.004 ± 0.000	-
Cu	-	0.001 ± 0.000	0.001 ± 0.000
Fe	0.001 ± 0.000	0.012 ± 0.002	0.018 ± 0.002
La	0.001 ± 0.000	0.089 ± 0.011	-
Li	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Mg	0.001 ± 0.000	0.011 ± 0.001	0.006 ± 0.001
Mo	0.012 ± 0.006	0.024 ± 0.013	0.008 ± 0.007
Na	0.028 ± 0.001	0.001 ± 0.000	0.010 ± 0.002
Ni	-	0.927 ± 0.119	-
Pb	-	0.006 ± 0.000	0.001 ± 0.000
Si	0.005 ± 0.001	0.003 ± 0.001	0.010 ± 0.001
Th	0.001 ± 0.000	-	-
V	0.002 ± 0.001	0.004 ± 0.002	0.001 ± 0.001
Yb	-	0.008 ± 0.001	-
Zn	0.001 ± 0.000	0.005 ± 0.001	-

[a] Measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) following method reported in Mortimer et al.[4] All data are shown as mean ±SE (n = 3). [b] The following metals were analyzed for, but were not detected: Ag, Ba, Be, Cd, Eu, Ho, Mn, Sb, Sc, Se, Sr, Tl, U. [c] Below detection limit.

Table S3. Soybean plant final stem length and stem elongation rate constant, according to treatment[a]

Treatment	Final Stem Length (cm)	Stem Elongation Rate Constant (d^{-1})[b]	End of Period (day) for Regression	Average R^2
Ctrl	85.7 ± 2.5	0.114 ± 0.008	11 – 14	0.997
CB_Low	82.1 ± 2.1	0.118 ± 0.010	11 – 18	0.996
CB_Med	83.8 ± 1.4	0.109 ± 0.005	11 – 14	0.997
CB_High	83.1 ± 1.8	0.114 ± 0.009	11 – 14	0.995
MWCNT_Low	73.1 ± 2.7*	0.111 ± 0.010	11 – 14	0.989
MWCNT_Med	84.7 ± 1.4	0.110 ± 0.007	11 – 14	0.995
MWCNT_High	87.3 ± 3.6	0.125 ± 0.007	11	0.998
GNP_Low	82.1 ± 1.7	0.118 ± 0.006	11 – 14	0.994
GNP_Med	84.5 ± 3.8	0.120 ± 0.008	7 – 14	0.998
GNP_High	83.7 ± 3.9	0.127 ± 0.009	11 – 14	0.988

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments). [b] Plant stem length elongation rate constant was calculated as the slope of a regression line from the linear region of the plot of the natural logarithm of stem length versus time (Figure 2A–C), where the initial time point was day 0 post transplantation and the final time point is the "End of Period (day) for Regression" (4^{th} column from the left). *P < 0.05, as compared to the control.

Table S4. Soybean plant total leaf area at the final harvest, maximum leaf cover, and leaf cover expansion rate constant, according to treatment[a]

Treatment	Final Total Leaf Area (cm^2)	Max. Leaf Cover (% coverage of soil)	Leaf Cover Expansion Rate Constant (d^{-1})[b]	End of Period (day) for Regression	Average R^2
Ctrl	562.6 ± 23.4	185.9 ± 20.2	0.197 ± 0.005	14 – 22	0.996
CB_Low	519.0 ± 51.3	143.7 ± 21.7	0.167 ± 0.017	14 – 28	0.994
CB_Med	518.3 ± 35.5	183.3 ± 17.5	0.172 ± 0.012	14 – 22	0.986
CB_High	519.0 ± 61.4	165.3 ± 21.3	0.152 ± 0.016	14 – 28	0.971
MWCNT_Low	428.0 ± 41.7*	137.8 ± 15.7	0.145 ± 0.012*	14 – 28	0.942
MWCNT_Med	464.8 ± 7.7	177.2 ± 16.3	0.173 ± 0.009	14 – 22	0.993
MWCNT_High	518.7 ± 27.9	170.6 ± 18.3	0.181 ± 0.013	14 – 22	0.977
GNP_Low	558.1 ± 17.2	164.2 ± 23.7	0.164 ± 0.015	14 – 28	0.982
GNP_Med	477.7 ± 24.6	186.8 ± 34.6	0.162 ± 0.011	14 – 28	0.989
GNP_High	569.7 ± 54.6	182.9 ± 19.6	0.183 ± 0.008	14 – 22	0.994

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments). [b] Leaf cover expansion rate constant was calculated as the slope of a regression line from the linear region of the plot of the natural logarithm of leaf cover versus time (Figure 2D–F), where the initial time point is day 0 post transplantation and the final time point is the "End of Period (day) for Regression" (5^{th} column from the left).. *$P < 0.05$, as compared to the control.

Table S5. Soybean plant pod (total: all pods; mature: seed containing pods) count per plant (Figure S2), average seed count per pod, pod length, and pod width at the final harvest, according to treatment[a]

Treatment	Pod No. Per Plant		Seed Count Per Pod	Pod Length (cm)	Pod Width (cm)
	Total	Mature			
Ctrl	9.8 ± 0.5	8.3 ± 0.3	1.6 ± 0.1	3.66 ± 0.17	1.11 ± 0.05
CB_Low	10.0 ± 0.3	10.0 ± 0.3**	1.8 ± 0.0	3.78 ± 0.10	1.25 ± 0.02
CB_Med	10.6 ± 0.5	9.8 ± 0.2*	1.8 ± 0.1	3.60 ± 0.19	1.15 ± 0.04
CB_High	11.8 ± 0.3*	10.3 ± 0.5**	1.6 ± 0.0	3.58 ± 0.10	1.14 ± 0.05
MWCNT_Low	8.5 ± 0.9	8.0 ± 0.4	1.6 ± 0.1	3.76 ± 0.10	1.19 ± 0.07
MWCNT_Med	10.4 ± 0.9	9.4 ± 0.5	1.6 ± 0.1	3.52 ± 0.17	1.12 ± 0.03
MWCNT_High	14.4 ± 3.2	8.0 ± 1.5	1.3 ± 0.3	2.98 ± 0.48	0.97 ± 0.13
GNP_Low	11.2 ± 0.5	9.8 ± 0.5	1.5 ± 0.1	3.54 ± 0.13	1.11 ± 0.05
GNP_Med	9.8 ± 0.4	9.2 ± 0.5	1.7 ± 0.1	3.75 ± 0.08	1.16 ± 0.02
GNP_High	10.8 ± 0.4	9.4 ± 0.7	1.6 ± 0.1	3.60 ± 0.19	1.13 ± 0.06

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments). *P < 0.05, **P < 0.01, as compared to the control.

Table S6. Soybean plant part and total dry biomass (g plant^{-1}) at the intermediate harvest, according to treatment[a]

Treatment	Stem	Leaf	Pod	Root	Nodule	Total
Ctrl	0.70 ± 0.04	1.34 ± 0.07	0.003 ± 0.002[b]	0.21 ± 0.01	0.013 ± 0.001	2.27 ± 0.10
CB_Low	0.78 ± 0.03	1.57 ± 0.06	-	0.23 ± 0.01	0.005 ± 0.001**	2.58 ± 0.08
CB_Med	0.72 ± 0.05	1.40 ± 0.11	-	0.23 ± 0.02	0.004 ± 0.001**	2.36 ± 0.15
CB_High	0.79 ± 0.06	1.59 ± 0.12	-	0.23 ± 0.02	0.009 ± 0.002	2.62 ± 0.18
MWCNT_Low	0.66 ± 0.05	1.28 ± 0.15	-	0.19 ± 0.03	0.002 ± 0.000*	2.14 ± 0.22
MWCNT_Med	0.68 ± 0.01	1.25 ± 0.05	-	0.21 ± 0.01	0.010 ± 0.004	2.15 ± 0.07
MWCNT_High	0.71 ± 0.04	1.38 ± 0.04	-	0.23 ± 0.01	0.009 ± 0.002	2.33 ± 0.09
GNP_Low	0.73 ± 0.10	1.38 ± 0.19	0.005[c]	0.23 ± 0.03	0.007 ± 0.002	2.34 ± 0.31
GNP_Med	0.82 ± 0.01	1.49 ± 0.03	0.012[c]	0.30 ± 0.03	0.012 ± 0.003	2.62 ± 0.03
GNP_High	0.70 ± 0.02	1.39 ± 0.07	-	0.22 ± 0.01	0.013 ± 0.003	2.33 ± 0.09

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 3 plants, except that [b] n = 2 and [c] n = 1 individual plants formed pods at intermediate harvest). *P < 0.05, **P < 0.01, as compared to the control. - indicates that there were no pods present at the intermediate harvest.

Table S7. Soybean plant part and total dry biomass (g plant^{-1}) at the final harvest, according to treatment[a]

Treatment	Stem	Leaf	Pod	Root	Nodule	Total
Ctrl	1.35 ± 0.03	2.29 ± 0.09	2.92 ± 0.06	0.32 ± 0.01	0.18 ± 0.01	7.06 ± 0.17
CB_Low	1.34 ± 0.04	2.41 ± 0.11	3.09 ± 0.16	0.32 ± 0.01	0.06 ± 0.02***	7.21 ± 0.29
CB_Med	1.36 ± 0.06	2.56 ± 0.14	3.10 ± 0.08	0.29 ± 0.03	0.04 ± 0.01***	7.36 ± 0.22
CB_High	1.50 ± 0.07	2.53 ± 0.08	3.16 ± 0.08	0.30 ± 0.04	0.12 ± 0.01[b]	7.61 ± 0.20
MWCNT_Low	1.14 ± 0.13	2.15 ± 0.25	2.57 ± 0.33	0.21 ± 0.03*	0.01 ± 0.01***	6.08 ± 0.70
MWCNT_Med	1.35 ± 0.03	2.28 ± 0.08	2.77 ± 0.08	0.35 ± 0.03	0.09 ± 0.02**	6.84 ± 0.21
MWCNT_High	1.39 ± 0.11	2.42 ± 0.25	2.52 ± 0.45	0.36 ± 0.03	0.08 ± 0.01***	6.77 ± 0.21
GNP_Low	1.32 ± 0.12	2.36 ± 0.19	2.98 ± 0.06	0.37 ± 0.07	0.06 ± 0.02**	7.09 ± 0.38
GNP_Med	1.28 ± 0.12	2.19 ± 0.18	2.83 ± 0.22	0.27 ± 0.04	0.11 ± 0.03	6.69 ± 0.56
GNP_High	1.38 ± 0.07	2.36 ± 0.07	2.98 ± 0.06	0.32 ± 0.02	0.19 ± 0.01	7.23 ± 0.11

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments). *P < 0.05, **P < 0.01, ***P < 0.001, as compared to the control. [b] P = 0.06, as compared to the control.

Table S8. Soybean plant moisture content (g H_2O g^{-1} wet biomass) by each tissue type at the intermediate harvest, according to treatment[a]

Treatment	Stem	Leaf	Pod	Root	Nodule
Ctrl	0.79 ± 0.01	0.80 ± 0.01	0.83 ± 0.03[b]	0.79 ± 0.02	0.80 ± 0.01
CB_Low	0.83 ± 0.00	0.81 ± 0.01	-	0.77 ± 0.08	0.79 ± 0.04
CB_Med	0.80 ± 0.02	0.81 ± 0.01	-	0.85 ± 0.02	0.83 ± 0.01
CB_High	0.81 ± 0.01	0.80 ± 0.00	-	0.84 ± 0.02	0.82 ± 0.01
MWCNT_Low	0.83 ± 0.02	0.82 ± 0.01	-	0.86 ± 0.01	0.84 ± 0.01*
MWCNT_Med	0.81 ± 0.00	0.81 ± 0.00	-	0.78 ± 0.07	0.82 ± 0.00
MWCNT_High	0.81 ± 0.01	0.80 ± 0.01	-	0.83 ± 0.03	0.83 ± 0.00
GNP_Low	0.81 ± 0.01	0.81 ± 0.01	0.79[c]	0.83 ± 0.02	0.82 ± 0.02
GNP_Med	0.81 ± 0.01	0.81 ± 0.00	0.76[c]	0.82 ± 0.02	0.83 ± 0.01
GNP_High	0.80 ± 0.00	0.81 ± 0.00	-	0.80 ± 0.01	0.80 ± 0.01

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 3 plants, except that [b] n = 2 and [c] n = 1 individual plants formed pods at the intermediate harvest). *$P < 0.05$, as compared to the control. - indicates that there were no pods present at the intermediate harvest.

Table S9. Soybean plant moisture content (g H_2O g^{-1} wet biomass) by each tissue type at the final harvest, according to treatment[a]

Treatment	Stem	Leaf	Pod	Root	Nodule
Ctrl	0.67 ± 0.00	0.76 ± 0.01	0.77 ± 0.00	0.78 ± 0.05	0.77 ± 0.01
CB_Low	0.67 ± 0.00	0.74 ± 0.00	0.77 ± 0.00	0.78 ± 0.02	0.76 ± 0.02
CB_Med	0.67 ± 0.00	0.74 ± 0.01	0.78 ± 0.00	0.81 ± 0.02	0.77 ± 0.01
CB_High	0.66 ± 0.00	0.74 ± 0.00	0.78 ± 0.00	0.80 ± 0.04	0.78 ± 0.01
MWCNT_Low	0.71 ± 0.01	0.74 ± 0.01	0.78 ± 0.01	0.81 ± 0.04	0.80 ± 0.01
MWCNT_Med	0.67 ± 0.00	0.75 ± 0.00	0.78 ± 0.00	0.79 ± 0.03	0.77 ± 0.01
MWCNT_High	0.67 ± 0.01	0.74 ± 0.02	0.77 ± 0.01	0.81 ± 0.03	0.78 ± 0.00
GNP_Low	0.68 ± 0.01	0.74 ± 0.01	0.78 ± 0.00	0.79 ± 0.03	0.79 ± 0.01
GNP_Med	0.68 ± 0.01	0.75 ± 0.01	0.78 ± 0.01	0.76 ± 0.05	0.78 ± 0.01
GNP_High	0.68 ± 0.01	0.75 ± 0.00	0.78 ± 0.00	0.81 ± 0.02	0.77 ± 0.01

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments).

Table S10. Soybean plant nodule count per plant, dry biomass per nodule, N_2 fixation potential (normalized to dry nodule biomass) and whole-plant N_2 fixation potential, at the intermediate harvest, according to treatment[a]

Treatment	Nodule Count (No. per plant)	Dry Biomass Per Nodule (mg)	N_2 Fixation Potential (E-8 mol ethylene min^{-1} g^{-1})	Whole-plant N_2 Fixation Potential (E-9 mol ethylene min^{-1} $plant^{-1}$)[b]
Ctrl	44.3 ± 2.3	0.30 ± 0.01	9.35 ± 1.91	1.26 ± 0.33
CB_Low	27.0 ± 3.5**	0.20 ± 0.02	1.83 ± 1.83	0.12 ± 0.12
CB_Med	28.3 ± 3.2*	0.13 ± 0.02**	2.00 ± 2.00	0.11 ± 0.11
CB_High	33.7 ± 1.8	0.27 ± 0.04	8.21 ± 3.47	0.83 ± 0.46
MWCNT_Low	13.0 ± 1.2**	0.12 ± 0.02*	0**	0*
MWCNT_Med	41.3 ± 1.8	0.23 ± 0.08	3.06 ± 1.59	0.33 ± 0.18
MWCNT_High	38.7 ± 8.4	0.23 ± 0.03	5.06 ± 1.59	0.51 ± 0.22
GNP_Low	45.0 ± 2.0	0.16 ± 0.03	5.28 ± 3.23	0.50 ± 0.34
GNP_Med	43.3 ± 6.4	0.28 ± 0.05	12.61 ± 5.30	1.86 ± 0.88
GNP_High	41.7 ± 9.0	0.32 ± 0.07	6.84 ± 3.45	0.88 ± 0.44

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 3 plants). [b] Calculated as the product of the dry nodule biomass per plant (Table S6) and the N_2 fixation potential which had been normalized to dry nodule biomass at the intermediate harvest. Calculation was made for each individual plant, with the mean and SE calculated for each treatment. *$P < 0.05$, **$P < 0.01$, as compared to the control.

Table S11. Soybean plant nodule count per plant, dry biomass per nodule, N_2 fixation potential (normalized to dry nodule biomass) and whole-plant N_2 fixation potential, at the final harvest, according to treatment[a]

Treatment	Nodule Count (No. per plant)	Dry Biomass per Nodule (mg)	N_2 Fixation Potential (E-7 mol ethylene min^{-1} g^{-1})	Whole-plant N_2 Fixation Potential (E-8 mol ethylene min^{-1} $plant^{-1}$)[b]
Ctrl	50.0 ± 4.8	3.6 ± 0.2	5.08 ± 1.19	9.28 ± 2.60
CB_Low	35.6 ± 4.9	1.5 ± 0.3***	1.51 ± 0.51**	0.77 ± 0.37***
CB_Med	27.4 ± 3.8*	1.4 ± 0.2***	2.18 ± 0.42*	0.86 ± 0.24***
CB_High	41.5 ± 4.3	2.9 ± 0.1	4.06 ± 0.31	4.94 ± 0.80
MWCNT_Low	24.0 ± 4.3*	0.4 ± 0.1***	1.01 ± 0.56**	0.21 ± 0.18***
MWCNT_Med	45.6 ± 6.2	1.9 ± 0.3***	2.98 ± 0.53	2.56 ± 0.63**
MWCNT_High	46.0 ± 3.8	1.8 ± 0.1***	2.25 ± 0.61	1.80 ± 0.49**
GNP_Low	26.4 ± 3.5[c]	2.3 ± 0.5	3.97 ± 0.14	3.55 ± 2.20
GNP_Med	48.4 ± 8.9	2.2 ± 0.5	2.30 ± 0.71	2.96 ± 0.94
GNP_High	65.4 ± 4.8	2.9 ± 0.2	2.64 ± 0.68	4.98 ± 1.35

[a] Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. All data are shown as mean ±SE (n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments).

[b] Calculated as the product of the dry nodule biomass per plant (Table S7) and the N_2 fixation potential which had been normalized to dry nodule biomass at the final harvest. Calculation was made for each individual plant, with the mean and SE calculated for each treatment. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, as compared to the control. [c] $P = 0.07$, as compared to the control.

Table S12. Soil characteristics

Characteristic	Result[a]	Analytical Method Reference
Saturation water content (%)	41.00	[5]
pH	7.38 (7.36–7.40)	[6]
Estimated Soluble Salts (EC, dS m^{-1})	2.65 (2.65–2.65)	[5]
Ca, (saturated paste extract, meq L^{-1})	8.01 (7.97–8.05)	[7-8]
Mg, (saturated paste extract, meq L^{-1})	16.67 (16.58–16.75)	[7-8]
Na, (saturated paste extract, meq L^{-1})	3.63 (3.61–3.65)	[7-8]
Cl, (saturated paste extract, meq L^{-1})	4.34 (4.30–4.37)	[5]
B, (saturated paste extract, mg L^{-1})	0.12	[7-8]
HCO_3^-, (saturated paste extract, meq L^{-1})	0.4	[9]
CO_3^{2-}, (saturated paste extract, meq L^{-1})	<0.1	[9]
Total N (%)	0.152 (0.149–0.154)	[10]
Total C (%)	1.53 (1.52–1.54)	[10]
NH_4^+ (N, extractable, ppm)	1.82	[11-12]
NO_3^- (N, extractable, ppm)	44.45	[12-13]
P (Olsen, extractable, ppm)	15.1 (14.7–15.4)	[14-15]
K (exchangeable, ppm)	498 (495–501)	[16]
K (exchangeable, meq per 100 g)	1.28 (1.27–1.28)	[16]
Na (exchangeable, ppm)	91 (90–91)	[16]
Na (exchangeable, meq per 100 g)	0.39 (0.39–0.39)	[16]
Ca (exchangeable, meq per 100 g)	8.85 (8.82–8.88)	[16]
Mg (exchangeable, meq per 100 g)	12.1 (12.0–12.1)	[16]
Cation Exchange Capacity (CEC, meq per 100 g)	22.6 (22.4–22.7)	[16]
Organic Matter (loss on ignition, LOI, %)	3.03 (3.02–3.03)	[17]
Zn (DTPA extraction, ppm)	0.8 (0.7–0.8)	[18]
Mn (DTPA extraction, ppm)	108 (102–113)	[18]
Cu (DTPA extraction, ppm)	0.9 (0.9–0.9)	[18]
Fe (DTPA extraction, ppm)	146 (142–150)	[18]
Zn (Total, ppm)	55 (54–56)	[19]
Mn (Total, ppm)	685 (675–695)	[19]
Fe (Total, ppm)	31250 (30500–32000)	[19]
Cu (Total, ppm)	28 (27–28)	[19]
Sand (%)	55	[20]
Silt (%)	25	[20]
Clay (%)	20	[20]

[a] Data are shown as mean with results from duplicate measurements (of separate soil subsamples) in parentheses. For saturation water content, B, HCO_3^-, CO_3^{2-}, NH_4^+, NO_3^-, sand, silt and clay, only a single measurement was made.

Table S13. Zeta (ζ) potential and electrophoretic mobility of the filtered soil extract, or of filtered soil extract containing 10 mg L^{-1} of either CB, MWCNTs or GNPs[a]

	ζ Potential (mV)	Electrophoretic Mobility (10^{-8} m^2 V^{-1} s^{-1})
Soil Extract Only	-17.4 ± 0.7	-1.364 ± 0.052
10 mg L^{-1} CB	-21.8 ± 0.3	-1.707 ± 0.025
10 mg L^{-1} MWCNTs	-20.4 ± 0.3	-1.600 ± 0.026
10 mg L^{-1} GNPs	-17.4 ± 0.2	-1.364 ± 0.015

[a] All data are shown as mean ±SE (n=6). CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets.

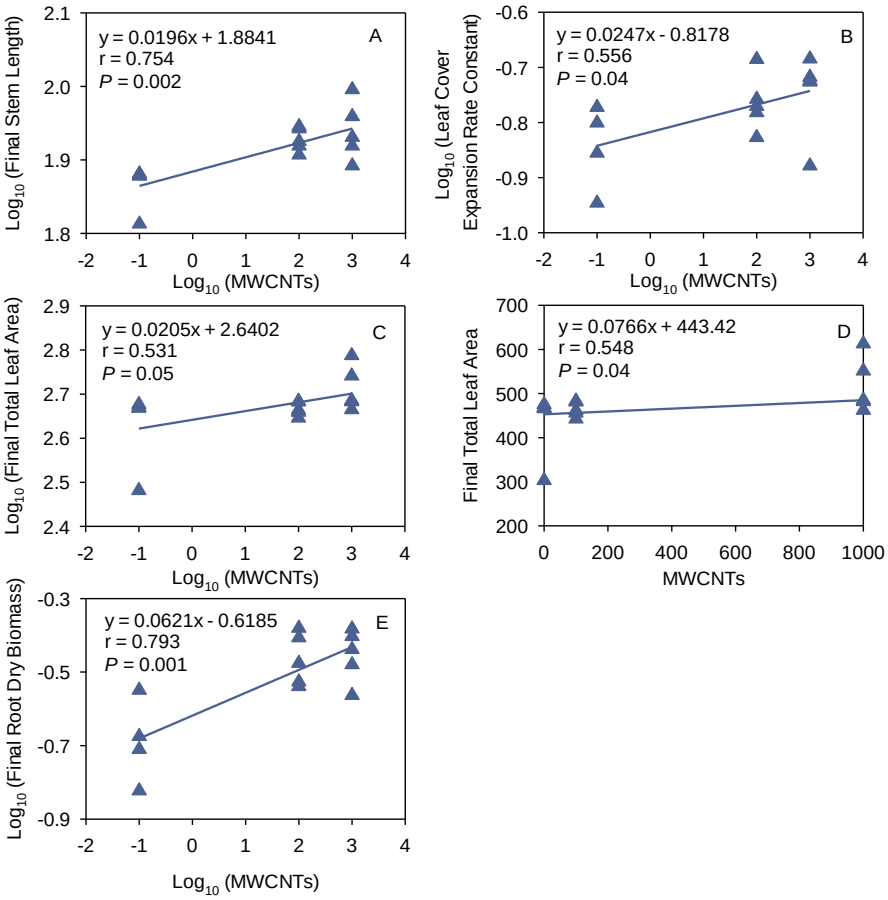

Figure S1. Summary of significant inverse dose-response relationships between multi-walled carbon nanotube (MWCNT) soil concentration and (A) final stem length, (B) leaf cover expansion rate constant, (C–D) final total leaf area, and (E) final dry root biomass. In all graphs (A–E), the control is excluded. MWCNT concentrations were 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. For each treatment, n = 5 plants, except n = 4 for the 0.1 mg kg^{-1} MWCNT treatment. Solid regression lines and the associated line equations within each graph provide the correlation results, including correlation coefficients (r) with significances (*P*). For the final stem length (A), leaf cover expansion rate constant (B) and final dry root biomass (E), correlations were significant when data were fitted by power models; hence data for both the x and y-axes of (A), (B) and (E) were logarithmically transformed. For the final total leaf area, the correlations were significant when fitted by either a power (C) or a linear (D) function.

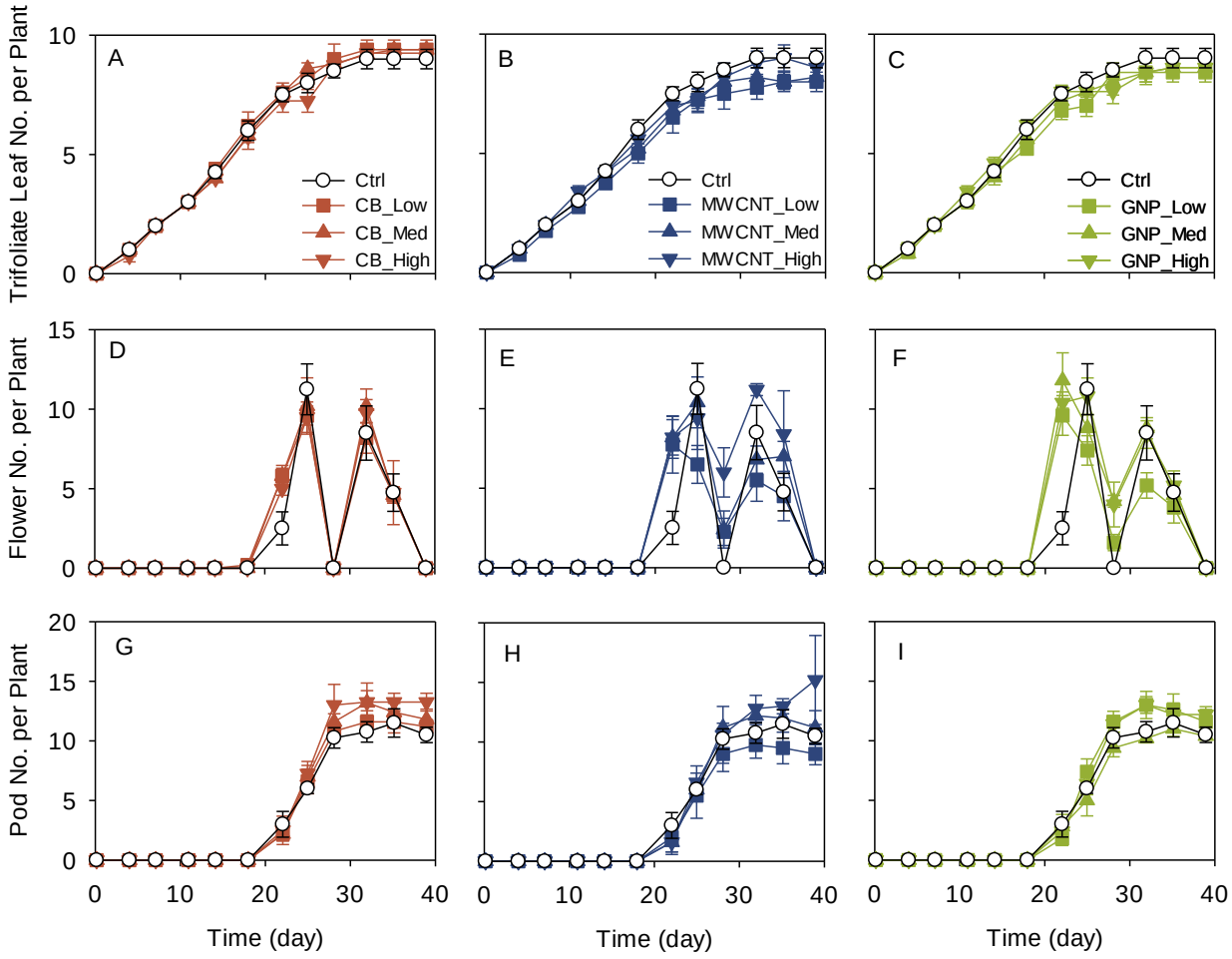

Figure S2. Time course of soybean plant vegetative and reproductive development post transplantation according to either (A–C) trifoliate leaf, (D–F) flower or (G–I) pod count per plant. Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. Error bars are ±SE (n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments).

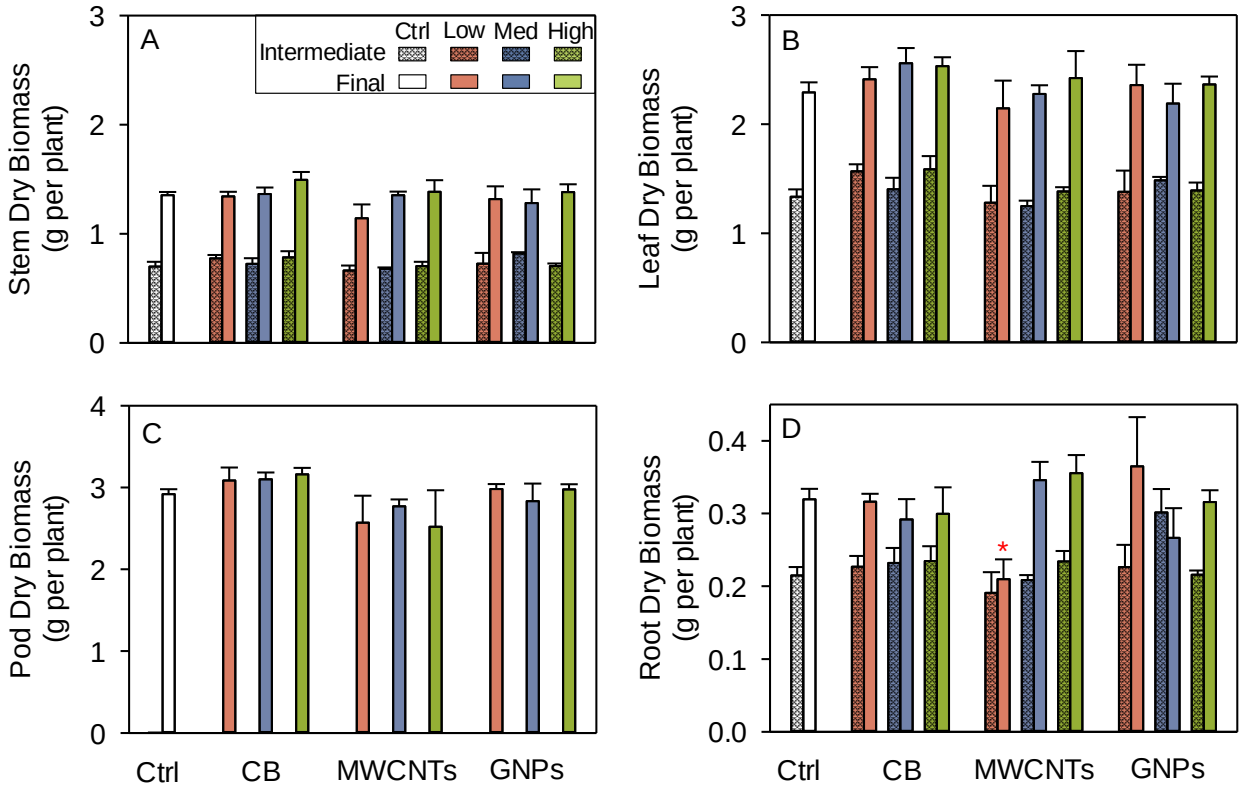

Figure S3. Soybean (A) stem, (B) leaf, (C) pod and (D) root dry biomass according to treatment, at the intermediate (Table S6) and final (Table S7) harvests, respectively. Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. Error bars are ±SE (n = 3 plants at intermediate harvest; n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments at the final harvest). *$P < 0.05$, as compared to the control (red *: final harvest).

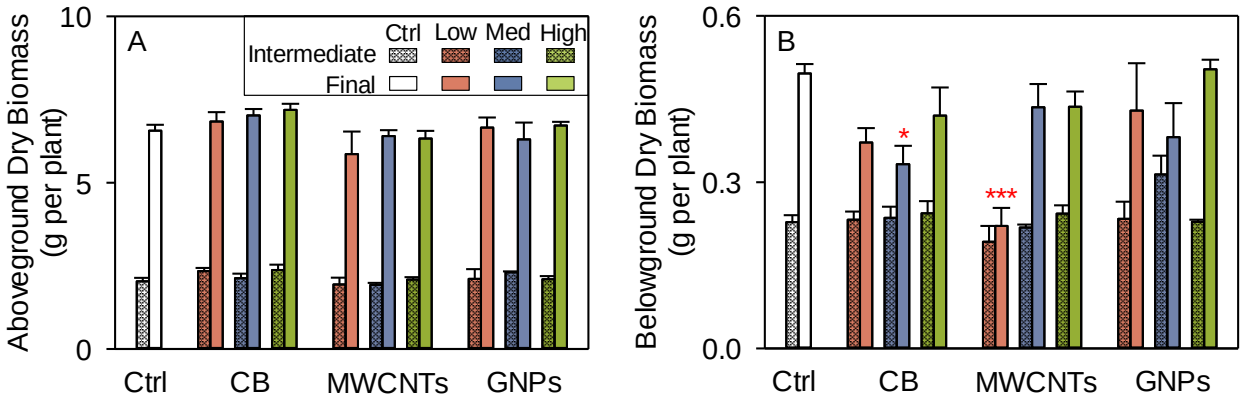

Figure S4. Soybean (A) aboveground and (B) belowground dry biomass at the intermediate and final harvests, respectively. Aboveground biomass was calculated as the sum of stem, leaf, and pod biomasses (Tables S6 and S7). Belowground biomass was calculated as the sum of root and nodule biomasses (Tables S6 and S7). Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. Error bars are ±SE (n = 3 plants at the intermediate harvest; n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments at the final harvest). *P < 0.05, ***P < 0.001, as compared to the control (red *: final harvest).

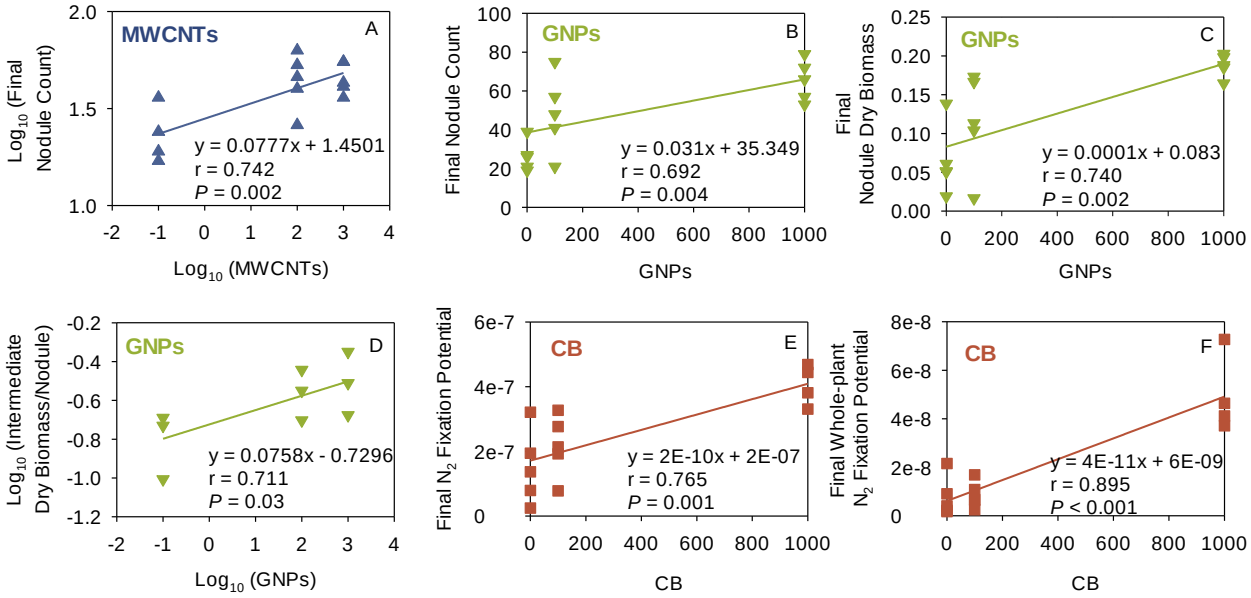

Figure S5. Summary of significant inverse dose-response relationships (ANOVA, $P < 0.05$) between CNM soil concentration and (A–B) final nodule count (Table S11), (C) final dry nodule biomass per plant (Table S7), (D) intermediate dry biomass per nodule (Table S10), (E) final N_2 fixation potential, and (F) final whole-plant N_2 fixation potential (Table S11). In all graphs, the control is excluded. CB = carbon black (red squares), MWCNTs = multi-walled carbon nanotubes (blue up triangles), and GNPs = graphene nanoplatelets (green down triangles). CNM concentrations were 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. For each treatment, n = 3 plants at the intermediate harvest; n = 5 plants, except n = 4 for the 1000 mg kg^{-1} CB and 0.1 mg kg^{-1} MWCNT treatments at the final harvest. Solid regression lines and the associated line equations within each graph provide the correlation results, including correlation coefficients (r) with significances (*P*). For the CB treatments, both the significant correlations were fitted by linear models (E and F). However, for the MWCNT treatments, the correlation was significant when data were fitted by a power model; hence data for both the x and y-axes of the figure (A) for MWCNT treatments were logarithmically transformed. For the GNP treatments, the final nodule count (B) and final dry nodule biomass (C) were fitted by linear models, while the intermediate dry biomass per nodule (D) was better fitted by a power function.

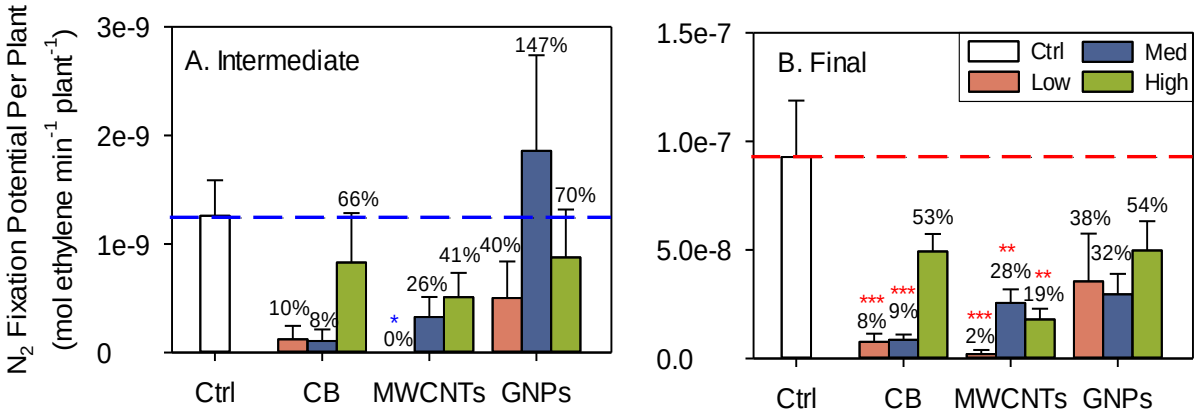

Figure S6. Whole-plant N_2 fixation potential (mol ethylene min^{-1} $plant^{-1}$), calculated as the product of the dry nodule biomass per plant (Figure 3B; Tables S6 and S7) and the N_2 fixation potential which had been normalized to dry nodule biomass (Figure 3D; Tables S10 and S11), according to treatment, at (A) the intermediate and (B) the final harvests. Ctrl = control without nanomaterial amendment, CB = carbon black, MWCNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. Low, Med (medium), and High concentrations correspond to 0.1, 100, and 1000 mg kg^{-1} nanomaterial on a dry soil basis. Error bars are ±SE (n = 3 plants at the intermediate harvest; n = 5 plants, except n = 4 for the Ctrl, CB_High and MWCNT_Low treatments at the final harvest). Calculation of the whole-plant N_2 fixation potential was made for each individual plant, with the mean and SE calculated for each treatment. Dashed lines indicate control levels, with numbers above each bar representing the percentage relative to the control. *P < 0.05, **P < 0.01, ***P < 0.001, as compared to the control (blue *: intermediate, red *: final).

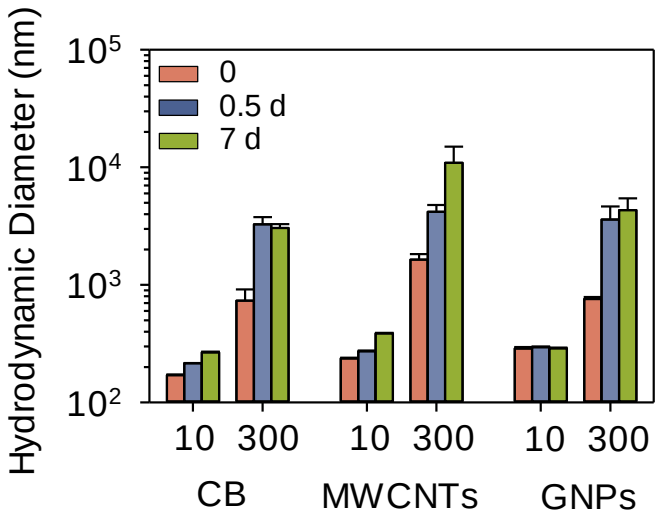

Figure S7. Hydrodynamic diameters of 10 and 300 mg L^{-1} carbon black (CB), multi-walled carbon nanotubes (MWCNTs) or graphene nanoplatelets (GNPs) in the filtered soil extract as measured by dynamic light scattering (DLS) over 7 d. Error bars are ±SE (n = 3).

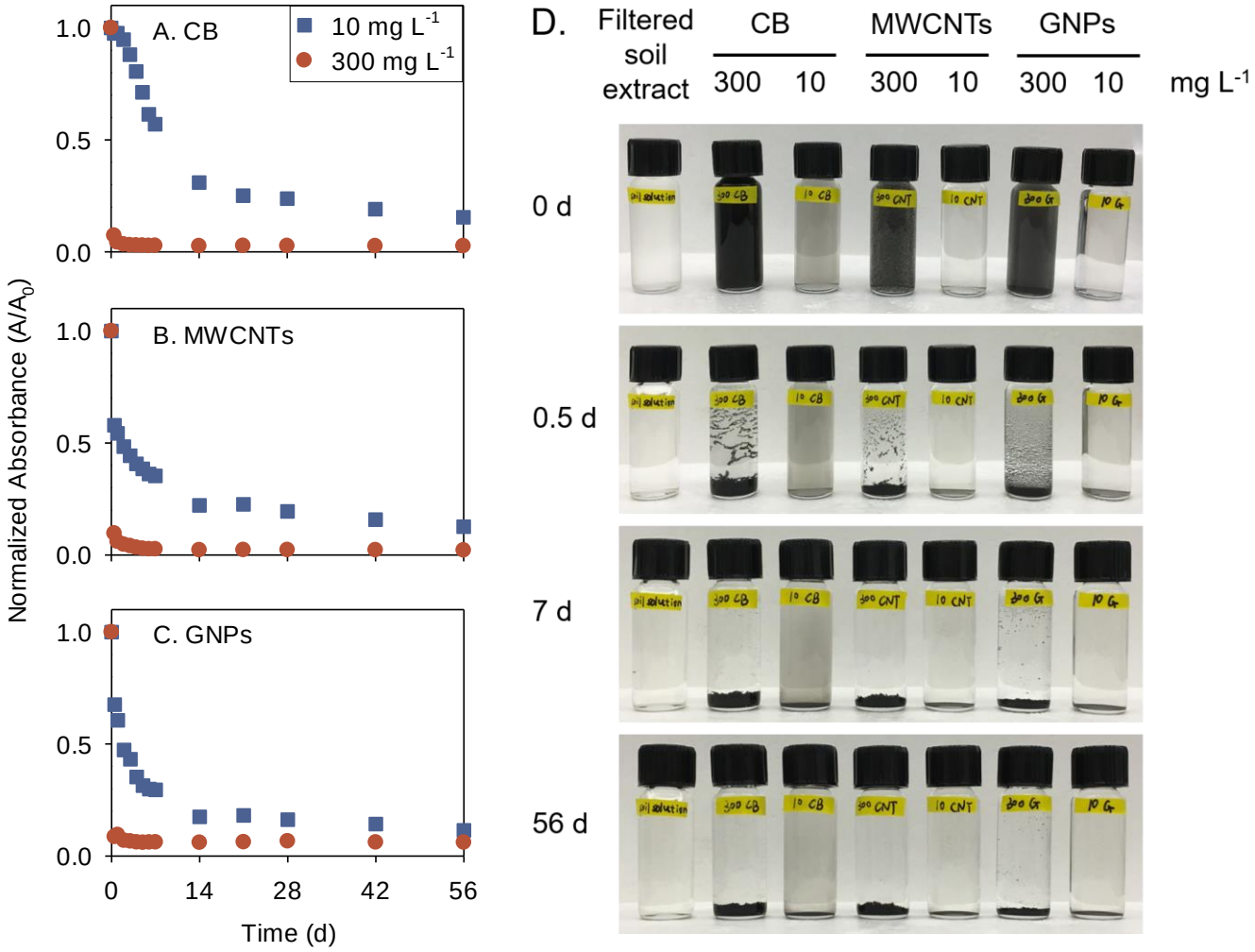

Figure S8. Stability of 10 or 300 mg L^{-1} CNMs in the filtered soil extract as indicated by (A–C) the time course to 56 d of normalized nanomaterial suspension absorbance at 600 nm (A/A_0, where A_0 was at time 0 of the experiment; the suspension absorbance at 600 nm used as a proxy for suspended nanomaterial concentration), and as indicated visually (D) at t = 0, 0.5, 7 or 56 days. CB = carbon black, MWCNTs = multi-walled carbon nanotubes, and GNPs = graphene nanoplatelets.

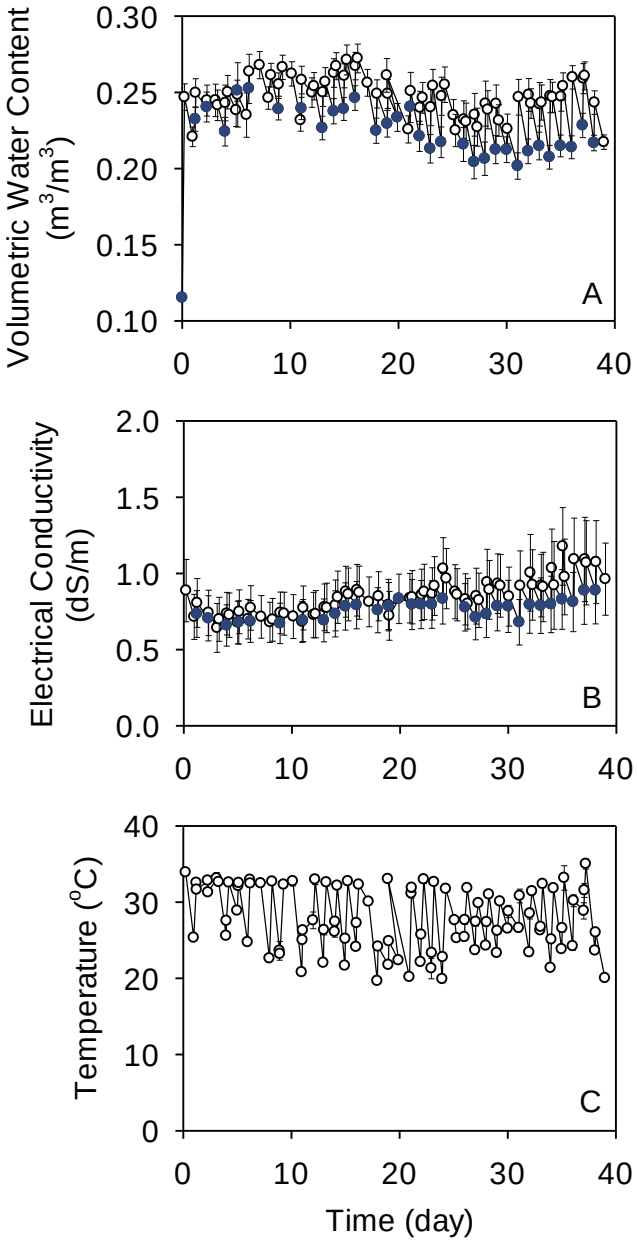

Figure S9. Soil environmental characteristics in planted soybean mesocosms over time, by *in situ* measurements (all circles; using Decagon Model 5TE sensors) in seven pots distributed across the treatments and replicates (see Methods): (A) volumetric water content, (B) electrical conductivity, and (C) temperature. Filled circles indicate that irrigation occurred immediately after the measurement. Error bars are ±SE (n = 7).

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