10.1071/EN19047_AC

Environmental Chemistry 2019, 16(6), 482-493

Supplementary Material

Effects of Carbonaceous Nanomaterials on Soil-Grown Soybeans under Combined Heat and Insect Stresses

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Number of Pages: 15

Number of Tables: 8

Number of Figures: 6

Table S1. CNM physicochemical characteristics[A]

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

[A]Data have been reported previously (Wang et al. 2017; Wang et al. 2018). CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets. [B]Reported by the manufacturers. [C]Measured by thermogravimetric analysis (TGA). [D]Measured from transmission electron microscopy (TEM) images. N/A not available.

Table S2. Soil characteristics

Characteristic	Result
Saturation water content (%)	28
pH	7.26
Estimated Soluble Salts (EC, dS m^{-1})	2.73
Ca, (saturated paste extract, meq L^{-1})	12.75
Mg, (saturated paste extract, meq L^{-1})	5.94
Na, (saturated paste extract, meq L^{-1})	7.53
Cl, (saturated paste extract, meq L^{-1})	7.54
B, (saturated paste extract, mg L^{-1})	0.43
HCO_3^-, (saturated paste extract, meq L^{-1})	1.2
CO_3^{2-}, (saturated paste extract, meq L^{-1})	<0.1
Total N (%)	0.073
Total C (%)	0.74
NH_4^+ (N, extractable, ppm)	1.14
NO_3^- (N, extractable, ppm)	18.7
P (Olsen, extractable, ppm)	48.3
K (exchangeable, ppm)	296
K (exchangeable, meq per 100 g)	0.76
Na (exchangeable, ppm)	109
Na (exchangeable, meq per 100 g)	0.47
Ca (exchangeable, meq per 100 g)	7.38
Mg (exchangeable, meq per 100 g)	2.22
Cation Exchange Capacity (CEC, meq per 100 g)	10.8
Organic Matter (loss on ignition, LOI, %)	1.41
Zn (DTPA extraction, ppm)	5.9
Mn (DTPA extraction, ppm)	60.1
Cu (DTPA extraction, ppm)	3.3
Fe (DTPA extraction, ppm)	111.1
Zn (Total, ppm)	71
Mn (Total, ppm)	336
Fe (Total, ppm)	14900
Cu (Total, ppm)	23
Sand (%)	66
Silt (%)	22
Clay (%)	12

Table S3. Soybean plant stem elongation rate constant, according to treatment[A]

	Linear Growth[B]				Exponential Growth[C]			
	Rate Constant (cm d^{-1})	**Time Period (d)**	**R^2**	***P***	**Rate Constant (d^{-1})**	**Time Period (d)**	**R^2**	***P***
Ctrl_1	0.32	0–28	0.983	0.001	0.03	0–28	0.977	0.001
Ctrl_2	0.15	0–35	0.962	0.001	0.02	0–35	0.948	0.001
Ctrl_3	0.26	0–35	0.970	0.000	0.02	0–35	0.917	0.003
Ctrl_4	0.26	0–35	0.927	0.002	0.06	0–14	0.864	*0.240*[D]
Ctrl_5	0.27	0–42	0.977	0.000	0.03	0–35	0.979	0.001
CB_1	0.26	0–14	0.996	0.042	0.02	0–14	0.987	*0.072*
CB_2	0.34	0–14	0.997	0.038	0.03	0–14	**1.000**[E]	0.006
CB_3	0.31	0–35	0.943	0.001	0.02	0–35	0.873	0.006
CB_4	0.36	0–35	0.942	0.001	0.07	0–14	0.906	*0.198*
CB_5	0.40	0–35	0.972	0.000	0.04	0–28	0.986	0.001
CNT_1	0.21	0–35	0.937	0.002	0.02	0–35	0.881	0.006
CNT_2	0.30	0–35	0.810	0.014	0.08	0–14	0.886	*0.219*
CNT_3	0.28	0–21	0.956	0.022	0.03	0–21	**0.980**	0.010
CNT_4	0.30	0–28	0.951	0.005	0.04	0–21	0.891	*0.056*
CNT_5	0.33	0–35	0.993	0.000	0.03	0–35	0.941	0.001
GNP_1	0.29	0–35	0.973	0.000	0.02	0–35	0.953	0.001
GNP_2	0.24	0–35	0.968	0.000	0.03	0–35	0.951	0.001
GNP_3	0.27	0–35	0.947	0.001	0.03	0–28	0.897	0.015
GNP_4	0.25	0–42	0.890	0.001	0.07	0–14	0.854	*0.250*
GNP_5	0.41	0–21	0.983	0.009	0.05	0–21	0.925	0.038

[A]Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. Values are for individual plants that served as independent replicates (i.e., 1, 2, 3, 4, and 5) for each treatment. Each replicate plant was examined separately to calculate the growth rate constant. [B]Zero-order stem elongation rate constant was calculated as the slope of a regression line from the linear region of the plot of stem length *versus* time (Fig. 1A). [C]First-order stem 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. [D]Italicized significances (*P* values) indicate not significant ($P > 0.05$). [E]Bold coefficient of determination (R^2) values indicate for that replicate plant, although correlations were significant using both models ($P < 0.05$), the exponential growth model was a better fit (with a higher R^2 and a lower *P* value) than the linear growth model for the stem length data. Overall, the correlation results suggested the zero-order (linear) model was a more significant and representative fit for the stem length data. Mean values and standard errors (SEs) of stem elongation rate constants for each treatment are summarized in Table S4.

Table S4. Summary of average stem elongation and leaf cover expansion rate constants, maximum leaf cover, and total leaf area at harvest for soybean plants, according to treatment[A]

Treatment	Linear Growth		Exponential Growth		Max. Leaf Cover (% coverage of soil)	Final Total Leaf Area (cm^2)
	Stem Elongation Rate Constant ($cm\ d^{-1}$)[B]	Leaf Cover Expansion Rate Constant (% d^{-1})[C]	Stem Elongation Rate Constant (d^{-1})[B]	Leaf Cover Expansion Rate Constant (d^{-1})[C]		
Ctrl	0.25 ± 0.03	0.43 ± 0.08	0.03 ± 0.01	0.05 ± 0.01	31.7 ± 5.7	31.2 ± 5.2
CB	0.33 ± 0.02	0.47 ± 0.11	0.04 ± 0.01	0.10 ± 0.05	39.3 ± 6.5	36.3 ± 2.5
CNT	0.29 ± 0.02	0.57 ± 0.11	0.04 ± 0.01	0.06 ± 0.01	40.9 ± 6.6	29.6 ± 1.3
GNP	0.30 ± 0.03	0.45 ± 0.11	0.04 ± 0.01	0.06 ± 0.02	31.1 ± 2.3	38.5 ± 2.6

[A]Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. All data are shown as mean ± SE (n = 5 plants). [B]Stem elongation rate constants for individual plants for each treatment are shown in Table S3. [C]Leaf cover expansion rate constants for individual plants for each treatment are shown in Table S5. Stem elongation rate constant, leaf cover expansion rate constant, maximum leaf cover, and final total leaf area did not vary significantly between treatments ($P > 0.05$).

Table S5. Soybean plant leaf cover expansion rate constant, according to treatment[A]

	Linear Growth[B]				Exponential Growth[C]			
	Rate Constant (% d^{-1})	**Time Period (d)**	**R^2**	***P***	**Rate Constant (d^{-1})**	**Time Period (d)**	**R^2**	***P***
Ctrl_1	0.282	0–35	0.893	0.004	0.023	0–35	0.886	0.005
Ctrl_2	0.265	0–14	0.931	*0.170*	0.042	0–14	0.960	*0.127*[D]
Ctrl_3	0.639	0–42	0.876	0.002	0.040	0–42	**0.891**[E]	0.001
Ctrl_4	0.343	0–14	0.997	0.033	0.050	0–14	0.978	*0.095*
Ctrl_5	0.637	0–49	0.869	0.001	0.074	0–49	**0.925**	0.000
CB_1	0.289	0–14	0.917	*0.187*	0.029	0–14	0.938	*0.160*
CB_2	0.258	0–42	0.784	0.008	0.022	0–42	0.752	0.011
CB_3	0.499	0–14	0.995	0.045	0.055	0–14	**0.998**	0.025
CB_4	0.876	0–42	0.914	0.001	0.106	0–14	**1.000**	0.000
CB_5	0.419	0–14	0.994	0.049	0.273	0–14	0.897	*0.208*
CNT_1	0.427	0–49	0.933	0.000	0.036	0–49	0.889	0.000
CNT_2	0.419	0–14	1.000	0.006	0.044	0–14	0.991	*0.061*
CNT_3	0.730	0–42	0.918	0.001	0.056	0–42	0.917	0.001
CNT_4	0.355	0–14	1.000	0.004	0.054	0–14	0.990	*0.064*
CNT_5	0.907	0–42	0.942	0.000	0.118	0–14	1.000	0.007
GNP_1	0.243	0–14	0.996	0.040	0.034	0–14	**1.000**	0.003
GNP_2	0.262	0–42	0.695	0.020	0.029	0–42	0.565	0.051
GNP_3	0.475	0–14	0.991	*0.060*	0.048	0–14	0.967	*0.117*
GNP_4	0.437	0–14	0.999	0.019	0.074	0–14	0.971	*0.109*
GNP_5	0.830	0–28	0.987	0.001	0.130	0–21	0.963	0.019

[A]Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. Values are for individual plants that served as independent replicates (i.e., 1, 2, 3, 4, and 5) for each treatment. Each replicate plant was examined separately to calculate the growth rate constant. [B]Zero-order leaf cover expansion rate constant was calculated as the slope of a regression line from the linear region of the plot of leaf cover *versus* time (Fig. 1B). [C]First-order 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. [D]Italicized significances (*P* values) indicate not significant ($P > 0.05$). [E]Bold coefficients of determination (R^2) values indicate for that replicate plant, although correlations were significant using both models ($P < 0.05$), the exponential growth model was a better fit (with a higher R^2 and a lower *P* value) than the linear growth model for the leaf cover data. Overall, the correlation results suggested the zero-order (linear) model was a more significant and representative fit for the leaf cover data. Mean values and SEs of leaf cover expansion rate constants for each treatment are summarized in Table S4.

Table S6. Soybean plant average seed count per pod, pod length, and pod width at harvest, according to treatment[A]

Treatment	Seed Count Per Pod	Pod Length (cm)	Pod Width (cm)
Ctrl	1.7 ± 0.2	3.0 ± 0.2	0.8 ± 0.0
CB	1.2 ± 0.1	2.4 ± 0.1	0.7 ± 0.0
CNT	1.5 ± 0.2	2.7 ± 0.2	0.8 ± 0.0
GNP	1.6 ± 0.2	2.8 ± 0.2	0.7 ± 0.0

[A]Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. All data are shown as mean ± SE (n = 5 plants). Seed count and pod size (both length and width) did not vary significantly between treatments ($P > 0.05$).

Table S7. Soybean plant moisture content (g H_2O g^{-1} wet biomass) by each tissue type at harvest, according to treatment[A]

Treatment	Stem	Leaf	Pod	Root
Ctrl	0.71 ± 0.02	0.58 ± 0.04	0.66 ± 0.02	0.78 ± 0.02
CB	0.69 ± 0.02	0.60 ± 0.04	0.69 ± 0.01	0.75 ± 0.03
CNT	0.68 ± 0.01	0.64 ± 0.02	0.65 ± 0.01	0.79 ± 0.01
GNP	0.69 ± 0.00	0.51 ± 0.05	0.64 ± 0.01	0.79 ± 0.01

[A]Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. All data are shown as mean ± SE (n = 5 plants). Moisture content of each tissue type did not vary significantly between treatments ($P > 0.05$).

Table S8. Soybean nodule count per plant and nodule wet biomass (g plant^{-1}) at harvest, according to treatment[A]

	Nodule Count Per Plant	Nodule Wet Biomass (g plant^{-1})
Ctrl_1	0	0
Ctrl_2	2	0.0016
Ctrl_3	4	0.0017
Ctrl_4	0	0
Ctrl_5	0	0
CB_1	1	0.0002
CB_2	1	0.0010
CB_3	1	0.0006
CB_4	0	0
CB_5	0	0
CNT_1	0	0
CNT_2	2	0.0005
CNT_3	0	0
CNT_4	0	0
CNT_5	0	0
GNP_1	1	0.0002
GNP_2	0	0
GNP_3	0	0
GNP_4	0	0
GNP_5	0	0

[A]Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. Values are for individual plants that served as independent replicates (i.e., 1, 2, 3, 4, and 5) for each treatment.

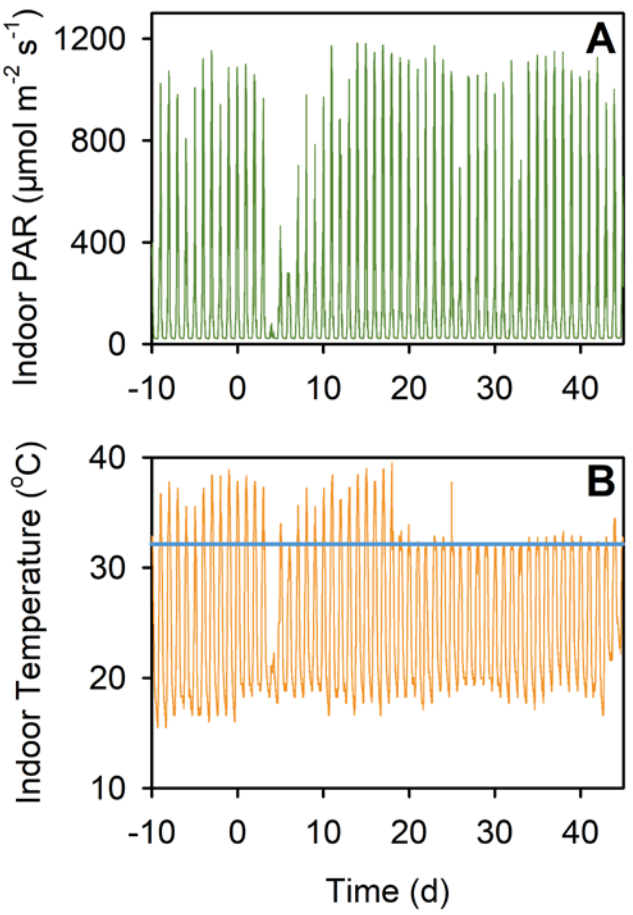

Figure S1. Greenhouse indoor climatic conditions over time: (A) photosynthetically active radiation (PAR) and (B) temperature. Day 0 = transplantation to pots; Day -10 = 10 days prior to transplantation (i.e., seed sowing into pellets). The blue horizontal line in (B) indicates the nominal maximum temperature set in the greenhouse (32 °C). Temperatures that were considerably higher than this set nominal maximum temperature occurred inside the greenhouse routinely during Day -10 to Day 18 (18 days post-transplanting).

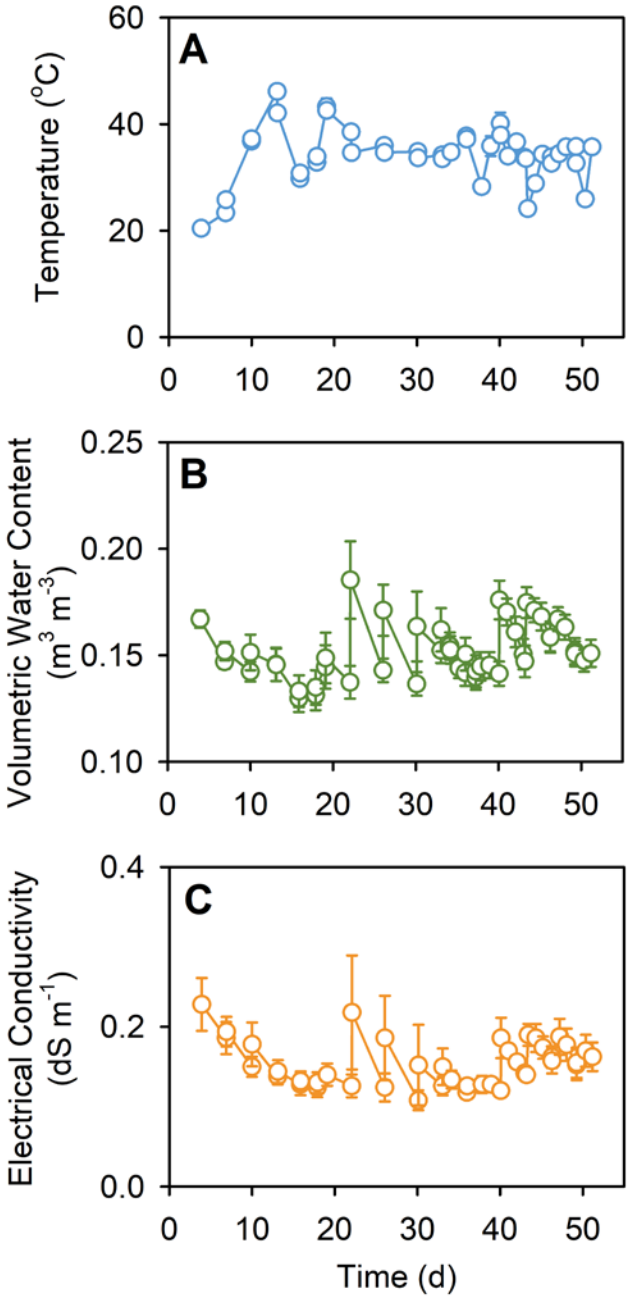

Figure S2. Soil environmental characteristics in planted soybean mesocosms over time, by *in situ* measurements using Decagon Model 5TE sensors in pots distributed across the treatments and replicates (see Experimental): (A) temperature, (B) volumetric water content, and (C) electrical conductivity. Error bars are ± SE (n = 5). To maintain an average soil water content of 0.15 m^3 m^{-3}, irrigation took place on Day 7, 10, 13, 16, 18, 19, 22, 26, 30, 33, 36, 37, 40, 43, 46, and 49 post-transplanting, respectively.

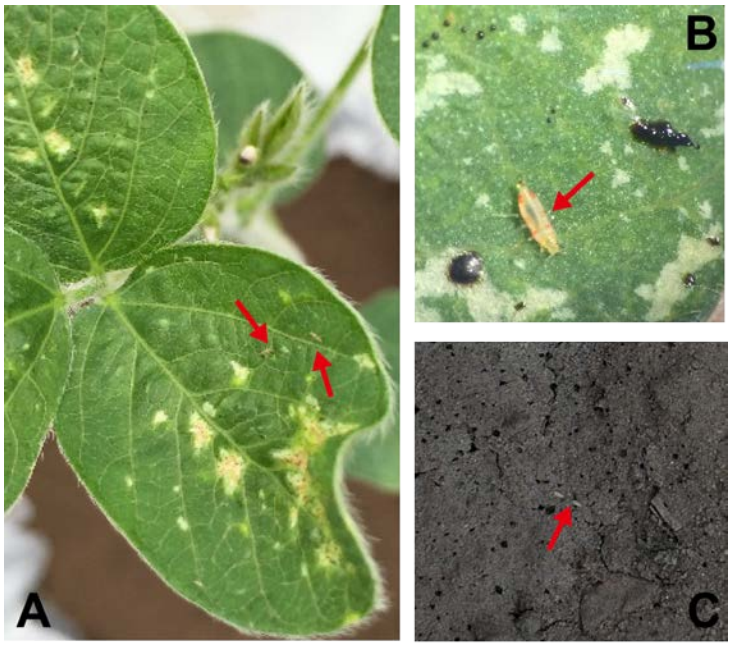

Figure S3. Photographs of thrips (indicated by arrows) observed (A) on a leaf of a soybean plant grown in the greenhouse, (B) on a soybean leaf viewed under a dissecting microscope, and (C) on surface soil in a pot inside the greenhouse.

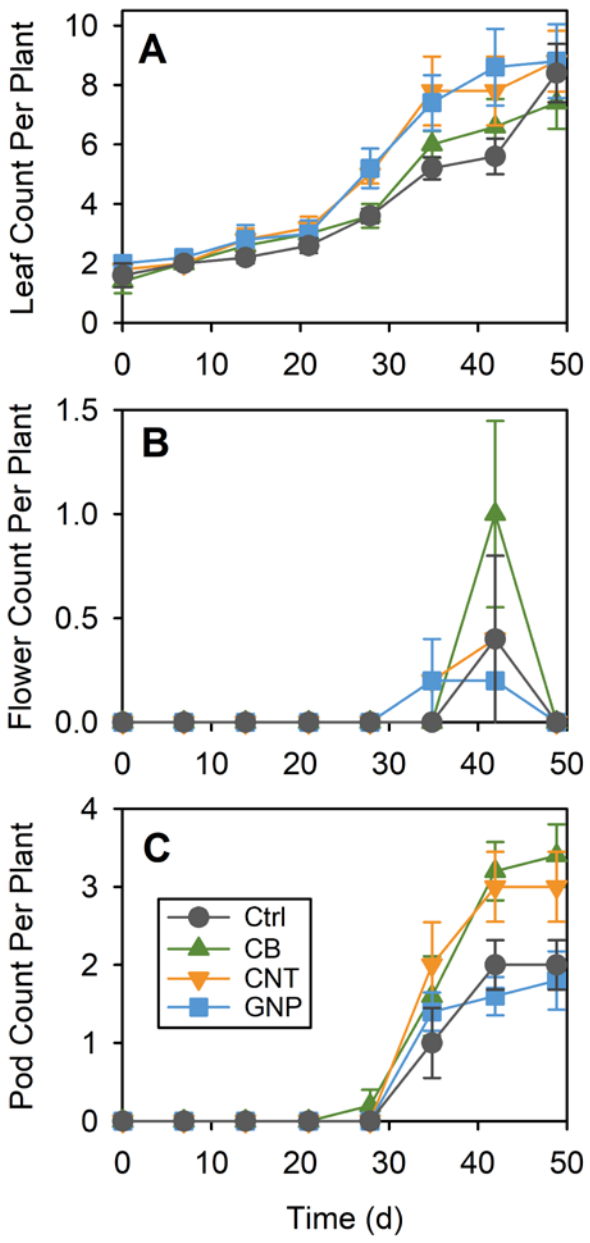

Figure S4. Time course of soybean plant vegetative and reproductive development post-transplantation according to either (A) leaf, (B) flower, or (C) pod count per plant. Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. Error bars are ± SE (n = 5 plants).

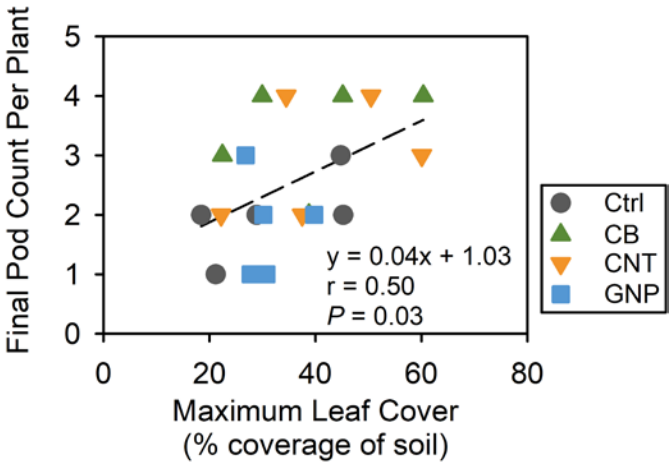

Figure S5. Significant linear correlation between soybean plant final pod count (Fig. S4C) and maximum leaf cover (Table S4) for all replicate plants across all treatments. Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis.

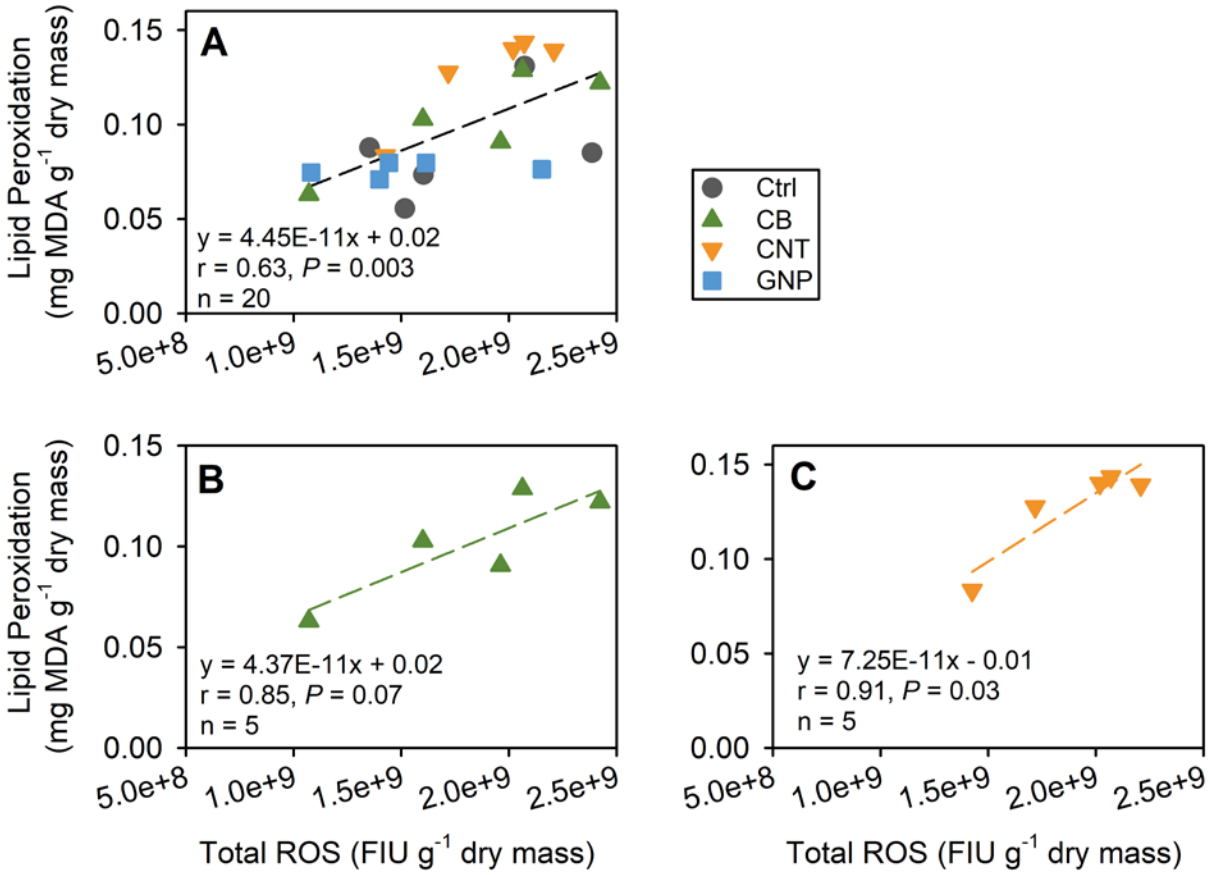

Figure S6. Linear correlations between soybean plant leaf lipid peroxidation (expressed as malondialdehyde (MDA) concentration normalized to dry leaf biomass) and leaf total reactive oxygen species (ROS, expressed as fluorescence intensity units (FIU) normalized to dry leaf biomass) measured at harvest for all replicate plants (A) across all treatments, or within the (B) CB or (C) CNT treatment. The relationships were not significant when only evaluating the control or GNP treatment. Ctrl = control without nanomaterial amendment; CB = carbon black, CNT = multi-walled carbon nanotubes, and GNP = graphene nanoplatelets, all with a concentration of 1000 mg kg^{-1} nanomaterial on a dry soil mass basis. MDA and ROS concentrations are as per Table 2.

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