Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information: Agricultural soil pore waters reduce copper oxide nanoparticle-induced root shortening in wheat

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Table S1: Experimental setup for non-*Pc*O6 experiment; + = present and - = absent.

Group	Treatment	Wheat	CuO	*PcO6*	3.4 mM $Ca(NO_3)_2$	AgrM SPW	OrgM SPW	GarM SPW
Wheat controls	1	+	-	-	+	-	-	-
	2	+	-	-	-	+	-	-
	3	+	-	-	-	-	+	-
	4	+	-	-	-	-	-	+
CuO NPs controls	5	-	+	-	+	-	-	-
	6	-	+	-	-	+	-	-
	7	-	+	-	-	-	+	-
	8	-	+	-	-	-	-	+
SPW controls	9	-	-	-	+	-	-	-
	10	-	-	-	-	+	-	-
	11	-	-	-	-	-	+	-
	12	-	-	-	-	-	-	+
CuO NPs treatments	13	+	+	-	+	-	-	-
	14	+	+	-	-	+	-	-
	15	+	+	-	-	-	+	-
	16	+	+	-	-	-	-	+

Table S2: Experimental setup for plants with *Pc*O6 colonization; + = present and - = absent.

PcO6 experiment setup								
Group	Treatment	Wheat	CuO	*PcO6*	3.4 mM $Ca(NO_3)_2$	AgrM SPW	OrgM SPW	GarM SPW
Wheat controls	1	+	-	+	+	-	-	-
	2	+	-	+	-	+	-	-
	3	+	-	+	-	-	+	-
	4	+	-	+	-	-	-	+
CuO NPs controls	5	-	+	+	+	-	-	-
	6	-	+	+	-	+	-	-
	7	-	+	+	-	-	+	-
	8	-	+	+	-	-	-	+
SPW controls	9	-	-	+	+	-	-	-
	10	-	-	+	-	+	-	-
	11	-	-	+	-	-	+	-
	12	-	-	+	-	-	-	+
CuO NPs treatments	13	+	+	+	+	-	-	-
	14	+	+	+	-	+	-	-
	15	+	+	+	-	-	+	-
	16	+	+	+	-	-	-	+

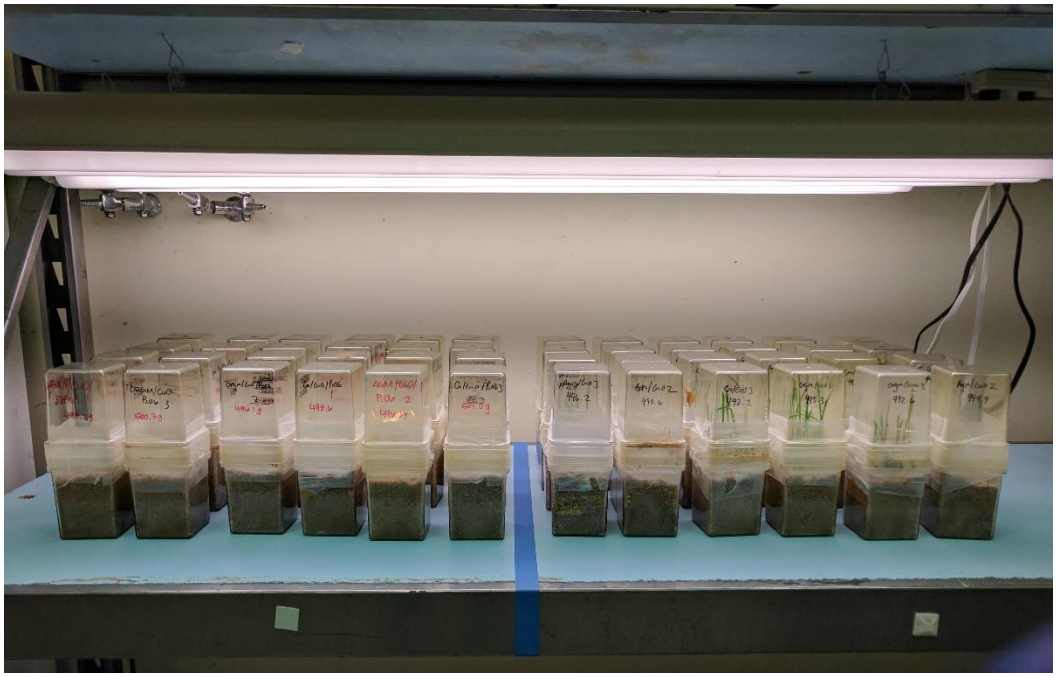

Fig. S1: Setup of 48 magenta boxes under grow lights, with/without wheat.

Table S3: Characteristics of soils. Soil samples were collected in 2014 for preliminary experiments in 2015-2016 and tested at a laboratory certified under the North American Proficiency Testing Program for Agricultural Labs. Soils were re-collected in 2016 for use in this study.

Soil characteristics			
Soil abbreviation	OrgM	AgrM	GarM
Name origin	Organic farm, Millville	Agricultural field, Millville	Community garden, Millville
Soil series, texture	Millville silt loam	Millville silt loam	Millville silt loam
Particle size distribution (% sand/silt/clay)	19/56/26	22/56/23	13/59/28
Cultivation	organic certified	commercial production	unknown amendments
Crop	continuous green cover	winter wheat	varied (community garden)
pH	7.7	7.8	7.8
EC (μS/cm)	1040	500	600
Phosphorus (mg/kg)	52.1	10.1	19.3
Potassium (mg/kg)	434	111	369
Ammonium (mg/kg N)	2.01	2.43	< 1.25
Nitrate (mg/kg N)	31.8	11.5	10.4
Sulfate (mg/kg S)	6.5	3.6	3.3
Organic matter (% of whole soil)	5.6	3.0	4.1
Cation exchange capacity (cmol/kg)	20.0	13.8	21.0
Calcium carbonate (%)	14.6	14.1	16.1
Saturation point (%)	46.5	41.0	45.5
DTPA – Fe (mg/kg)	9.8	8.95	10.5
DTPA – Cu (mg/kg)	1.44	1.29	2.72
DTPA – Mn (mg/kg)	16.3	14.1	13.8
DTPA – Zn (mg/kg)	3.07	1.66	1.62

Table S4: Full characterization of SPWs. Measurements = average of 3 replicates. "<" = below detection, followed by detection limit.

Soil name	OrgM	AgrM	GarM
Na (mg/L)	11.8	9.4	27.5
Mg (mg/L)	55.7	17.9	145.9
Al (µg/L)	8.3	6.9	<4
K (mg/L)	28.7	4.2	299.1
Ca (mg/L)	167.6	97.4	372.3
V (µg/L)	5.2	5.4	7.5
Cr (µg/L)	9.6	1.1	1.5
Mn (µg/L)	5.5	12.4	118.0
Fe (µg/L)	67.1	14.6	53.9
Co (µg/L)	1.6	1.5	11.1
Ni (µg/L)	5.7	6.7	20.3
Cu (µg/L)	13.4	22.8	48.4
Zn (µg/L)	51.1	34.1	48.7
As (µg/L)	7.2	6.1	18.8
Se (µg/L)	1.0	4.3	1.8
Sr (µg/L)	668.7	97.7	1124.0
Ba (µg/L)	402.0	161.6	640.4
Gluconate (mg/L)	1.9	3.9	< 0.5
Lactate (mg/L)	< 0.5	< 0.5	< 0.5
Acetate (mg/L)	0.7	< 0.5	< 0.5
Isobutyrate (mg/L)	< 0.5	< 0.5	<0.5
Butyrate (mg/L)	< 0.5	< 0.5	1.03
Isovalerate (mg/L)	< 0.5	< 0.5	<0.5
Valerate (mg/L)	< 0.5	< 0.5	< 0.5
Chloride (mg/L)	50.2	5.6	61.6
Nitrite (mg/L N)	5.7	11.8	2.80
Nitrate (mg/L N)	148.6	12.6	573.8
Sulfate (mg/L)	36.8	18.4	194.8
Oxalate (mg/L)	< 0.5	< 0.5	< 0.5
Phosphate (mg/L P)	< 0.5	< 0.5	1.99
Citrate (mg/L)	< 0.5	< 0.5	< 0.5
Alkalinity (mg /L $CaCO_3$)	340	450	490
EC (µS/cm)	735	391	3380
DOC (mg/L C)	42.7	73.4	305
Humic acids (mg/L C)	<0.8	<0.8	4.3
Fulvic acids (mg/L C)	28.3	38.0	165

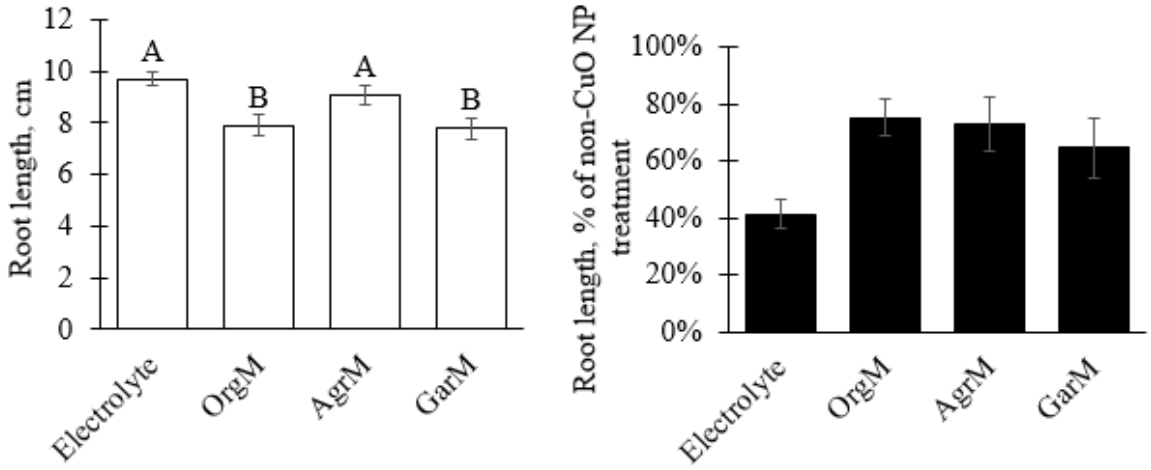

Fig. S2. Root length of wheat plants grown in non-CuO NP treatments (left) and root length of wheat plants grown with CuO NPs normalized to the non-CuO NP treatment root length. The error bars show 95% confidence intervals (n = 12 per bar) to illustrate the spread of data, but do not determine significant differences. Bars with differing letters (A, B, etc.) are statistically different ($p < 0.05$) by Tukey's HSD after two-way ANOVA. All root metals required a logarithmic transformation to maintain normal distribution of residuals.

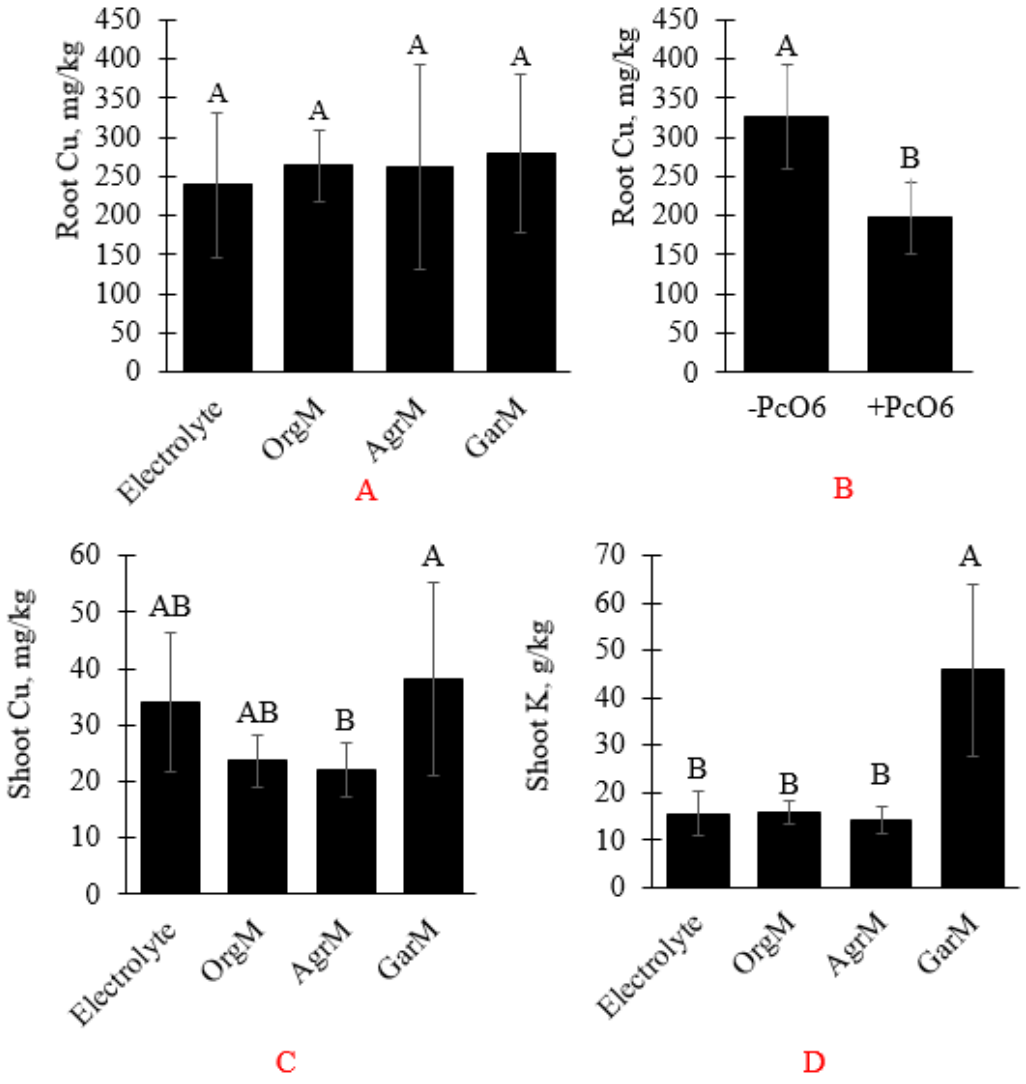

Fig. S3. Root and shoot metal contents in plants grown with CuO NPs as affected by RS (A, C, D) or *Pc*O6 (B). Bars are average of 12 (A, C, D) or 24 (B) pooled replicates. The error bars show 95% confidence intervals to illustrate the spread of data, but do not determine significant differences. Bars with differing letters (A, B, etc.) are statistically different ($p < 0.05$) by Tukey's HSD after two-way ANOVA. All root metals required a logarithmic transformation to maintain normal distribution of residuals.

Table S5 Number of contaminated boxes by RS and presence/absence of wheat, CuO NPs, and *Pc*O6. Each cell contains a maximum of six boxes.

	Planted				Unplanted				
	CuO NPs		Non-CuO NPs		CuO NPs		Non-CuO NPs		
RS	*Pc*O6	Non-*Pc*O6	*Pc*O6	Non-*Pc*O6	*Pc*O6	Non-*Pc*O6	*Pc*O6	Non-*Pc*O6	**Totals**
Control	0	5	0	6	0	0	0	3	14
OrgM	0	6	0	6	0	0	0	4	16
AgrM	0	5	1	5	0	0	0	3	14
GarM	0	6	0	6	0	1	0	3	16
Totals	0	22	1	23	0	1	0	13	60

Discussion of bacterial contamination in this study

*Pc*O6 formed wet, orange colonies on Luria Broth (LB) medium, whereas contaminating bacteria took many differing forms. Under these conditions, contamination in the growth boxes could not be completely avoided, even with autoclaving of the boxes and sand before use, thorough surface sterilization of the seeds and initial growth on sterile LB plates, and aseptic techniques. The contamination was spread evenly across RS types (29-35%), but were primarily found in planted samples compared to unplanted samples (48% versus 15%), non-*Pc*O6 samples compared to *Pc*O6 samples (61% versus 1%), and non-CuO NP samples compared to CuO NP samples (39% versus 24%) (Table S5). By a chi-squared test, RS type did not significantly impact contamination rates, but the presence of wheat ($p < 0.0001$), the lack of CuO NPs ($p = 0.0287$), and the lack of *Pc*O6 ($p < 0.0001$) increased contamination rates.

The even distribution of infection across all RSs indicated the SPWs were not the likely source. Most likely, endophytes living inside the seed grew after transplanting despite the described precautions. Endophytes are common in wheat and often these isolates have biocontrol activity (Díaz Herrera et al. 2016; Comby et al. 2017). The toxicity of CuO NPs to a variety of microbes (as seen in soils by Frenk et al. 2013, for example) and the competitive native *Pc*O6 against the microbes explains the lower rates of infection in treatments with each of those two variables.

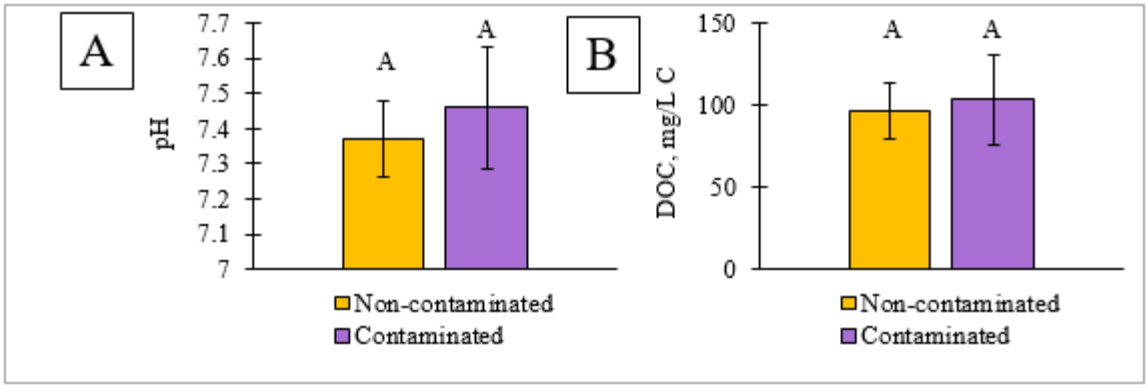

Fig. S4: pH (A) and DOC (B) in contaminated versus non-contaminated samples without wheat, *Pc*O6, or CuO NPs. Bars represent the mean of measurements and error bars represent Tukey HSD statistical significance. No significant changes occurred between sterile (non-*Pc*O6) and contaminated samples (typically also non-*Pc*O6).

Table S6 Correlations of PCA components in planted, non-*Pc*O6 samples with and without CuO NPs. Bolded correlations are significant ($p < 0.05$) and bolded, red correlations are $R > |0.5|$.

	Root length	Shoot length	Root Cu	Shoot Cu	Dissolved Cu	Free Cu^{2+}	EC	pH	DMA	Total DOC	Citrate	Malate
gluconate	**-0.365**	0.040	**0.601**	0.201	**0.656**	-0.003	0.160	0.141	0.246	**0.561**	**0.663**	**0.359**
malate	0.079	**-0.467**	**0.375**	-0.057	**0.433**	0.051	**-0.398**	**-0.356**	0.264	0.144	**0.467**	
citrate	-0.454	-0.078	**0.522**	**0.473**	**0.720**	0.112	0.078	-0.005	**0.527**	**0.486**		
Total DOC	**-0.340**	**0.297**	0.257	**0.319**	**0.506**	0.065	**0.565**	0.245	0.126			
DMA	**-0.499**	-0.212	**0.559**	**0.363**	**0.508**	**0.526**	-0.122	-0.250				
pH	-0.132	**0.623**	-0.058	0.023	0.001	**-0.463**	**0.742**					
EC	-0.244	**0.675**	0.025	0.251	0.107	-0.145						
Free Cu^{2+}	**-0.549**	-0.253	0.221	**0.436**	0.279							
Dissolved Cu	**-0.571**	-0.031	**0.791**	**0.421**								
Shoot Cu	**-0.598**	-0.048	0.140									
Root Cu	**-0.468**	-0.090										
Shoot length	-0.090											

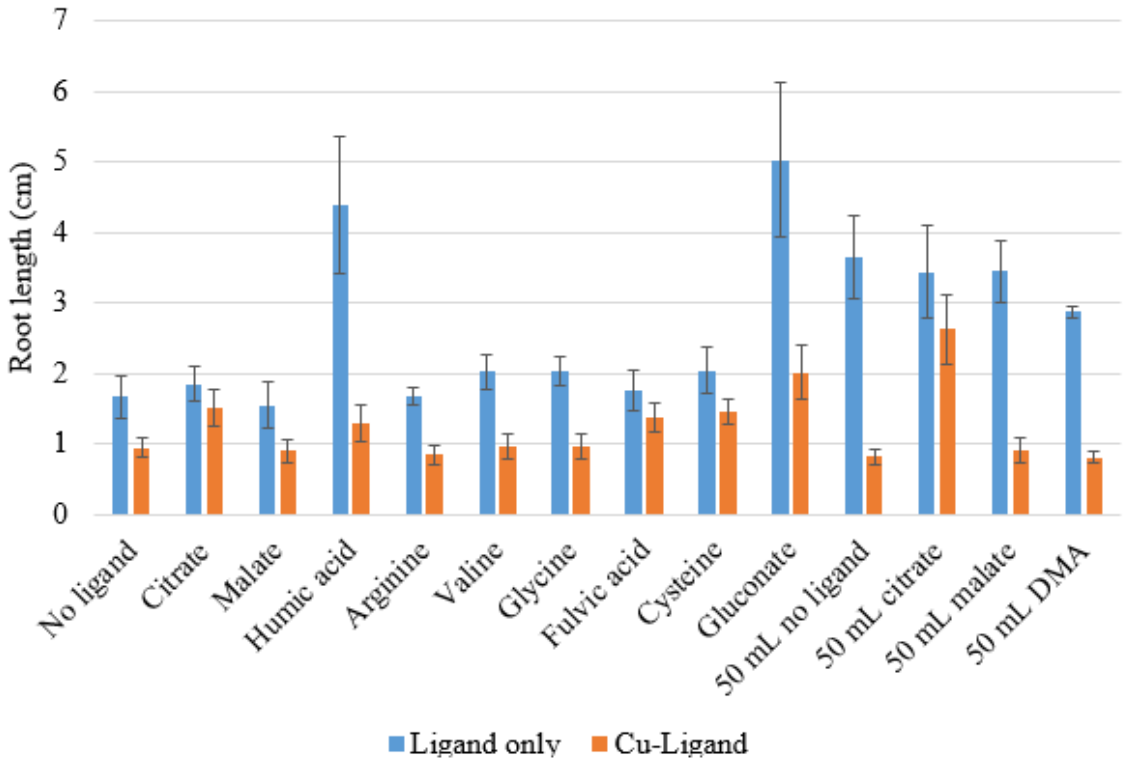

Fig. S5: Root lengths of wheat exposed for 48 h to 1.6 µM Cu and ligand (orange) or ligand only with no Cu (blue). Bars are average of 3 samples and error bars represent the standard error of the mean. NRE is calculated by dividing the (Cu-ligand root – 0.75 cm) by (ligand root – 0.75 cm), shown in Table 2 of the text. The test was conducted in 1 L solution except for the 50 mL samples on the far right. The 50 mL no ligand, malate, and citrate tests were conducted to show that while the smaller volume appeared to influence root length, the same NRE results were obtained in 50 mL as in 1 L. Ligand concentration and calculated Cu speciation are shown in Table 2 of the article.