Supplementary Information (SI) for Environmental Science: Nano.
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Supporting Information

Soil Species Sensitivity Distributions for terrestrial risk assessment of silver nanomaterials: the influence of nanomaterial characteristics and soil type

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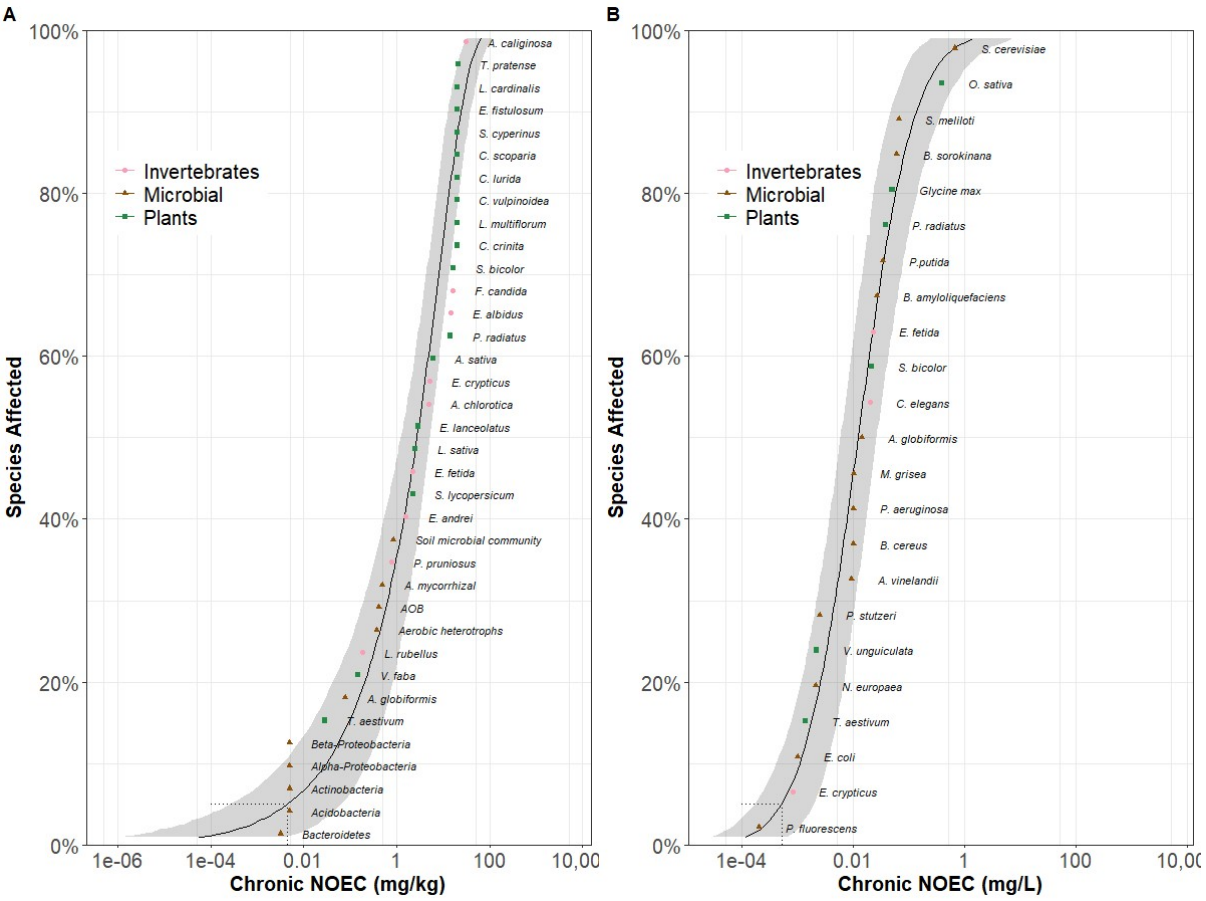
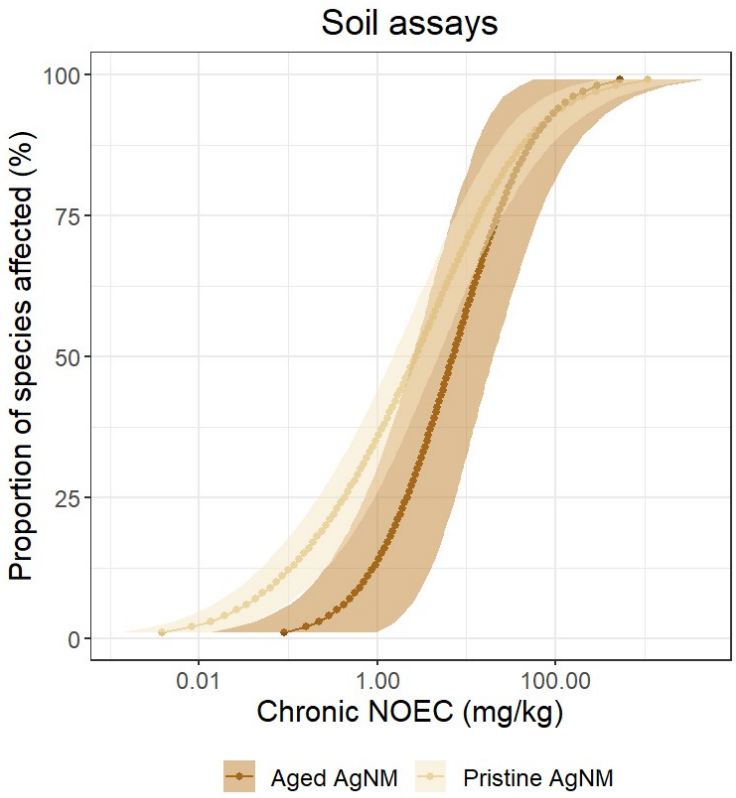
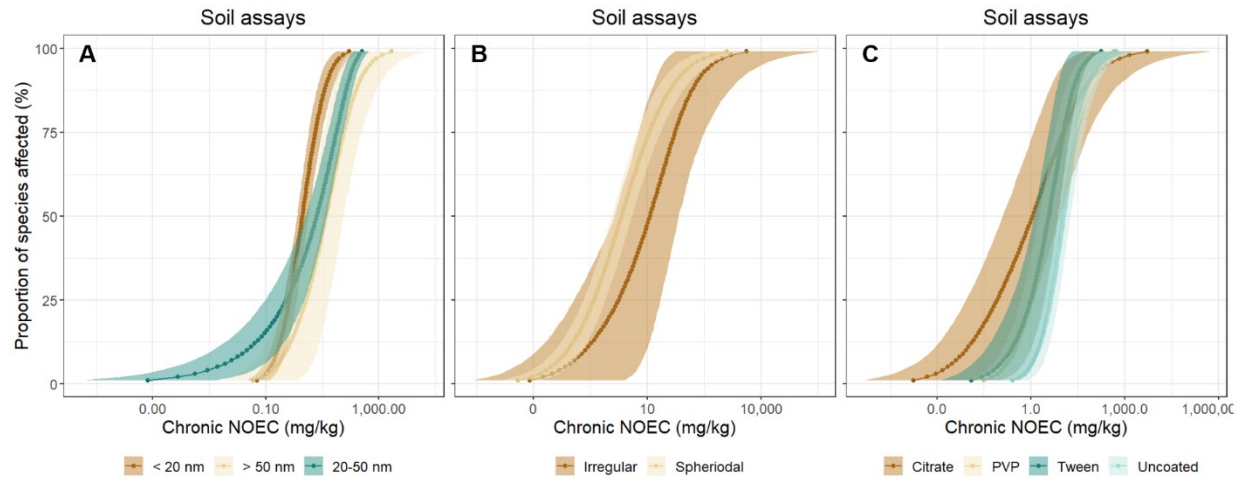


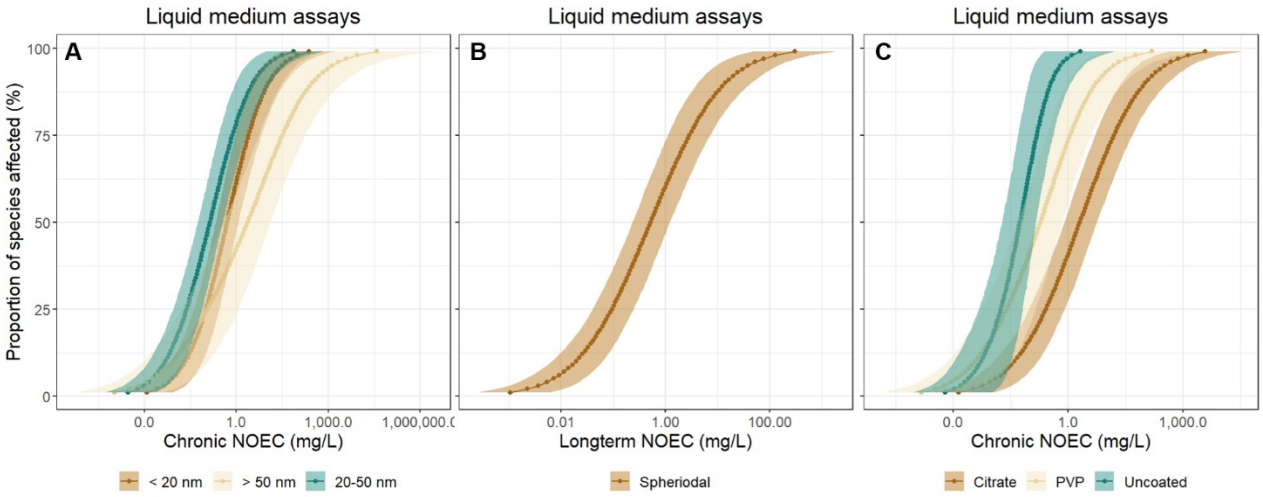
SI Figure 1: Soil species sensitivity distributions for Ag salt ($AgNO_3$) in (A) soil and (B) liquid medium assays.



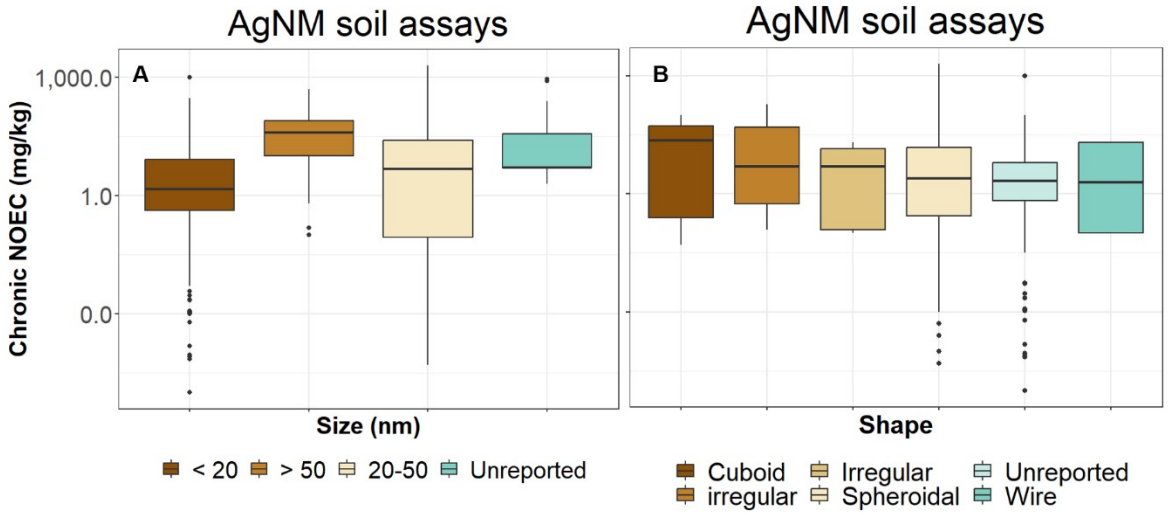
SI Figure 2: Soil species sensitivity distribution for pristine and aged AgNMs in soil assays.



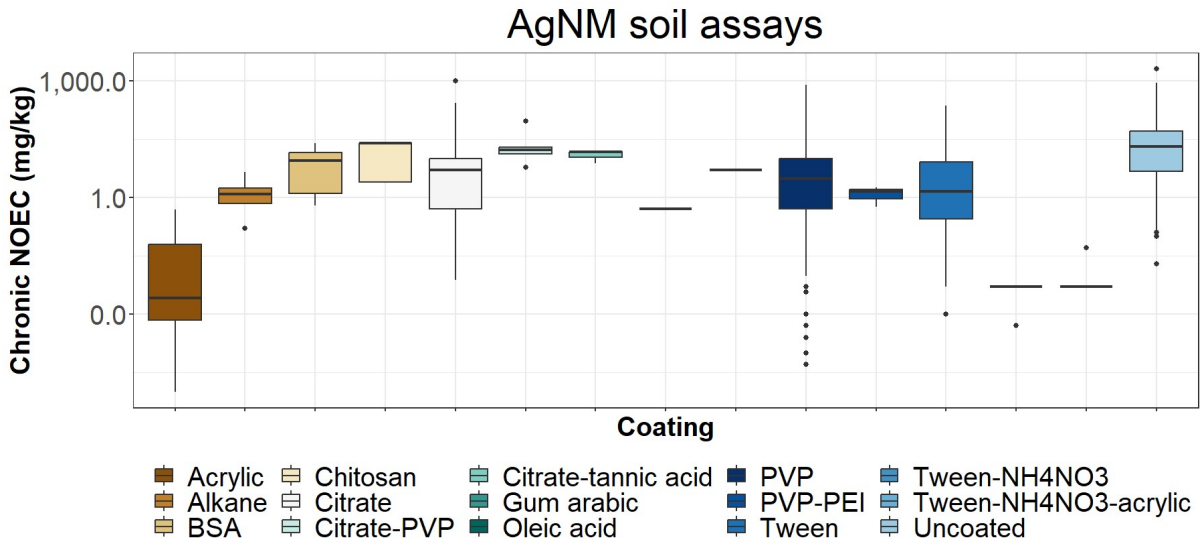
SI Figure 3: Soil species sensitivity distributions for AgNMs grouped by (A) size, (B) shape and (C) coating in soil assays.



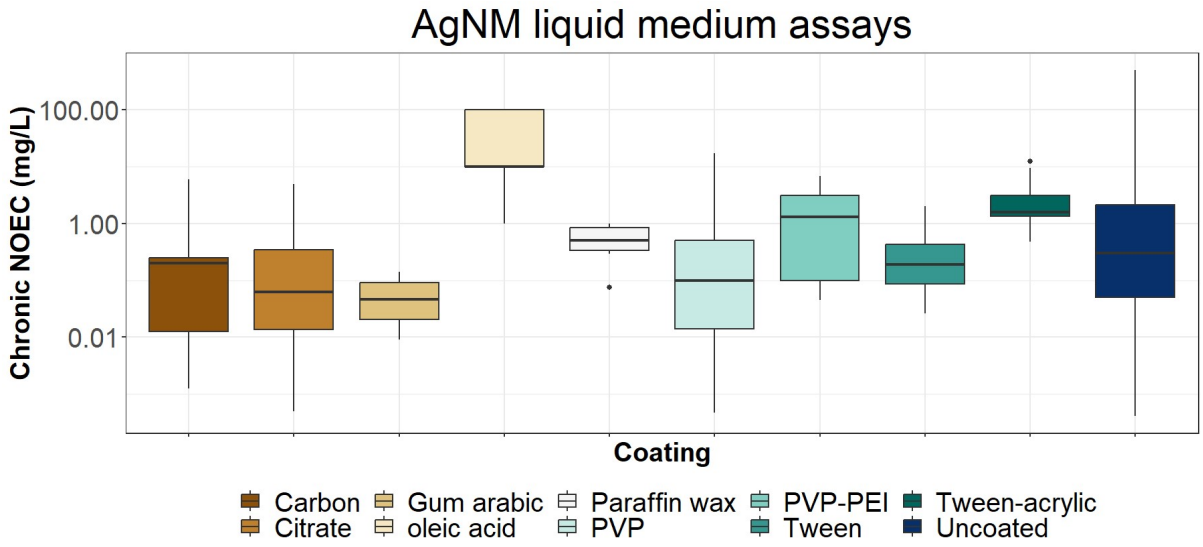
SI Figure 4: Soil species sensitivity distributions for AgNMs grouped by (A) size, (B) shape and (C) coating in liquid medium assays.



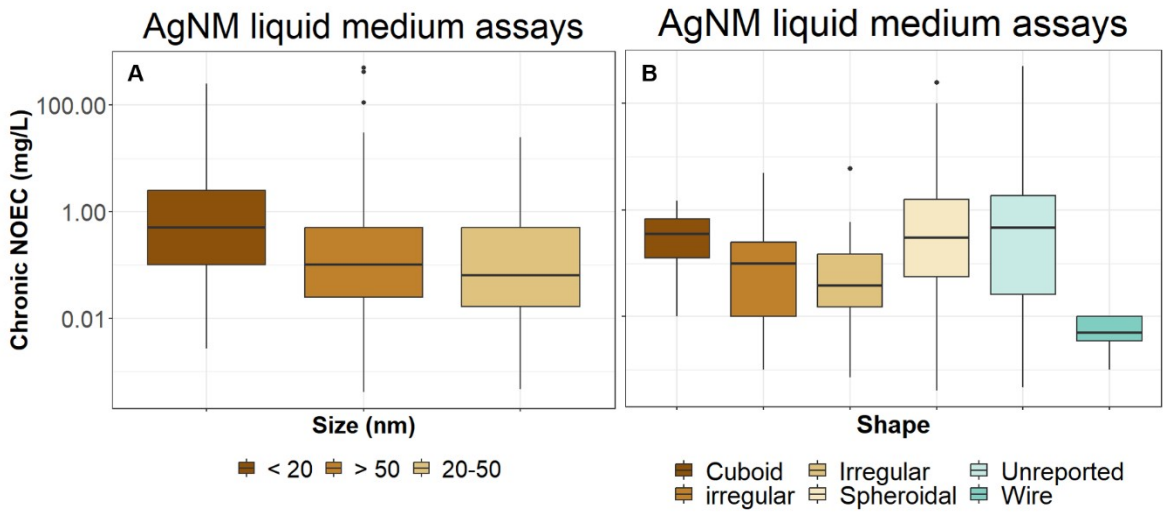
SI Figure 5: Variation in chronic NOEC values across AgNM (A) size and (B) shape groupings in soil assays.



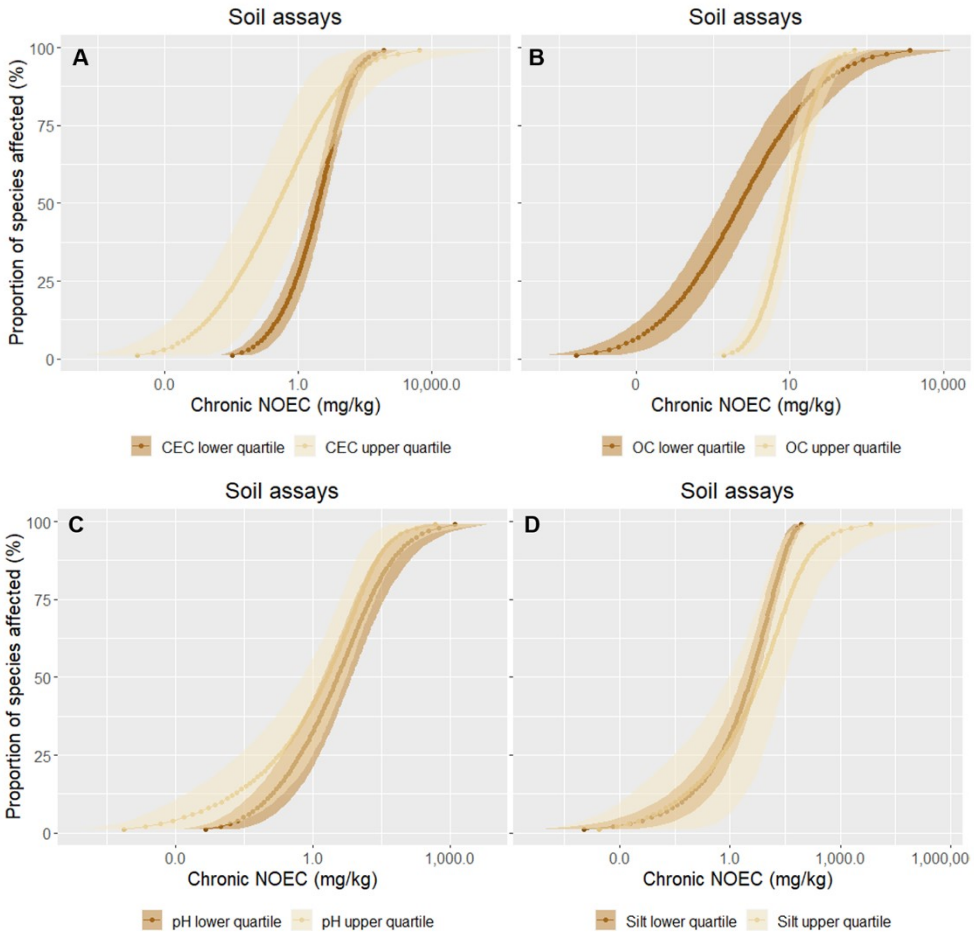
SI Figure 6: Variation in chronic NOEC values across AgNM coating in soil assays.



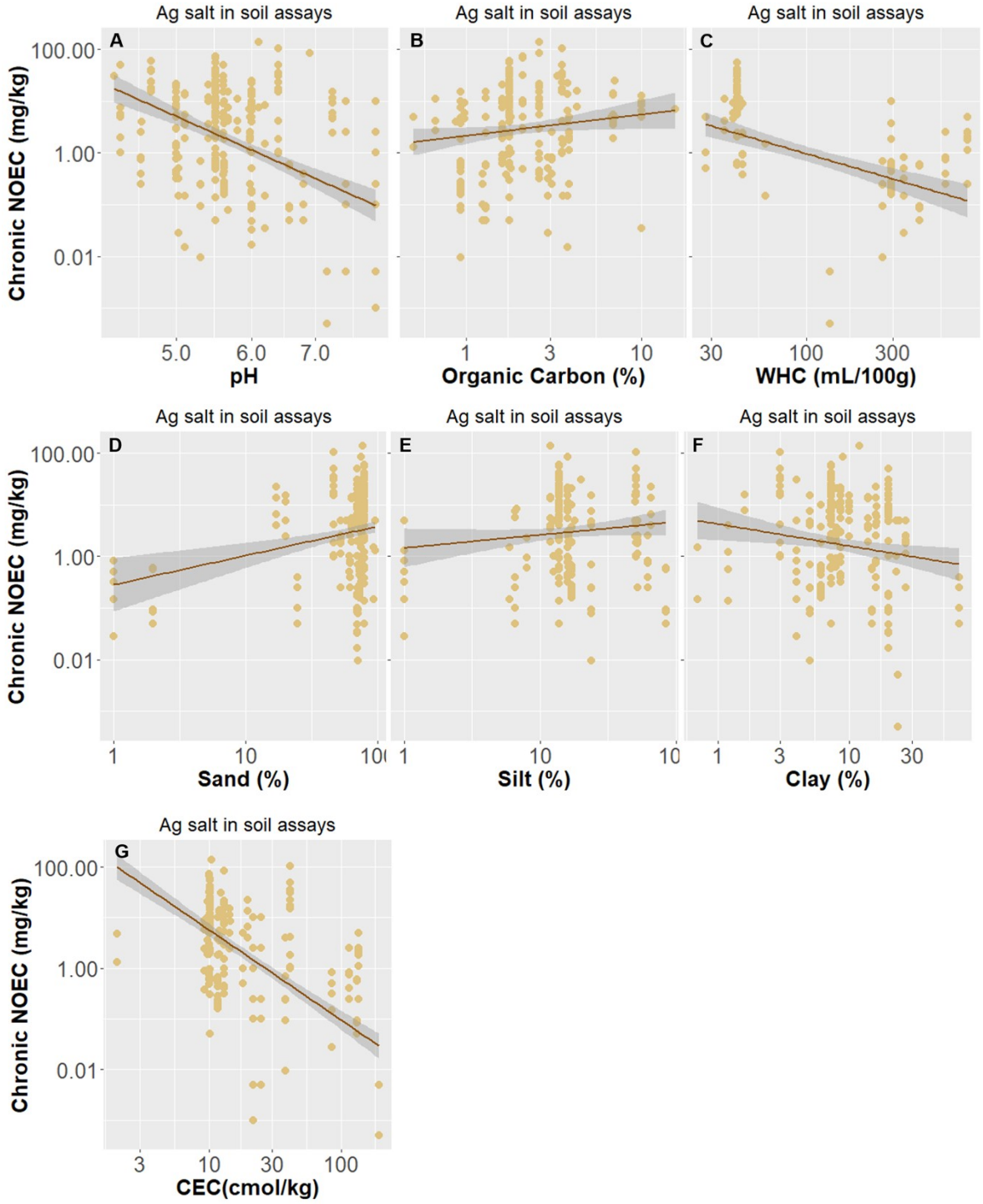
SI Figure 7: Variation in chronic NOEC values across AgNM coating in liquid assay exposures.



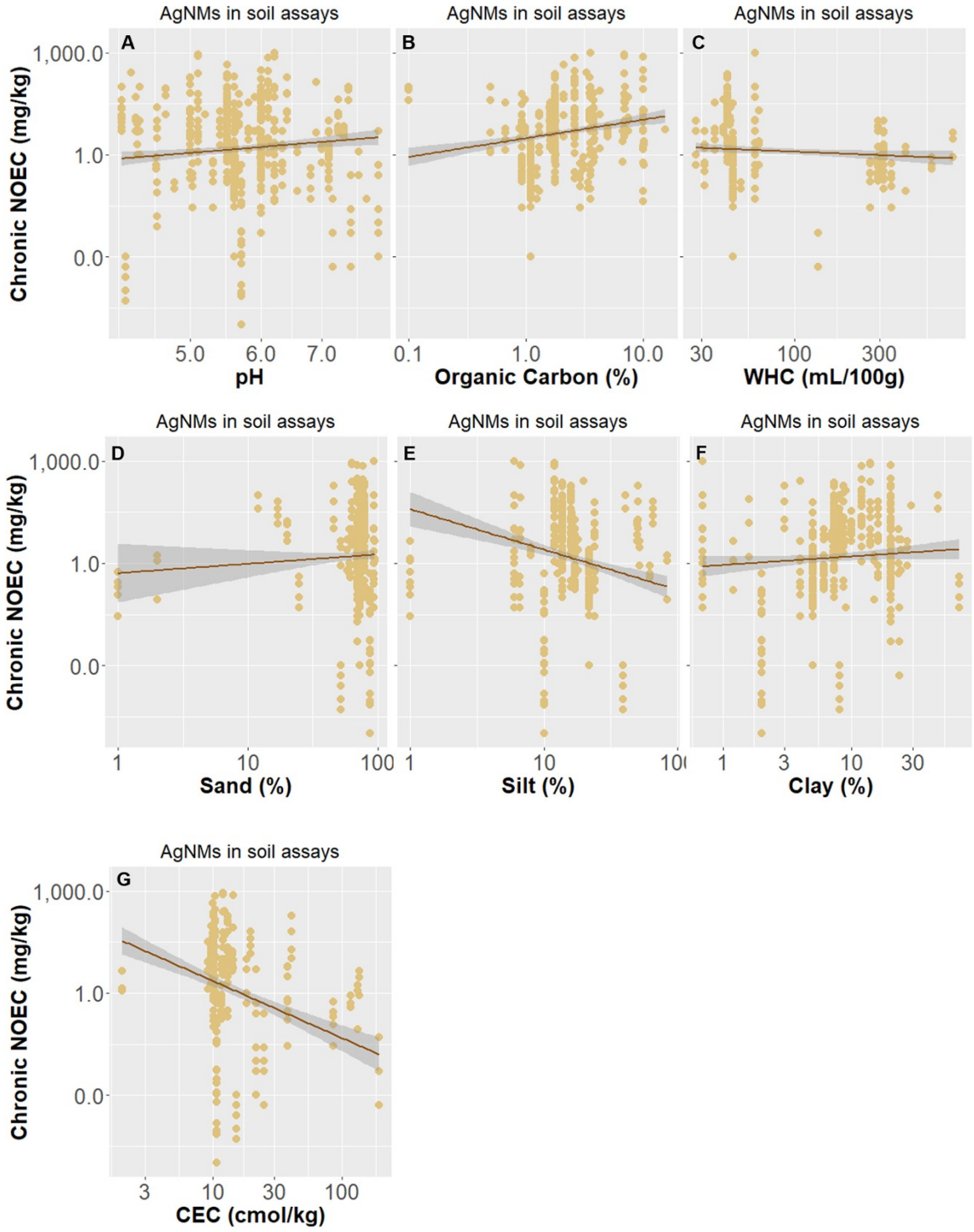
SI Figure 8: Variation in chronic NOEC values across AgNM (A) size and (B) shape groupings in liquid medium assays.


Soil assays
A
Proportion of species affected (%)
100
75
50
25
0
0.0
1.0
10,000.0
Chronic NOEC (mg/kg)
CEC lower quartile
CEC upper quartile
Soil assays
B
0
10
10,000
Chronic NOEC (mg/kg)
OC lower quartile
OC upper quartile
Soil assays
C
Proportion of species affected (%)
100
75
50
25
0
0.0
1.0
1,000.0
Chronic NOEC (mg/kg)
pH lower quartile
pH upper quartile
Soil assays
D
0.0
1.0
1,000.0
1,000,00
Chronic NOEC (mg/kg)
Silt lower quartile
Silt upper quartile

SI Figure 9: Soil species sensitivity distributions for AgNMs in soil assays with low and high values for (A) cation exchange capacity (CEC), (B) organic carbon content (OC), (C) pH and (D) silt content.


Ag salt in soil assays
A
B
C
D
E
F
G
Chronic NOEC (mg/kg)
100.00
1.00
0.01
5.0
6.0
7.0
pH
1
3
10
Organic Carbon (%)
30
100
300
WHC (mL/100g)
Sand (%)
Silt (%)
Clay (%)
CEC(cmol/kg)

SI Figure 10: Regression correlations between Ag salt chronic NOEC values and (A) pH, (B) organic carbon content, (C) water holding capacity, (D) sand content, (E) silt content, (F) clay content and (G) cation exchange capacity in soil assays.


AgNMs in soil assays
A
B
C
D
E
F
G
Chronic NOEC (mg/kg)
1,000.0
1.0
0.0
pH
5.0
6.0
7.0
Organic Carbon (%)
0.1
1.0
10.0
WHC (mL/100g)
30
100
300
Sand (%)
1
10
100
Silt (%)
Clay (%)
3
30
CEC (cmol/kg)

SI Figure 11: Regression correlations between AgNM chronic NOEC values and (A) pH, (B) organic carbon content, (C) water holding capacity, (D) sand content, (E) silt content, (F) clay content and (G) cation exchange capacity in soil assays.

SI Table 1: AgNM and soil property factors used to explore variation in toxicity values.

Factors	**Parameters**	**Parameter type/category**
AgNM property factors	Size	< 20 nm 20–40 nm > 50 nm
	Shape	Spheroidal Cuboid Irregular Wire Plate
	Coating	Uncoated Tween Tween-acrylic Tween-NH_4NO_3 Tween-NH_4NO_3-acrylic PVP PVP-PEI Acrylic Citrate Citrate-PVP Citrate-tannic acid Alkane Bovine Serum Albumin (BSA) Carbon Chitosan Oleic acid Citrate-PVP Gum arabic Paraffin wax
Soil property factors	pH	Upper quartile ≥ 6.9 Lower quartile ≤ 5.5
	OC	Upper quartile ≥ 3.5%

		Lower quartile ≤ 1.1%
	WHC	Upper quartile ≥ 293 mL/100g Lower quartile ≤ 45 mL/100g
	Sand	Upper quartile ≥ 77% Lower quartile ≤ 70%
	Silt	Upper quartile ≥ 22% Lower quartile ≤ 12%
	Clay	Upper quartile ≥ 14% Lower quartile ≤ 5%
	CEC	Upper quartile ≥16.0 cmol/kg Lower quartile ≤ 10.1 cmol/kg

SI Table 2: Proportion of AgNM toxicity values across soil taxonomic group endpoints from soil and liquid medium assays.

Endpoint	**Soil assays**			**Liquid medium assays**		
	Microbes	**Invertebrates**	**Plants**	**Microbes**	**Invertebrates**	**Plants**
Reproduction	(-)	39.2%	(-)	(-)	28.8%	(-)
Growth	(-)	11.4%	74.6%	(-)	24.0%	74.4%
Mortality	(-)	27.6%	4.0%	3.0%	19.6%	(-)
Enzyme activity	31.4%	2.1%	9.9%	6.9%	1.3%	(-)
Microbial biomass change	13.8%	(-)	(-)	78.3%	(-)	(-)
Nitrification	41.5%	(-)	(-)	4.9%	(-)	(-)
Behaviour	(-)	9.7%	(-)	(-)	9.9%	(-)
Carbon transformation	11.5%	(-)	(-)	(-)	(-)	(-)
Oxidative stress	(-)	8.1%	(-)	3.0%	5.8%	(-)
Development	(-)	1.2%	10.7%	(-)	(-)	17.9%
Cytotoxicity	(-)	(-)	(-)	3.4%	6.1%	(-)
Diversity	1.6%	(-)	(-)	(-)	(-)	(-)
Immobilization	(-)	0.6%	(-)	(-)	0.6%	(-)
Genotoxicity	(-)	0.2%	0.8%	(-)	3.8%	7.7%
Soil functions	0.1%	(-)	(-)	(-)	(-)	(-)
Decomposition	0.1%	(-)	(-)	0.5%	(-)	(-)

SI Table 3: SSD-derived HC_{50} and 95% confidence intervals (CI) for Ag salt ($AgNO_3$), AgNM (ungrouped, pristine and aged), soil properties and nanoforms for soil and liquid medium assays. Pristine refers to “as manufactured” and aged refers to AgNMs incubated in biosolids pre-exposure.

Exposure	Ag form	Grouping	Grouping	HC_{50}	HC_{50} Lower CI	HC_{50} Upper CI	Species
Soil assay	$AgNO_3$	Ungrouped	Ungrouped	2.74	1.22	5.23	36
	AgNM	Ungrouped	Ungrouped	3.06	1.74	5.21	74
		Pristine	Pristine	2.68	1.49	4.82	72
		Aged	Aged	7.05	2.48	20.00	11
		CEC	Lower quartile	3.92	2.33	6.34	65
			Upper quartile	0.24	0.03	1.87	14
		OC	Lower quartile	1.08	0.46	2.65	48
			Upper quartile	9.40	6.21	14.16	31
		pH	Lower quartile	3.42	1.62	7.03	47
			Upper quartile	1.98	0.60	5.35	24
		Silt	Lower quartile	3.72	2.06	6.01	64
			Upper quartile	6.76	0.94	33.54	10
		Size	<20 nm	1.98	1.27	3.21	42
			20 – 50 nm	6.41	2.48	14.08	38
			>50 nm	13.82	2.84	55.78	9
		Shape	Spheroidal	2.13	1.15	3.97	66
			Irregular	11.88	1.37	67.83	6
		Coating	PVP	3.66	1.82	7.76	25
			Citrate	1.14	0.12	8.35	11
			Tween	3.79	1.26	10.31	11
			Uncoated	11.55	7.33	18.36	39
Liquid medium assay	$AgNO_3$	Ungrouped	Ungrouped	0.01	0.01	0.03	23
	AgNM	Ungrouped	Ungrouped	0.70	0.32	1.64	54
		Size	<20 nm	0.51	0.21	1.19	31
			20 – 50 nm	0.14	0.06	0.36	27
			>50 nm	2.39	0.39	13.00	20
		Shape	Spheroidal	0.52	0.24	1.22	37
		Coating	PVP	0.19	0.05	0.68	17

			Citrate	2.04	0.80	5.62	9
			Uncoated	0.06	0.02	0.14	37

SI Table 4: SSD-derived HC_5 and 95% confidence intervals (CI) for Ag salt ($AgNO_3$), AgNM (ungrouped, pristine and aged), soil properties and nanoforms for soil and liquid medium assays. Pristine refers to "as manufactured" and aged refers to AgNMs incubated in biosolids pre-exposure.

Exposure	Ag form	Grouping	Grouping	HC_5	HC_5 Lower CI	HC_5 Upper CI	Species
Soil assay	$AgNO_3$	Ungrouped	Ungrouped	0.005	0.0003	0.07	36
	AgNM	Ungrouped	Ungrouped	0.03	0.01	0.08	74
		Pristine	Pristine	0.03	0.01	0.07	72
		Aged	Aged	0.35	0.08	2.14	11
		CEC	Lower quartile	0.06	0.03	0.17	65
			Upper quartile	0.0002	0.00001	0.01	14
		OC	Lower quartile	0.01	0.002	0.03	48
			Upper quartile	1.30	0.71	2.62	31
		pH	Lower quartile	0.03	0.01	0.11	47
			Upper quartile	0.001	0.0001	0.04	24
		Silt	Lower quartile	0.01	0.001	0.05	64
			Upper quartile	0.01	0.0002	0.72	10
		Size	<20 nm	0.15	0.07	0.31	42
			20 – 50 nm	0.002	0.0001	0.06	38
			>50 nm	0.22	0.02	3.60	9
		Shape	Spheroidal	0.03	0.01	0.08	66
			Irregular	0.07	0.003	5.91	6
		Coating	PVP	0.14	0.05	0.53	25
			Citrate	0.002	0.0001	0.08	11
			Tween	0.08	0.01	1.12	11
			Uncoated	0.94	0.46	2.08	39
Liquid medium assay	$AgNO_3$	Ungrouped	Ungrouped	0.001	0.0002	0.002	23
	AgNM	Ungrouped	Ungrouped	0.004	0.001	0.01	54
		Size	<20 nm	0.01	0.002	0.04	31
			20 – 50 nm	0.002	0.0004	0.01	27
			>50 nm	0.002	0.0002	0.04	20
		Shape	Spheroidal	0.01	0.002	0.03	37
		Coating	PVP	0.001	0.0002	0.01	17
			Citrate	0.01	0.003	0.06	9
			Uncoated	0.002	0.0005	0.02	37

SI Table 5: Geomean, standard deviation (SD) and coefficient of variance (CoV) of chronic NOECs (mg/kg or mg/L) for AgNM and Ag salt across soil species-endpoints for soil and liquid medium assays. A dissolved/particle ratio less than 1 indicates Ag salt is more toxic than AgNM, a ratio equal to 1 indicates similar toxicity for Ag salt and AgNM and a ratio more than 1 indicates Ag salt is less toxic than AgNM.

Exposure	Soil Biota	Endpoint	AgNM chronic NOEC (mg/kg or mg/L)			Ag salt chronic NOEC (mg/kg or mg/L)			Dissolved/Particle Ratio
			Geomean	SD	CoV	Geomean	SD	CoV	
Soil Assays	Microbes	Enzyme activity	0.17	121.44	70052.21	0.73	51.16	7029.50	4.20
		Nitrification	1.27	86.83	6825.54	0.48	16.80	3470.77	0.38
		Carbon transformation	0.43	34.34	7901.55	1.73	46.40	2684.60	3.98
		Microbial biomass change	0.12	47.79	40646.53	0.02	44.18	219643.45	0.17
		Diversity	1.21	212.09	17522.74	(-)	(-)	(-)	(-)
	Invertebrates	Reproduction	7.57	32.28	426.19	3.69	10.68	289.68	0.49
		Growth	10.21	111.56	1092.21	8.10	7.09	87.57	0.79
		Mortality	25.60	129.19	504.73	9.80	15.63	159.50	0.38
		Enzyme activity	1.92	3.51	182.72	2.89	1.64	56.95	1.50
		Behaviour	1.01	80.74	7986.02	0.10	0.16	163.02	0.09
		Oxidative stress	2.00	4.56	228.05	1.55	12.17	783.65	0.78
		Development	0.29	0.26	91.03	0.12	0.18	144.34	0.42
		Immobilization	0.37	0.17	47.02	(-)	(-)	(-)	(-)
	Plants	Growth	11.40	194.02	1701.20	3.96	22.81	575.63	0.35
		Mortality	8.27	1.73	20.89	6.12	4.42	72.17	0.74
		Enzyme activity	0.34	8.12	2393.42	(-)	(-)	(-)	(-)
		Development	10.94	39.50	361.17	1.39	4.56	327.85	0.13
Liquid medium assays	Microbes	Enzyme activity	1.36	0.89	65.40	0.97	1.96	200.82	0.72
		Cytotoxicity	0.36	0.84	237.28	0.04	0.02	54.25	0.11
		Microbial biomass change	0.49	98.70	20198.80	0.09	1.65	1895.28	0.18
		Oxidative stress	0.29	0.21	70.64	(-)	(-)	(-)	(-)
		Nitrification	0.04	0.22	487.54	(-)	(-)	(-)	(-)
	Invertebrates	Enzyme activity	0.003	0.002	70.20	(-)	(-)	(-)	(-)
		Growth	0.35	17.99	5135.23	0.08	0.65	845.46	0.22
		Reproduction	0.19	1.88	1006.93	0.02	0.05	303.56	0.08
		Cytotoxicity	0.27	1.16	435.07	0.02	0.37	1648.29	0.08
		Immobilization	0.32	0.21	67.08	(-)	(-)	(-)	(-)
		Genotoxicity	0.01	0.03	405.56	0.001	0.001	64.57	0.11
		Mortality	0.07	20.36	30710.92	0.01	0.14	1033.26	0.20
		Behaviour	0.07	0.19	267.61	(-)	(-)	(-)	(-)
		Oxidative stress	0.04	0.13	312.40	(-)	(-)	(-)	(-)
	Plants	Growth	0.44	55.30	12691.96	0.01	0.83	7290.49	0.03
		Genotoxicity	3.97	3.87	97.59	(-)	(-)	(-)	(-)
		Development	0.63	5.14	821.00	0.03	0.02	60.05	0.05

SI Table 6: Factorial Analysis of Variance (ANOVA) analysis between chronic NOEC values for different AgNM characteristics (size, shape and coating) for soil and liquid medium assays. (*) indicates significant differences in chronic NOEC values within AgNM property group and across AgNM property group combinations.

Particle properties	F value	p value
Soil exposures		
Size	14.29	< 0.01 *
Shape	0.93	0.46
Coating	2.19	0.01 *
Size: Shape	2.99	0.05 *
Size: Coating	8.15	< 0.01 *
Shape: Coating	0.26	0.96
Size: Shape: Coating	0.14	0.71
Liquid medium assays		
Size	8.74	< 0.01 *
Shape	0.89	0.47
Coating	5.96	< 0.01 *
Size: Shape	0.58	0.68
Size: Coating	2.13	0.05 *
Shape: Coating	0.46	0.63

SI Table 7: Factorial Analysis of Variance (ANOVA) and post hoc analysis output between chronic NOEC values for different AgNM characteristics (size, shape and coating) for soil and liquid medium assays. (*) indicates significant differences in chronic NOEC values within AgNM property group and across AgNM property group combinations and only significant relationships are displayed in the table below.

Factor	diff	P value	Exposures
20–50 nm - < 20 nm	32.6	< 0.01 *	Soil assay
Uncoated - PVP	40.4	< 0.01 *	Soil assay
>50 nm - < 20nm	-8.9	< 0.01 *	Liquid medium assay
20-50nm - < 20nm	-8.4	< 0.01 *	Liquid medium assay
Oleic acid - citrate	44.0	< 0.01 *	Liquid medium assay
Oleic acid – gum arabic	44.5	0.01*	Liquid medium assay
PVP - Oleic acid	-44.0	< 0.01 *	Liquid medium assay
Tween - Oleic acid	-44.2	< 0.01 *	Liquid medium assay
Tween-acrylic - Oleic acid	-41.8	< 0.01 *	Liquid medium assay
Uncoated - Oleic acid	-36.0	< 0.01 *	Liquid medium assay
Uncoated - PVP	7.9	0.04 *	Liquid medium assay

SI Table 8: Regression correlations between AgNM and Ag salt ($AgNO_3$) chronic NOEC and soil properties; pH, organic carbon (OC), water holding capacity (WHC), sand content, silt content, clay content and cation exchange capacity (CEC).

Ag form	Parameter	R^2	p value	β	Data points
AgNM	pH	0.01	< 0.01 *	0.10	951
	OC	0.04	< 0.01 *	0.19	682
	WHC	0.01	0.04 *	-0.09	486
	Sand	0.001	0.25	0.04	693
	Silt	0.05	< 0.01 *	-0.22	630
	Clay	0.004	0.08	0.06	752
	CEC	0.11	< 0.01 *	-0.33	486
Ag salt	pH	0.15	< 0.01 *	-0.39	381
	OC	0.01	0.04 *	0.12	310
	WHC	0.18	< 0.01 *	-0.42	182
	Sand	0.05	< 0.01 *	0.22	313
	Silt	0.009	0.10	0.09	275
	Clay	0.02	< 0.01 *	-0.14	345
	CEC	0.45	< 0.01 *	-0.67	291

SI Table 9: Pre-exposure and in-exposure measurements of AgNMs in soil and liquid medium assays. Proportion of data points in relation to all toxicity records are shown.

Exposure	NP measurement	Min	Max	Data points	Proportion
Pre-exposure measurements					
Soil assay	Hydrodynamic diameter (nm)	2.8	532	675	64.2%
	Polydispersity index	0.1	0.85	122	11.6%
	Surface Charge (mV)	-70.2	43.3	193	18.4%
Liquid medium assay	Hydrodynamic diameter (nm)	1.3	750.7	291	65.4%
	Polydispersity index	0.2	0.91	67	15.1%
	Surface Charge	-61.9	40.7	200	44.9%
In exposure measurements					
Soil assay	Hydrodynamic diameter (nm)	73.7	284.2	20	1.9%
	Polydispersity index	(-)	(-)	(-)	(-)
	Surface Charge (mV)	-45.2	19.2	25	2.4%
Liquid medium assay	Hydrodynamic diameter (nm)	11.16	1300	52	11.7%
	Polydispersity index	0.1	0.3	22.0	4.9%
	Surface Charge (mV)	-37	3.5	26	5.8%