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Bioturbation of Ag_2S-NPs in soil columns by earthworms

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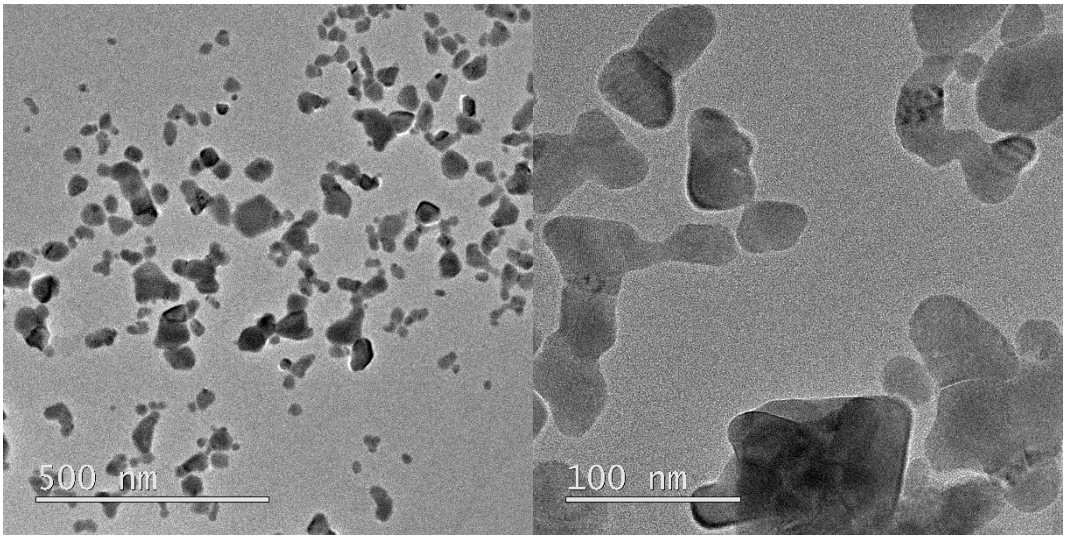
SUPPLEMENTAL MATERIALS

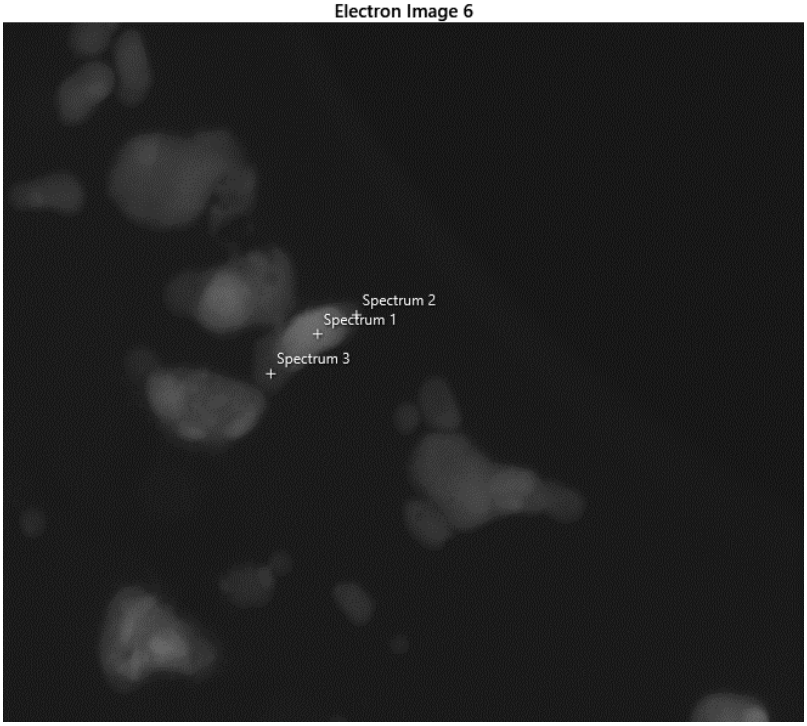
Paragraph S1

Bright field TEM pictures of the Ag_2S-NPs in stock solution.



STEM/EDX (scanning transmission electron microscope/energy dispersive X-ray) pictures of the Ag_2S-NPs in stock solution and relative Ag and S atomic %.

Electron Image 6



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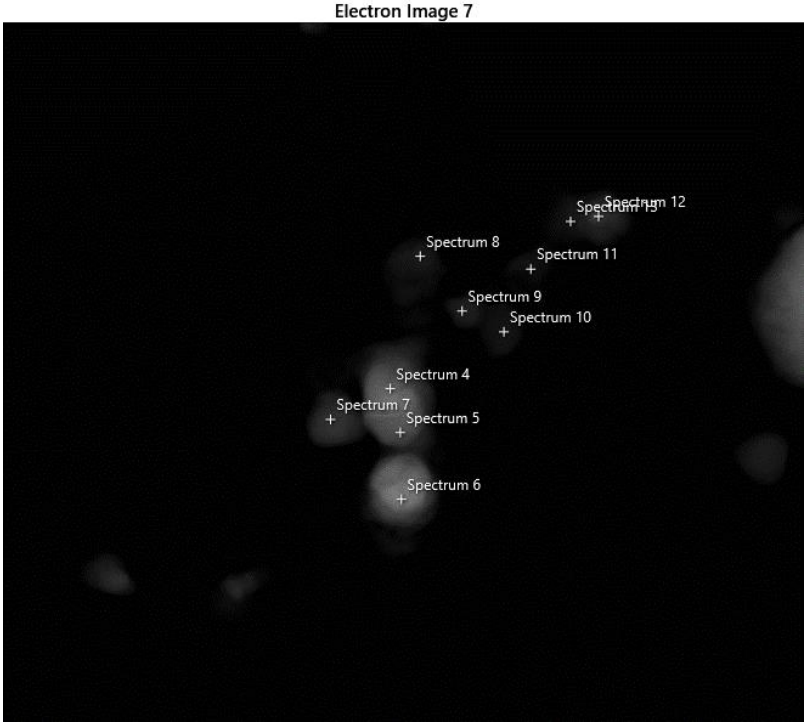
Electron Image 7



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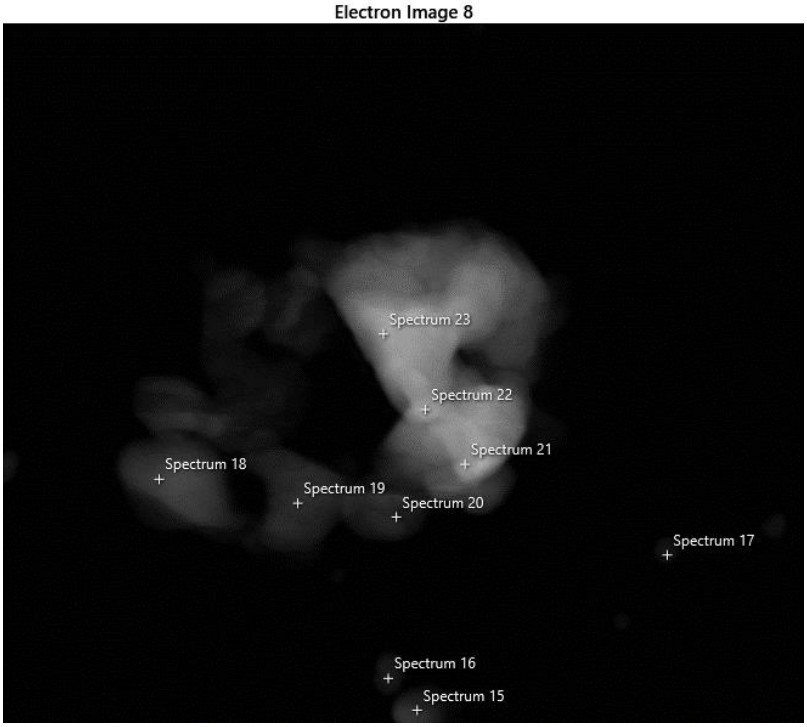

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Spectrum Label	S	Ag	Ag/S ratio
Spectrum 1	0	4.52	no sulphur signal, Ag only
Spectrum 2	0.47	2.72	5.8
Spectrum 3	0.81	4.33	5.3
Spectrum 4	9.81	28.54	2.9
Spectrum 5	12.09	16.69	1.4
Spectrum 6	3.34	30.11	9
Spectrum 7	9.63	22.66	2.4
Spectrum 8	6.79	24.53	3.6
Spectrum 9	5.54	14.38	2.6
Spectrum 10	5.65	14.92	2.6
Spectrum 11	6.61	13.28	2
Spectrum 12	6.24	18.07	2.9
Spectrum 13	3.71	9.56	2.6
Spectrum 15	5.57	19.79	3.6
Spectrum 16	7.89	18.14	2.3
Spectrum 17	1.84	17.14	9.3
Spectrum 18	9.17	18.66	2
Spectrum 19	4.92	14.44	2.9
Spectrum 20	5.22	17.42	3.3
Spectrum 21	6.27	18.13	2.9
Spectrum 22	2.79	21.21	7.6
Spectrum 23	0.83	22.73	27.4

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Table S1. Exchangeable base concentrations in the Kooijenburg soil used for the earthworm bioturbation experiment with Ag_2S-NPs.

Ammonium acetate extractable concentrations	
Mg 285.2	9.1 mg kg^{-1}
Ca 317.93	97.3 mg kg^{-1}
Mn 257.61	1.4 mg kg^{-1}
Na 589.6	2.2 mg kg^{-1}
K 766.5	16.0 mg kg^{-1}

Table S2. Phosphorus, manganese, aluminium and iron concentrations in the Kooijenburg soil used for the earthworm bioturbation experiment with Ag_2S-NPs.

Ammonium oxalate extraction	
P 213.6	777 mg kg^{-1}
Mn 257.6	142 mg kg^{-1}
Fe 259.9	3049 mg kg^{-1}
Al 308.2	3005 mg kg^{-1}

Table S3

Post hoc Tukey multiple comparison test between total Ag concentrations in earthworms exposed to Ag_2S-NPs in Kooijenburg soil treatments without rain and with rain at different time points following one way ANOVA ($F(5, 18) = 19.26$)). Positive confidence interval indicates that concentrations are higher in first factor, and vice versa.

Treatment without artificial rain

	Mean Diff.	95% CI of diff	P value
7 days vs 14 days	0.0002433	-0.7164 to 0.7169	>0.9999
7 days vs 21 days	-0.08338	-0.7469 to 0.5801	0.9796
7 days vs 28 days	-0.1711	-0.8878 to 0.5456	0.8827
14 days vs 21 days	-0.08362	-0.8003 to 0.6331	0.9835
14 days vs 28 days	-0.1714	-0.9375 to 0.5948	0.9008
21 days vs 28 days	-0.08774	-0.8044 to 0.6289	0.9811
Treatment with artificial rain			
	Mean Diff.	95% CI of diff	P value
7 days vs 14 days	-0.2201	-1.3160 to 0.8760	0.913
7 days vs 21 days	-0.3943	-1.4900 to 0.7018	0.7145
7 days vs 28 days	-1.591	-2.6870 to -0.4950	0.0048
14 days vs 21 days	-0.1742	-1.2700 to 0.9218	0.9638
14 days vs 28 days	-1.371	-2.4670 to -0.2749	0.0136
21 days vs 28 days	-1.197	-2.2930 to -0.1007	0.0311

Table S4

Two way ANOVA test between total Ag concentrations in earthworms exposed to Ag_2S-NPs in Kooijenburg soil in treatments without rain and with rain at different time points together.

Source of variation	F	P value
Treatment	12.91 (1, 22)	0.0016
Time	5.77 (3, 22)	0.0046
Interaction	3.74 (3, 22)	0.0261

Table S5

Three way ANOVA between percentage of the position of earthworms at three depths in Kooijenburg soil columns with and without application of artificial rain and the presence and absence of Ag_2S-NPs over time.

Source of variation	df	Mean square	F	P value
Layer * Ag_2S-NPs * time	6	0.840	0.103	0.995
Layer * Ag_2S-NPs * rain	2	4.771	0.638	0.534
Layer * rain * time	6	20.951	5.466	0.001

Table S6

Post hoc Tukey multiple comparison test between pore size distributions (expressed as number of pixels) in Kooijenburg soil in columns with Ag_2S-NPs and earthworms, with earthworms and without earthworms following one way ANOVA (F (2, 49) = 38.15). Positive confidence intervals indicate that concentrations are higher in first factor, and vice versa.

	Mean Diff.	95% CI of diff	P value
Ag_2S-NP + worm vs worm	25447	-250429 to 301323	0.9741
Ag_2S-NP + worm vs control	372289	96413 to 648165	0.0048
worm vs control	346842	70966 to 622718	0.0095

Table S7A

Multiple regression analysis of changes in porosity of Kooijenburg soil between treatments with earthworms and with earthworms in presence of Ag_2S-NPs over time (Adjusted R^2= 0.26, F(2, 30)=10.54 p value<0.01).

	Coefficients	Standard Error	P value
Intercept	1.039	0.035	<0.001
Time	0.008	0.002	<0.001
Treatment	-0.039	0.035	0.283

Table S7B

Multiple regression analysis of changes in porosity of Kooijenburg soil between treatments without earthworms and with earthworms over time (Adjusted R^2= 0.71, F(2, 30)=14.08, p value<0.01).

	Coefficients	Standard Error	P value
Intercept	0.975	0.026	<0.001
Time	0.004	0.001	0.002
Treatment	0.111	0.026	<0.001

Table S7C

Multiple regression analysis of changes in porosity of Kooijenburg soil between treatments without earthworms and with earthworms and Ag_2S-NP over time (Adjusted R^2= 0.26, F(2, 30)=6.05, p value<0.01).

	Coefficients	Standard Error	P value
Intercept	0.975	0.033	<0.001
Time	0.004	0.002	0.011
Treatment	0.036	0.017	0.039

Table S8

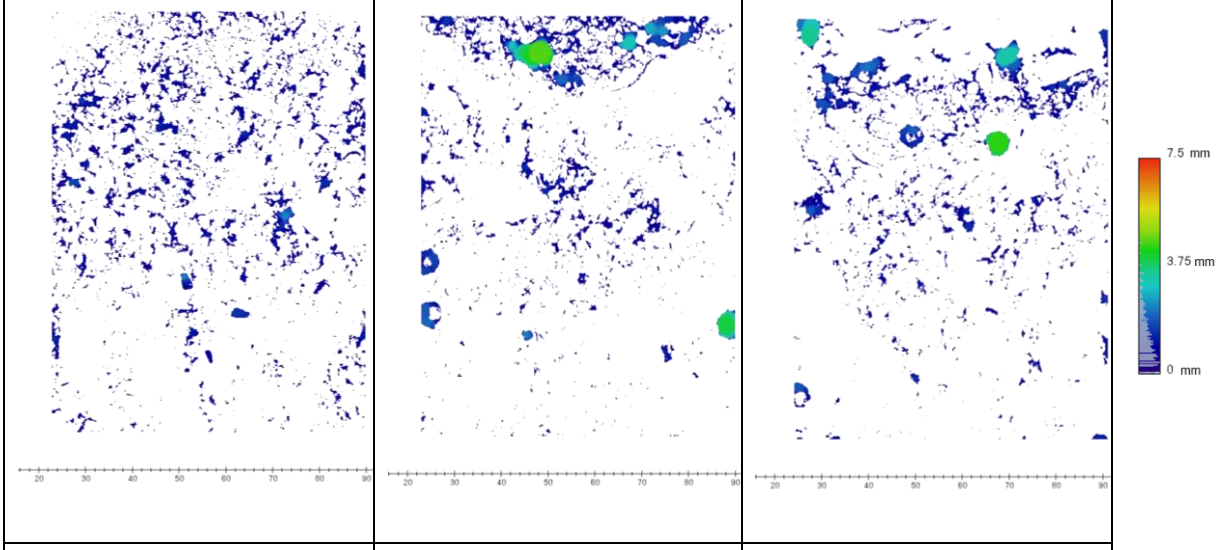
Two way ANOVA between changes of porosity of Kooijenburg soil (between day 7 and day 28) at three depths amongst treatments with and without Ag_2S-NPs and with and without earthworms.

	Interaction		layers		treatment	
	F	p value	F	p value	F	p value
Worm vs control	0.58	0.5744	0.09	0.9175	5.81	0.0329
Ag_2S-NP + worm vs control	0.11	0.8970	0.03	0.9658	3.40	0.0900
Worm vs Ag_2S-NP + worm	0.03	0.9694	0.17	0.8453	0.02	0.8954

Paragraph S9

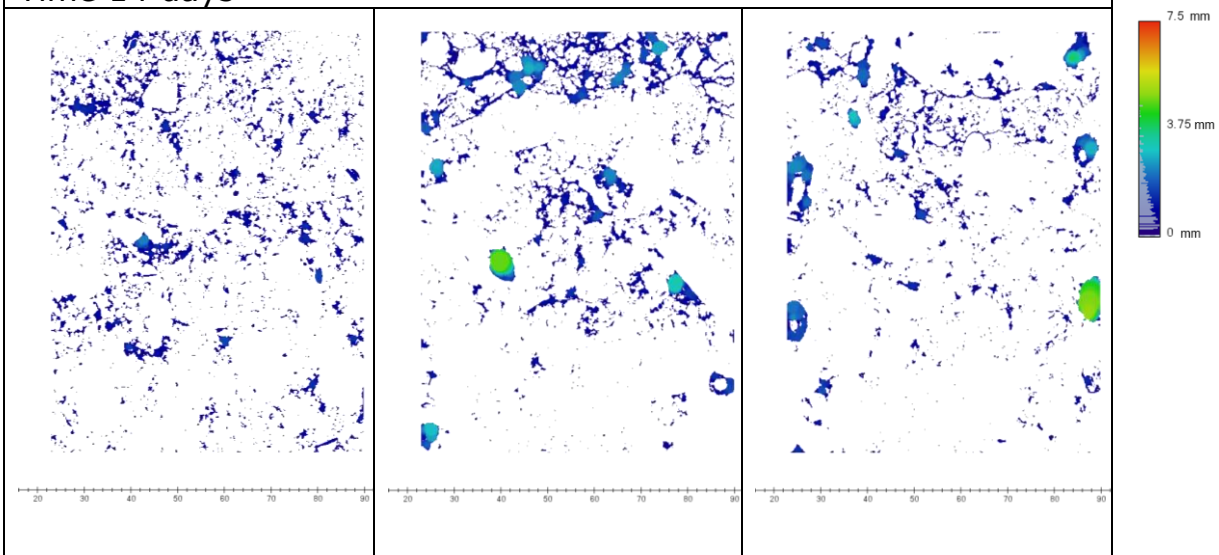
Colour maps of the pore size distribution in longitudinal profile of one soil column of the three treatments (with and without earthworms, with earthworms and Ag_2S-NPs) at day 7, 14 and 21.

Time 7 day



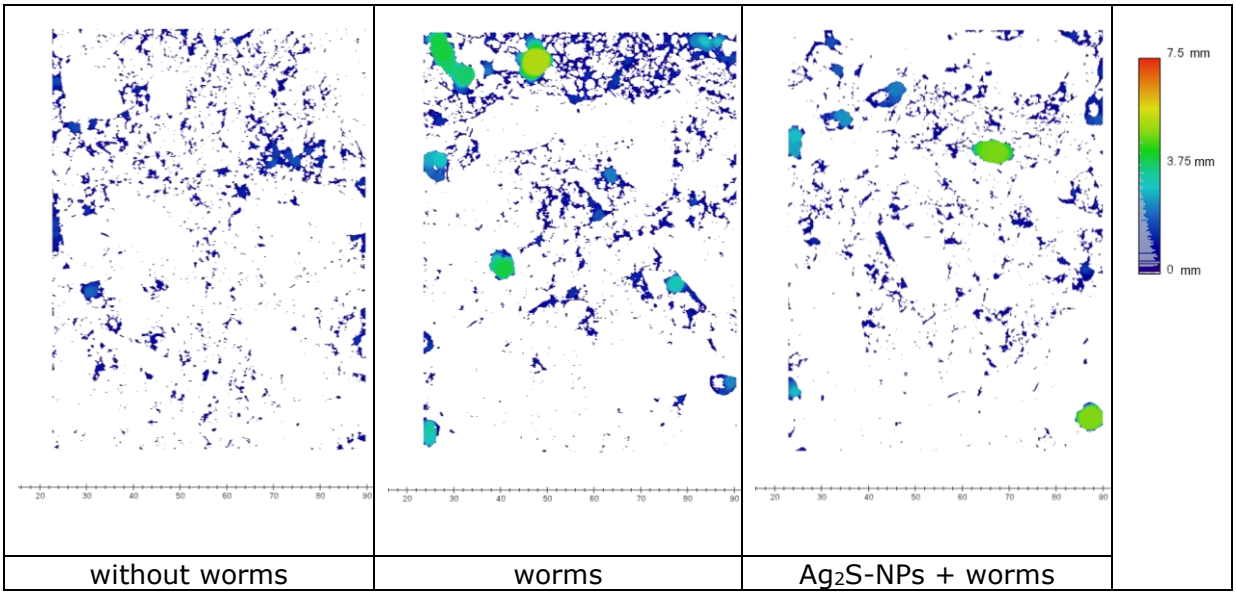
without worms	worms	Ag_2S-NPs + worms

Time 14 days



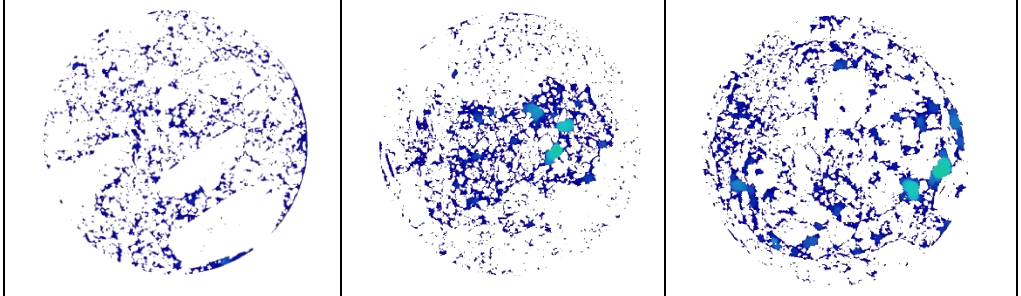
without worms	worms	Ag_2S-NPs + worms

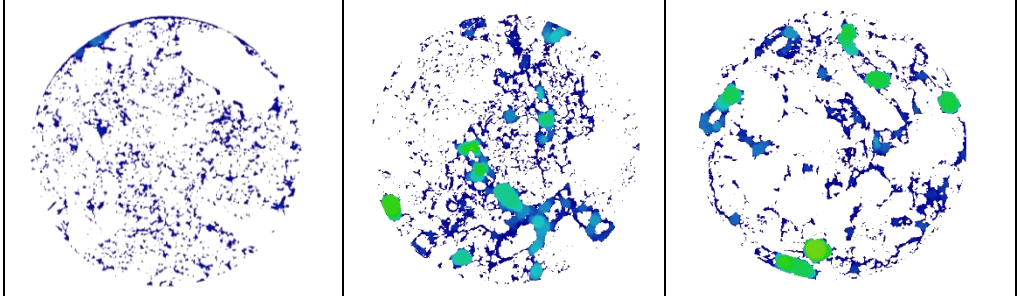
Time 21 day



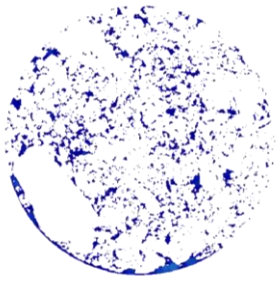
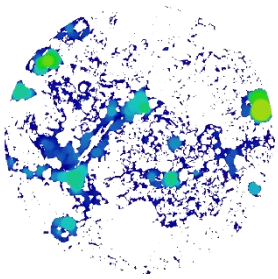
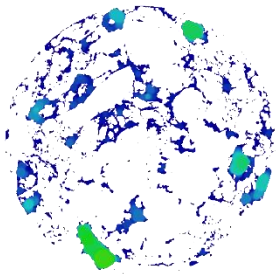
without worms	worms	Ag_2S-NPs + worms

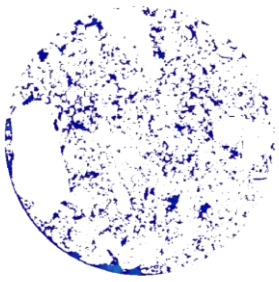
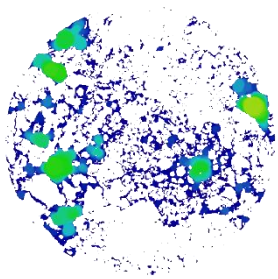
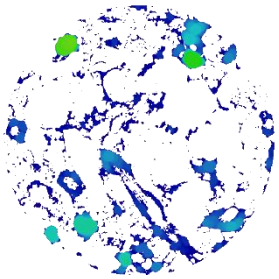
Colour maps of the pore size distribution in cross sections of one soil column of the three treatments (with and without earthworms, with earthworms and Ag_2S-NPs) at days 0, 7, 14, 21 and 28.

Time 0 day		
		
without worms	worms	Ag_2S-NPs + worms

Time 7 day		
		
without worms	worms	Ag_2S-NPs + worms

Time 14 days

		
without worms	worms	Ag_2S-NPs + worms

Time 21 day		
		
without worms	worms	Ag_2S-NPs + worms

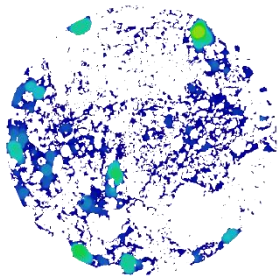
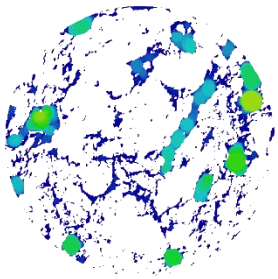
Time 28 day		
		
without worms	worms	Ag_2S-NPs + worms

Table S10

Two-way ANOVA between Ag soil concentrations in the middle and bottom depths of Kooijenburg soil columns with and without earthworms (2 layers x 2 treatments x 4 replicates). When the interaction leads to significant p value, "presence of worms" and "layer" factor are not reported.

Treatment without artificial rain

	Interaction	Presence of worms	layers
7 days without earthworms vs 7 days with earthworms	0.046	-	-
14 days without earthworms vs 14 days with earthworms	0.1605	0.0156	0.1644
21 days without earthworms vs 21 days with earthworms	0.9494	0.0015	0.9579
28 days without earthworms vs 28 days with earthworms	0.4541	<0.0001	0.4408
Treatment with artificial rain			
	Interaction	Presence of worms	layers
7 days without earthworms vs 7 days with earthworms	0.0060	-	-
14 days without earthworms vs 14 days with earthworms	0.0296	-	-
21 days without earthworms vs 21 days with earthworms	0.0018	-	-
28 days without earthworms vs 28 days with earthworms	<0.0001	-	-

Table S11

Two-way ANOVA between Ag soil concentrations in the middle and bottom depths of Kooijenburg soil columns with and without earthworms (2 layers x 2 treatments x 4 replicates).

Treatment without artificial rain			
	Mean square	F value	P value
Without earthworms	0.1398	0.822	0.5590
With earthworms	1.774	5.194	0.0006
Treatment with artificial rain			
	Mean Diff.	95% CI of diff	P value
Without earthworms	0.1431	1.667	0.1575
With earthworms	2.069	2.642	0.0316

Table S12

Two-way ANOVA between Ag soil concentrations in the middle and bottom depths of Kooijenburg soil columns with and without the application of ARW over time.

Treatment without earthworms			
	Interaction	Presence of ARW	layers
7 days without ARW vs 7 days with ARW	0.8330	0.0960	0.3854
14 days without ARW vs 14 days with ARW	0.4890	0.2440	0.7973
21 days without ARW vs 21 days with ARW	0.9900	0.3259	0.8505
28 days without ARW vs 28 days with ARW	0.8789	0.6915	0.2841
Treatment with earthworms			
	Interaction	Presence of ARW	layers
7 days without ARW vs 7 days with ARW	0.6869	0.4997	0.0025
14 days without ARW vs 14 days with ARW	0.8379	0.8384	0.0304
21 days without ARW vs 21 days with ARW	0.0217	0.3964	0.0184
28 days without ARW vs 28 days with ARW	0.0569	0.0954	0.0037

Figure S13

Moisture content at three depths in the Kooijenburg soil columns of the treatment with Ag_2S-NPs and with the application of ARW.