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


# Supplementary Information

## Effect of nanoplastics on the transport of platinum-based pharmaceuticals in water-saturated natural soil and their effect on a soil microbial community

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## 1. Characterization of the PS-NPs

Homogeneous, 50 nm diameter spherical, surface-modified (aminated and carboxylated) polystyrene nanoparticles (PS-NPs), that exhibit a low polydispersity index and form stable colloids in biological systems[1], were purchased from Megasphere, CA, USA. The particles are described by the supplier as "uniform nanospheres"; as such, PS-NPs with surface functional groups are assumed to be covered uniformly. The PS-NP sizes and zeta potentials were verified using a Malvern Zetasizer and the results are given in Table S1. Sizes were found to match the sizes reported by the manufacturer with polydispersity index values ≤ 0.06. We also verified the size, shape and size distribution by SEM analysis as shown in Fig. S1. For all PS-NPs, the concentration was 2.5% and the polymer density was 1.05 g/mL.

Table S1: Physical and chemical characteristics of PS-NPs used in the study

| Size reported by manufacturer (nm) | Average measured size (nm) | Measured zeta potential (mV) | Surface functional groups |
|---|---|---|---|
| 48 ± 10 | 52 | -40.3 | Carboxylated |
| 51 ± 15 | 57 | +36.6 | Aminated |

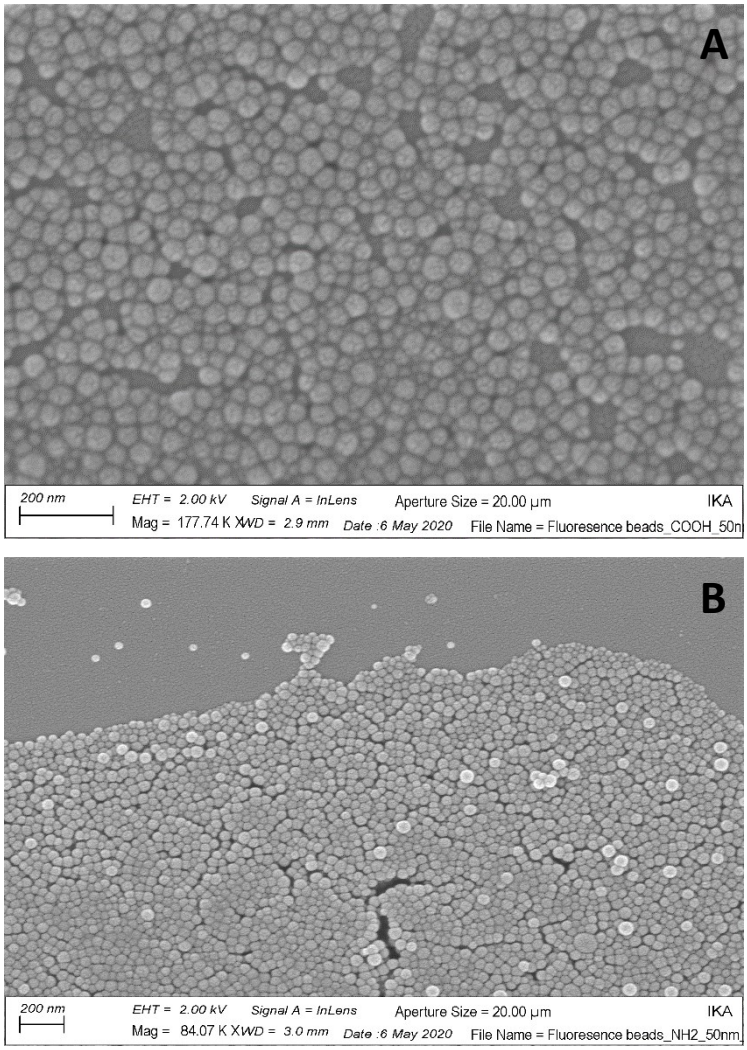


**Fig. S1.** SEM images of the carboxylated (A) and aminated (B) PS-NPs used in the experiment.

## 2. Transport of Pt-based drugs in soil columns

In column transport experiments, the transport of Pt-based drugs were studied in natural soil-water environment. Thus, to mimic the real environment the soil in the packed column was saturated first with DDW and then with simulated water (background solution), containing all kinds of different salts at concentrations relevant in natural water with ionic strength of 8.3 mM. The exact chemical composition of the background solution used in the experiments is presented in Table S2 below. The transport of the Pt-based drugs (carboplatin, Pt-complexes originating from cisplatin, oxaliplatin) was studied in both the presence and the absence of surface functionalized PS-NPs in duplicates. The results of one experiment in each set are presented as Figs. 2, 3 and 4 of the main manuscript. The results of the duplicate experiment of each set are presented below as Figs. S2, S3 and S4.

**Table. S2.** Composition of background solution used in soil column transport experiments of Pt-based drugs.

| Chemical | M. W. (g/mol) | Concentration (µg/mL) |
|---|---|---|
| $MgSO_4 \bullet 7H_2O$ | 246.47 | 157 |
| $K_2HPO_4$ | 174.17 | 4 |
| $NaHCO_3$ | 84.01 | 23 |
| $CaCl_2$ | 110.98 | 127 |
| $NH_4Cl$ | 53.50 | 6 |
| $KNO_3$ | 101.10 | 10 |
| KBr | 119 | 0.5 |
| $NaCH_3COO$ | 82.03 | 60 |
| MeOH | 32.04 | 10 mL/L |
| $Na_3C_6H_5O_7 \bullet 2H_2O$ | 294.10 | 1.78 |

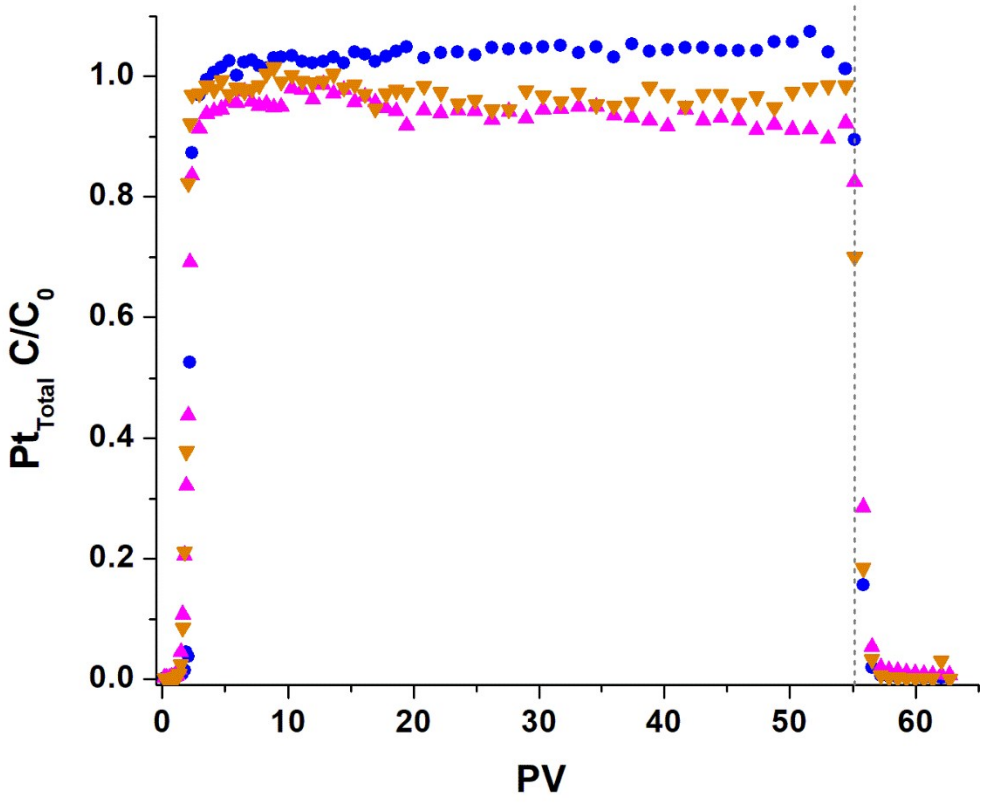


**Fig. S2.** Replicate breakthrough curve of carboplatin (●), carboplatin with aminated PS-NPs (▲), and carboplatin with carboxylated PS-NPs (▼) in water-saturated soil shown as a function of PV. Dashed gray line represents the beginning of the flushing phase.

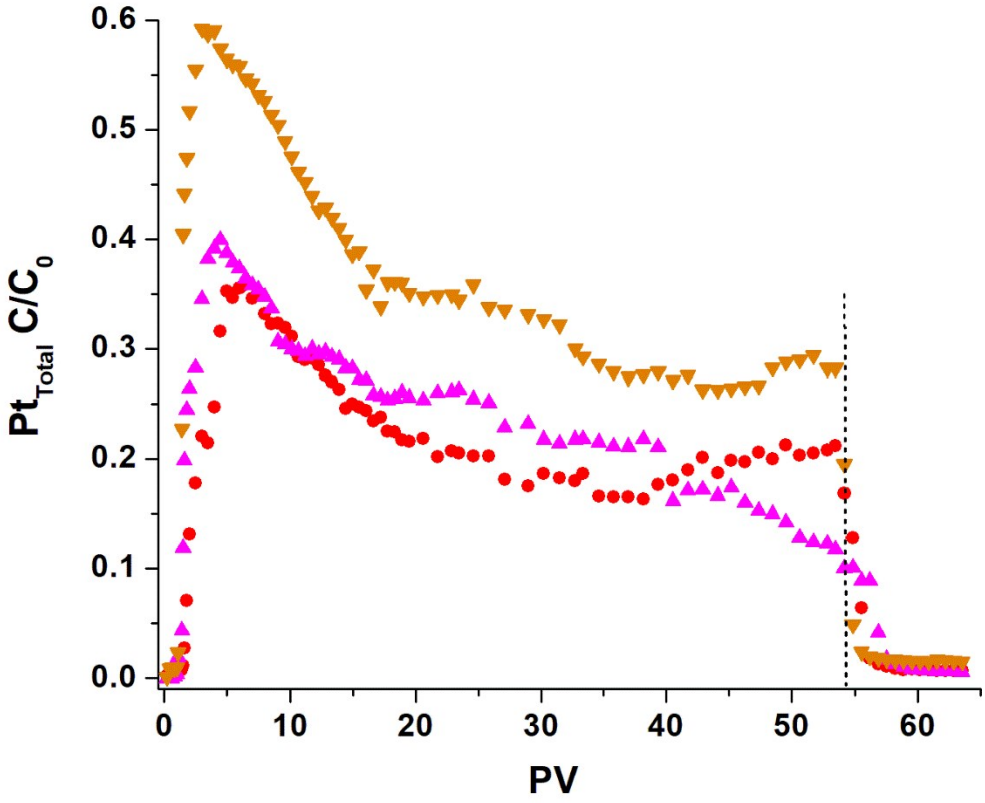


**Fig. S3.** Replicate breakthrough curve of Pt-complexes originating from cisplatin (●), Pt-complexes originating from cisplatin with aminated PS-NPs (▲), and Pt-complexes originating from cisplatin with carboxylated PS-NPs (▼) in water-saturated soil shown as a function of PV. Dashed gray line represents the beginning of the flushing phase.

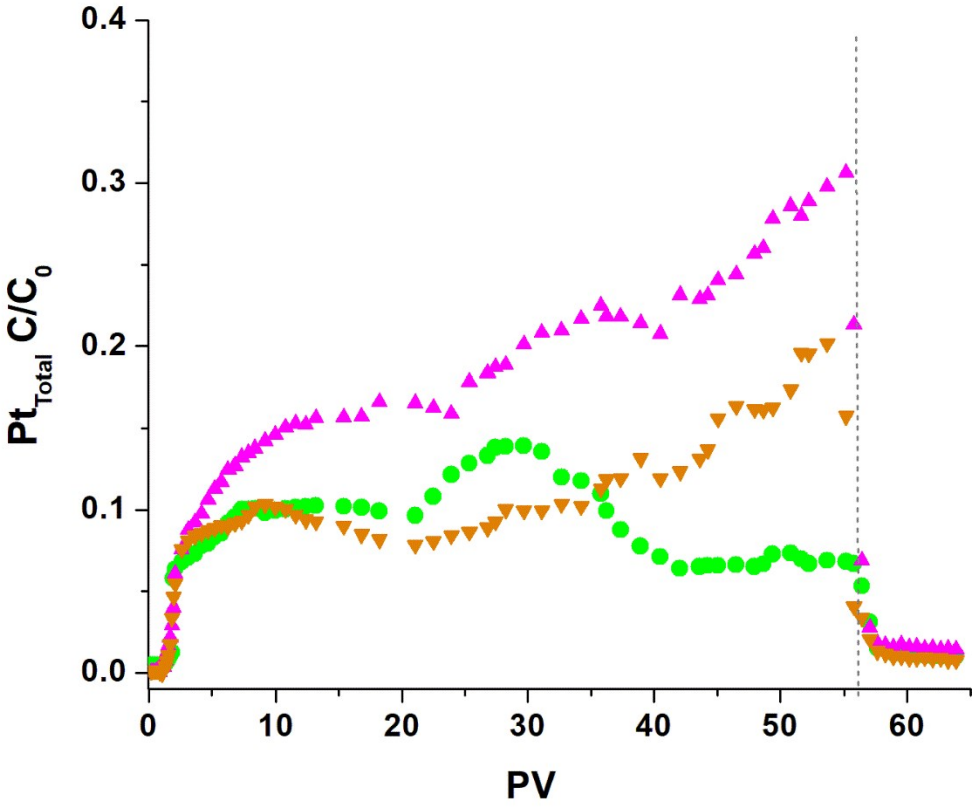


**Fig. S4.** Replicate breakthrough curve of oxaliplatin (●), oxaliplatin with aminated PS-NPs (▲) and oxaliplatin with carboxylated PS-NPs (▼) in water-saturated soil are shown as a function of PV. Dashed gray line represents the beginning of the flushing phase.

### 3. Sorption test of Pt-complexes on PS-NPs

The sorption capacity of carboplatin, Pt-complexes originating from cisplatin and oxaliplatin on the aminated and the carboxylated PS-NPs was tested. Solutions of 0.42, 0.33, and 0.335 mg/L of cisplatin, oxaliplatin and carboplatin respectively in the background solution (see Table S2 for solution composition) were prepared. To 100 mL of each of Pt- drug solution, 50 nm COOH-PS-NPs or 50 nm $NH_2$-PS-NPs at concentration of 5 mg/L were added and allowed to interact. After 1 week, a sample of each solution was moved to a dialysis bag (Cellu Sep H1 with nominal MWCO of 1000) and allowed to equilibrate for 24 h. The solutions were then collected from inside the analysis bag and from the background solution, and analyzed for Pt concentration by ICP-MS.

The results of the sorption experiment are summarized in Table S3. Carboplatin showed the lowest adsorption to the PS-NPs with only ~5% higher Pt concentration inside the dialysis bag compared to the background concentration. Pt-complexes originating from cisplatin showed the strongest adsorption with concentrations ~100% higher than the background solution. For the Pt-complexes originating from cisplatin, the PS-NPs with the carboxylic groups showed higher adsorption compared to the aminated PS-NPs (115.9% vs. 92.2 % increase compared to their respective background solution levels). Oxaliplatin showed 31.3% and 21.1% increases in concentration for the aminated and carboxylated PS-NPs, respectively. It is noted that a

reverse trend in the sorption behavior was found for the aminated and carboxylated PS-NPs, where aminated PS-NPs sorbed oxaliplatin more strongly than carboxylated PS-NPs, and vice versa for Pt-complexes originating from cisplatin (stronger sorption on the carboxylated PS-NPs than aminated PS-NPs). This sorption behavior is in accord with the transport behavior of the Pt-complexes described in the main text. Moreover, it indicates that the enhanced/modified mobility observed is related directly to sorption interactions between the functionalized PS-NPs and the Pt-complex, rather than to blocking of potential sorption sites in the soil. This behavior also demonstrates that the properties of the Pt complex and surface properties of the PS-NPs are involved in the interactions. It is further noted that the enrichment of Pt in the dialysis bag should be considered as supporting data that indicate sorption of the Pt complexes to PS-NPs. Note though that the values of the enrichment can change, as they depend on multiple parameters such as solution composition and sorption kinetics.

**Table S3.** Difference in Pt concentration between sorbed on PS-NPs (inside dialysis bag) and dissolved concentration in the background solution.

| Pt complex | PS-NP functional group | % added Pt in the dialysis bag |
|---|---|---|
| carboplatin | $NH_2$ | 4.6 |
| | COOH | 6.5 |
| cisplatin | $NH_2$ | 92.2 |
| | COOH | 115.9 |
| oxaliplatin | $NH_2$ | 31.3 |
| | COOH | 21.1 |

## 4. Speciation of the Pt-complexes

Carboplatin and oxaliplatin are considered relatively stable under the conditions applied in the experiment. However, cisplatin is relatively labile and is expected to undergo ligand exchange (Goykhman et al.[2] and references within). A chemical scheme for the ligand exchange of cisplatin is presented in Fig. S5. This process is continuous and starts when the inlet solution is prepared; it therefore leads to a constant change in the influent during the BTC experiment.

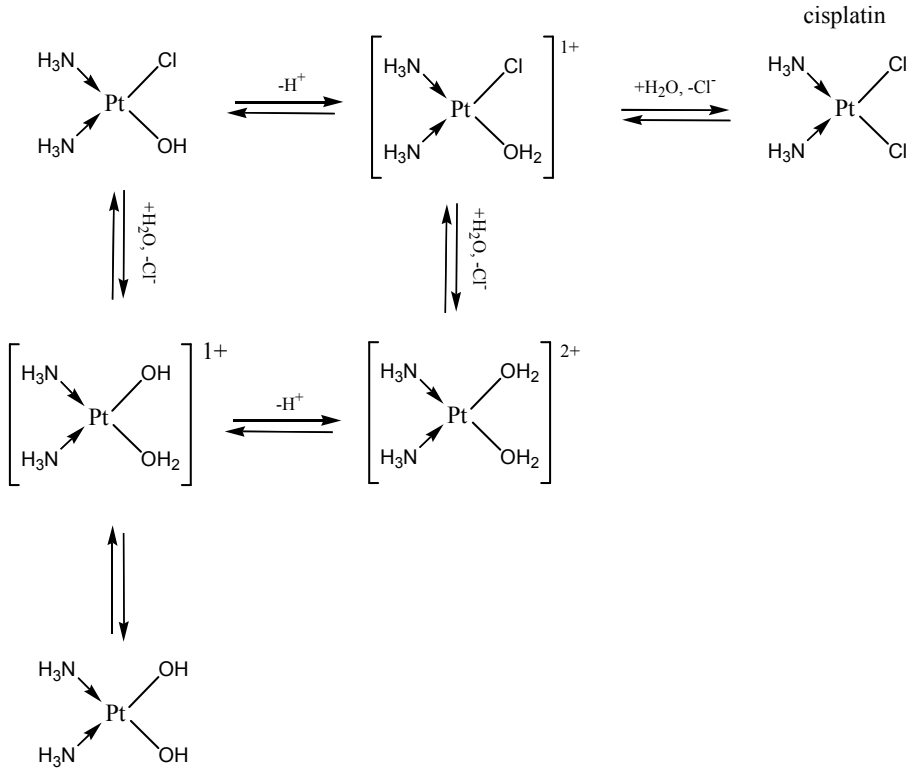


**Fig. S5.** Suggested speciation scheme for ligand exchange of cisplatin in solution.

## 5. Characteristics of soil used for plant cultivation

Soil from the Weizmann Institute of Science campus, Rehovot, Israel was used to study the transport of Pt-based drugs/PS-NPs, and then to study their effect on the soil microbial community. This soil was characterized by Goykhman et al.[2] according to 18 standard procedures[3]; the results are summarized in Table S4.

**Table S4.** Properties of soil from the Weizmann Institute of Science campus, Israel.

| Properties | Methods | Results |
|---|---|---|
| Composition | Hydrometer method | 89% ± 3% sand<br>7% ± 4% silt<br>3% ± 1% clay |
| pH | DDW | 7.97 ± 0.01 |
| | DDW+$CaCl_2$ | 7.41 ± 0.02 |
| Porosity | Saturated column method | 0.37 ± 0.03 |
| Organic matter (%) | Loss on ignition | 0.5% ± 0.2% |
| CEC composition [meq/100g] | $Na^+$ | 0.27 |
| | $K^+$ | 0.13 |

| | | |
|---|---|---|
| | $Mg^{2+}$ | 1.21 |
| | $Ca^{2+}$ | 8.71 |
| Total CEC [meq/100g] | Ammonium replacement method | 10.32 |
| Soil carbonates ($CO_3$-C%) | Gravimetric method | 0.14 ± 0.02 |
| Dissolved Organic Carbon [ppm] | UV at 254 nm | 1.0 ± 0.06 |

## 6. Effect of Pt-based drugs and PS-NPs on microbial community structure

Effects of Pt-based drugs (Pt-complexes originating from cisplatin, oxaliplatin and carboplatin) on soil microbial taxa are discussed in section 3.2 of main manuscript. Figure 5 of the main manuscript shows and compares the effect of these three drugs with respect to the relative abundance of data in control soil for only one of the three studied concentration of Pt-based drugs for better and clear comparison. Fig. S6, shows the effect of three different concentrations, viz. 0.1, 0.6 and 1 g/L of Pt-complexes originating from cisplatin (Fig. S6a), oxaliplatin (Fig. S6b) and carboplatin (Fig. S6c), on the relative abundance of all identified taxa. Figure S6 shows that except for 2-3 taxa, there is not much difference in the effects of three different concentrations.

Fig. S7a shows the replicate data for Fig. 5 of the main manuscript. While Fig. 5 and Fig. S7a show all data for 57 different taxa, Fig. S7b shows the frequency histogram of the same set of data (average of two replicates), to ease comparison of the effect of each Pt-compound, considering the concentration of the microbial taxa in control soil as 1.0 (normalized point). Figures S9, S10 and S11 show the effect of these three Pt-based drugs in the presence of aminated and carboxylated PS-NPs, which are discussed in detail in section 3.3 of the main paper.

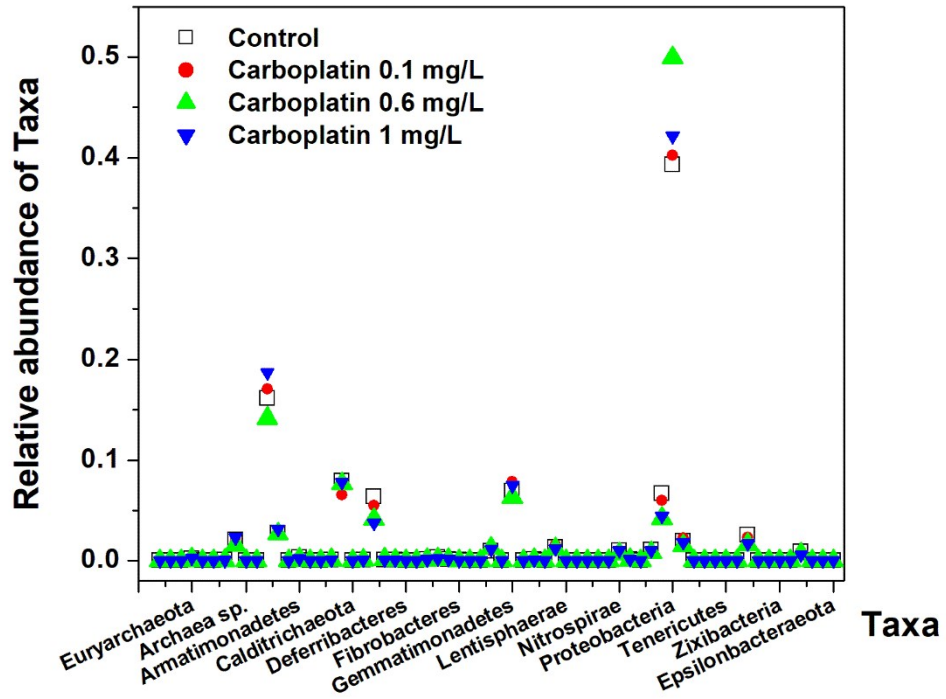


**Fig. S6a.** Effect of three different concentrations of carboplatin (shown as legends), on relative abundance of identified soil microbial taxa.

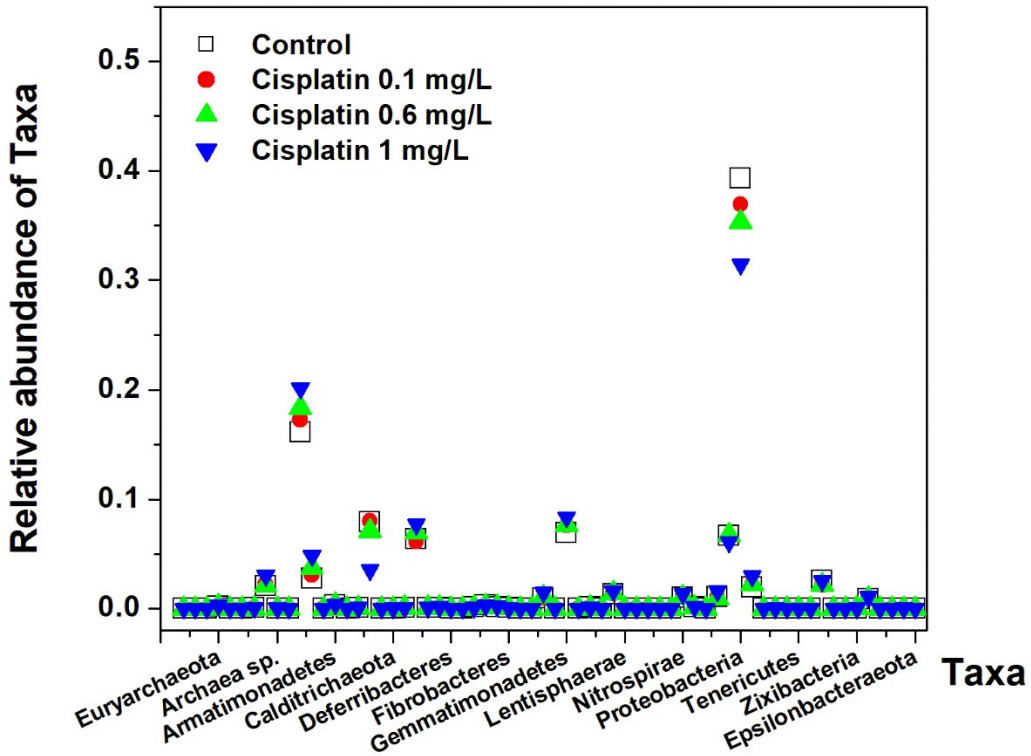

**Fig. S6b.** Effect of three different concentrations of Pt-complexes originating from cisplatin (shown as legends), on relative abundance of identified soil microbial taxa.

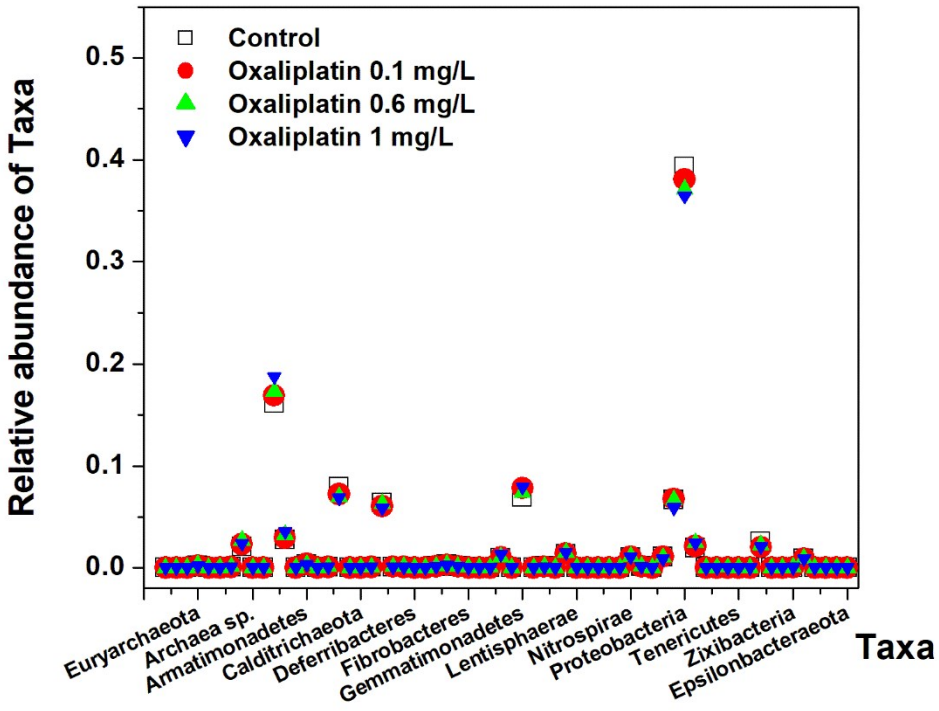


**Fig. S6c.** Effect of three different concentrations of oxaliplatin (shown as legends), on relative abundance of identified soil microbial taxa.

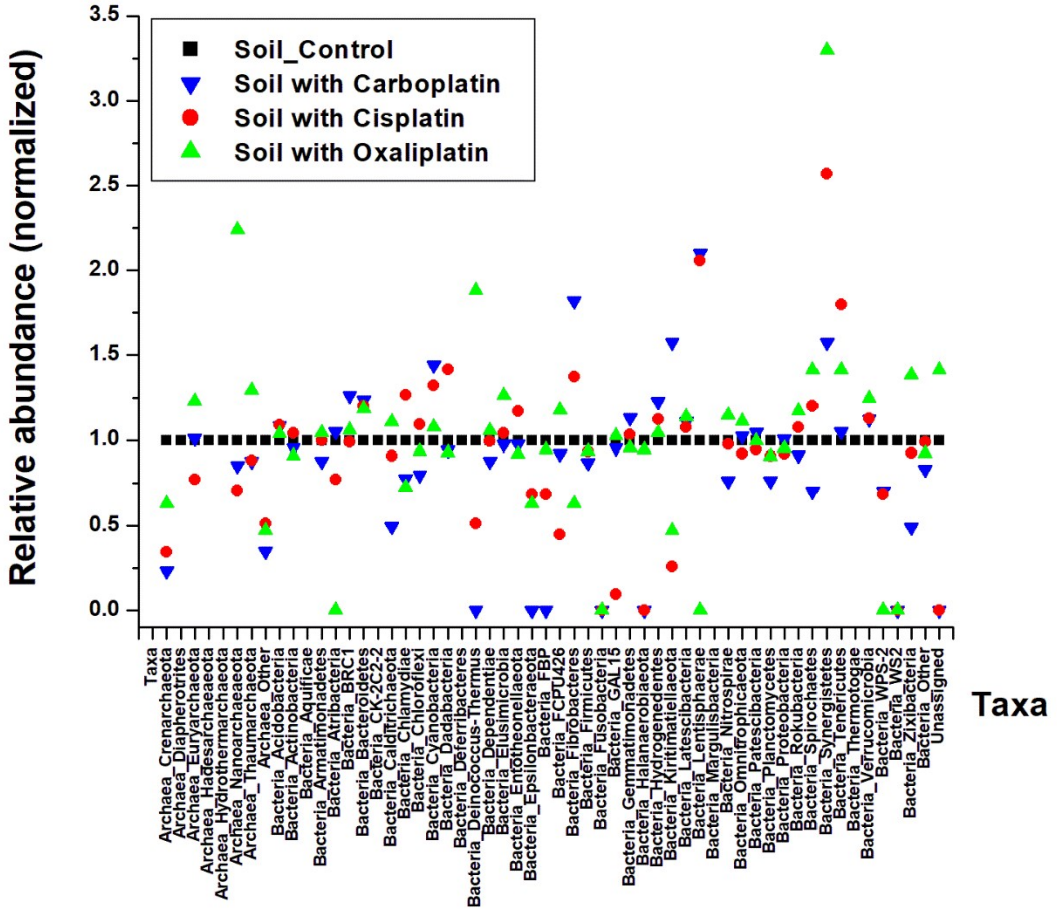


**Fig. S7a.** Replicate data showing the effect of carboplatin, Pt-complexes originating from cisplatin, and oxaliplatin, on Weizmann soil microbial communities (taxa) with respect to the microbial communities present in control (pristine) soil. The x-axis shows the individual taxa. The y-axis shows the average relative abundance of each taxon, normalized to the abundance of each taxon in control soil.

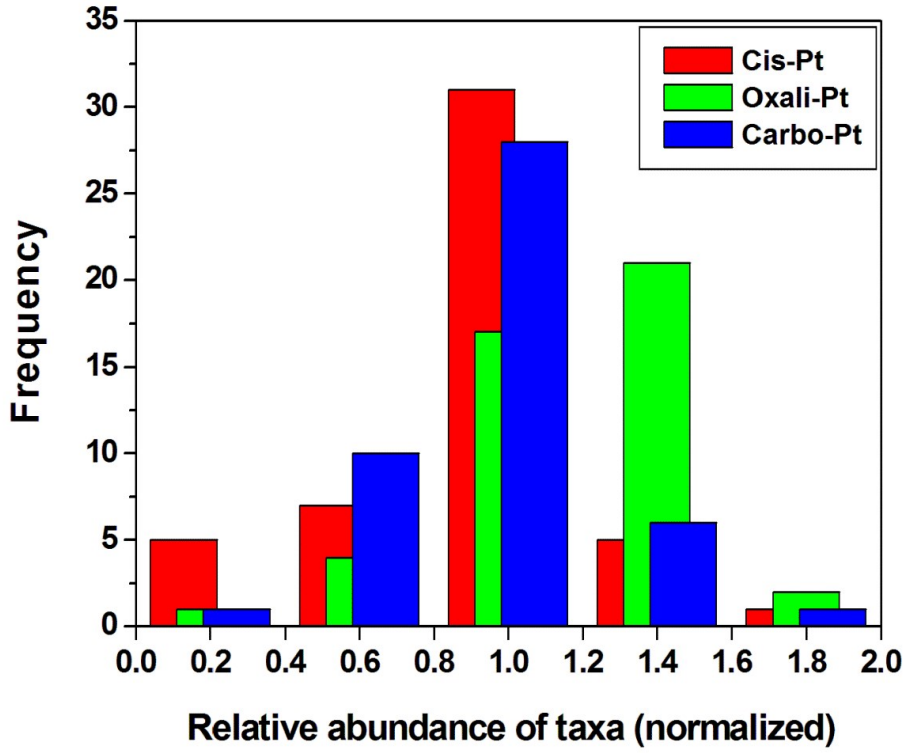


**Fig. S7b.** Frequency histogram for relative abundance of microbial taxa in soil, upon treatment with cisplatin, oxaliplatin and carboplatin. The x-axis shows the normalized relative abundance of taxa (average data from set of two replicate experiments) after treatment with Pt-compounds, normalized to the abundance of microbial taxa in the control soil. The y-axis shows the frequency of relative abundance of taxa, i.e., the ratio of the number of taxa between treated and non-treated control soils.

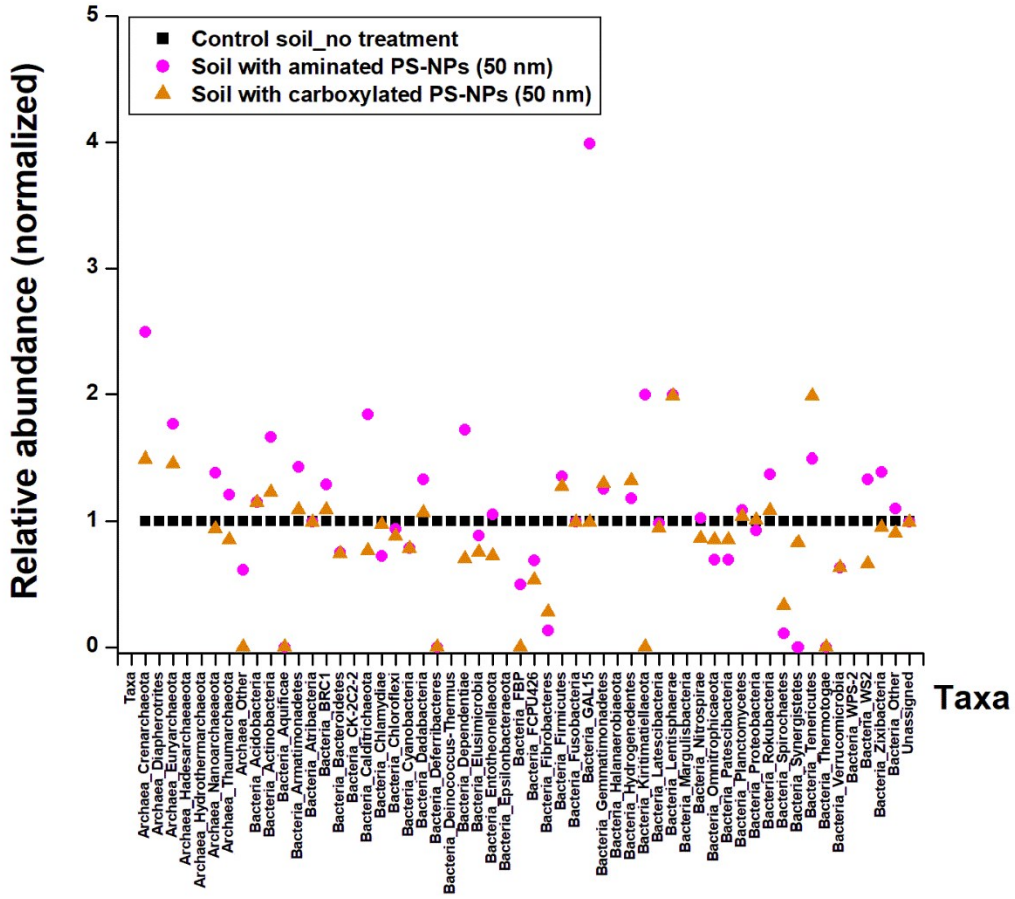


**Fig. S8.** Replicate data showing the effect of 50 nm aminated and carboxylated polystyrene nanoplastics (PS-NPs) on soil microbial taxa with respect to the control soil. The x-axis shows the individual taxa. The y-axis shows the relative average abundance of each taxon, normalized to the abundance of each taxon in control soil.

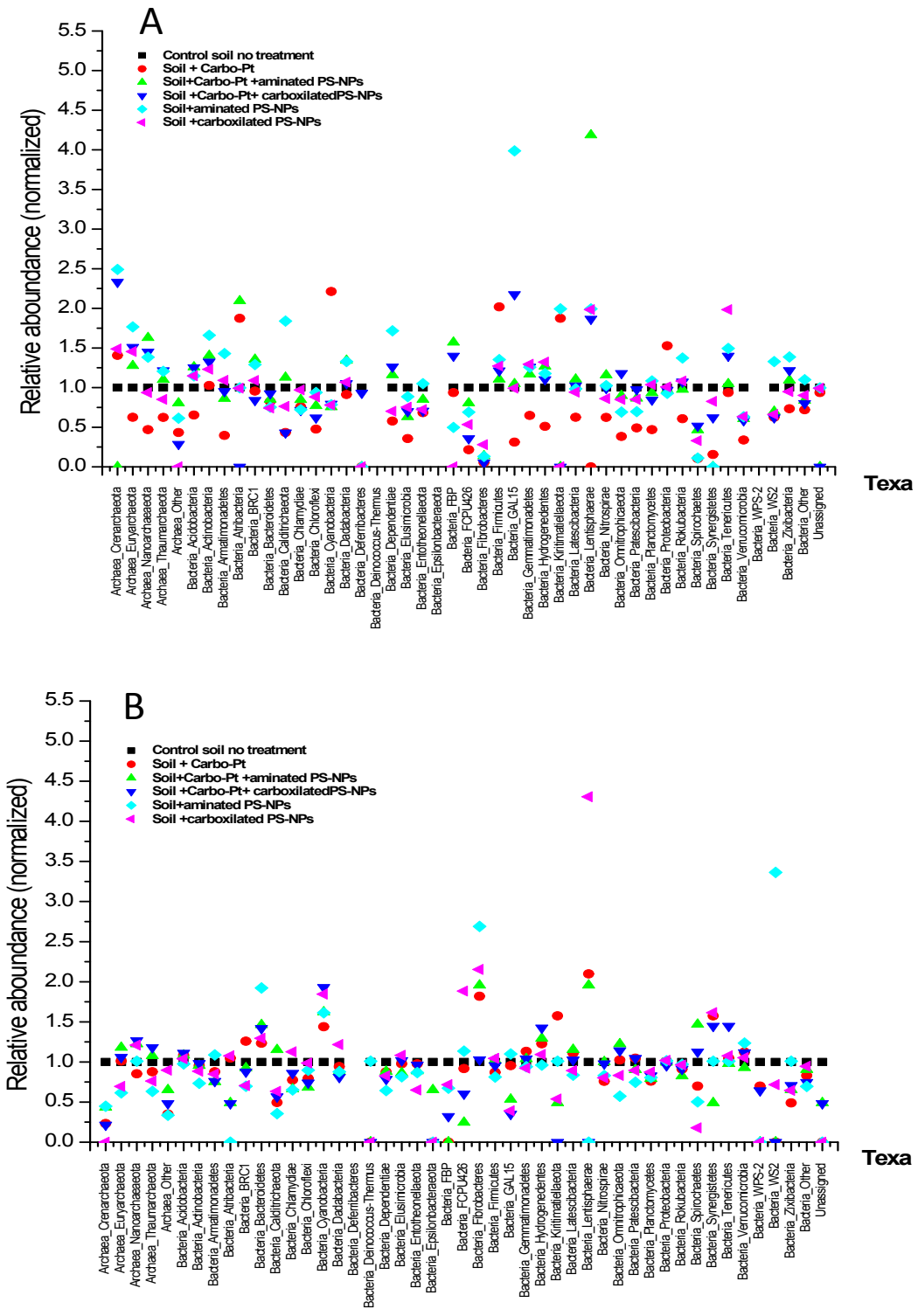


**Fig. S9.** The effect of carboplatin together with aminated and carboxylated PS-NPs on the native microbial communities (taxa) in Weizmann Institute campus soil. The x-axis shows the individual taxa. The y-axis shows the relative abundance of each taxon, normalized to the abundance of each taxon in control soil. Panels (A) and (B) are two replicates of the experiment.

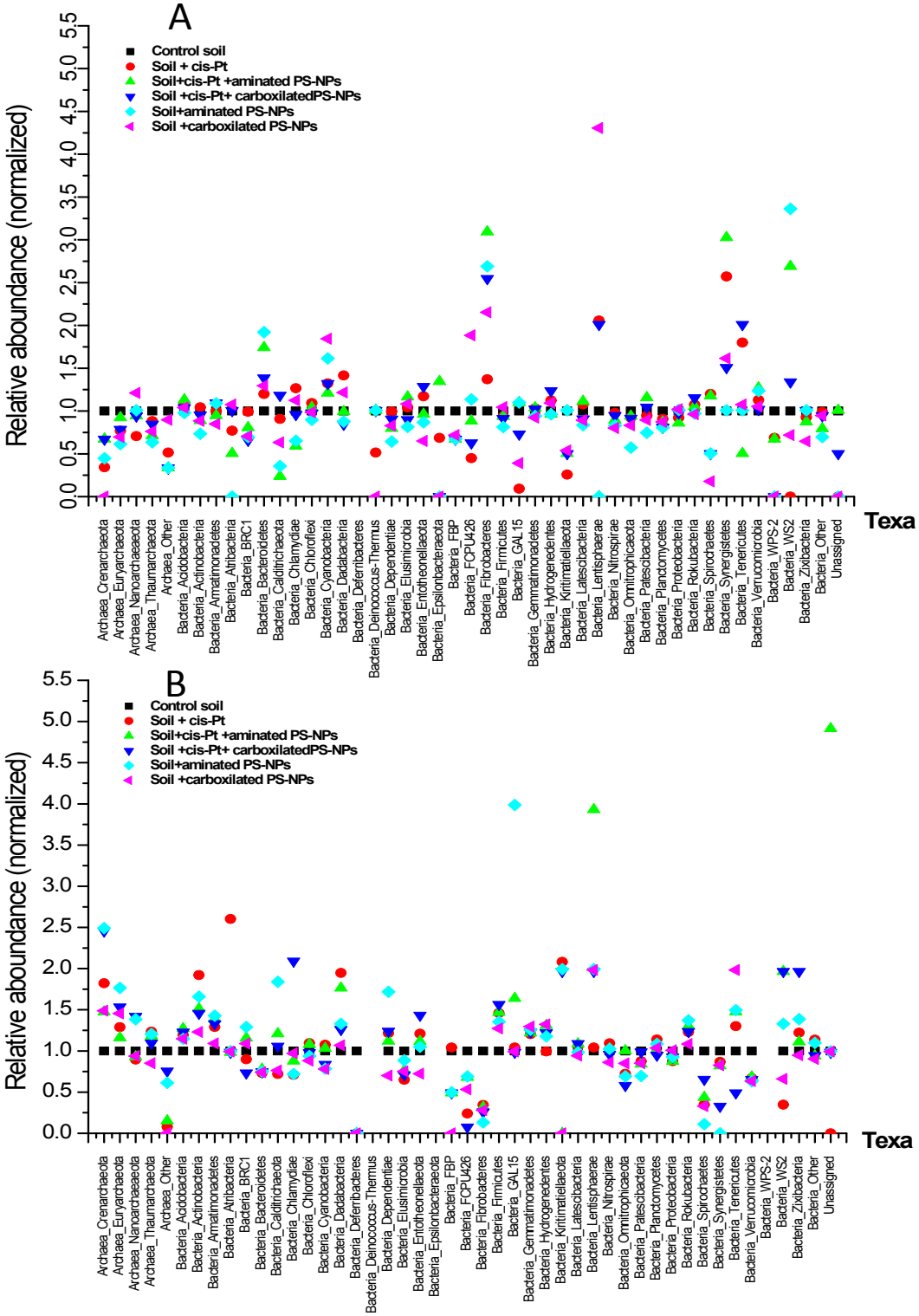


**Fig. S10.** The effect of Pt-complexes originating from cisplatin together with aminated and carboxylated PS-NPs on the native microbial communities (taxa) in Weizmann Institute campus soil. The x-axis shows the individual taxa. The y-axis shows the relative abundance of

each taxon, normalized to the abundance of each taxon in control soil. Panels (A) and (B) are two replicates of the experiment.

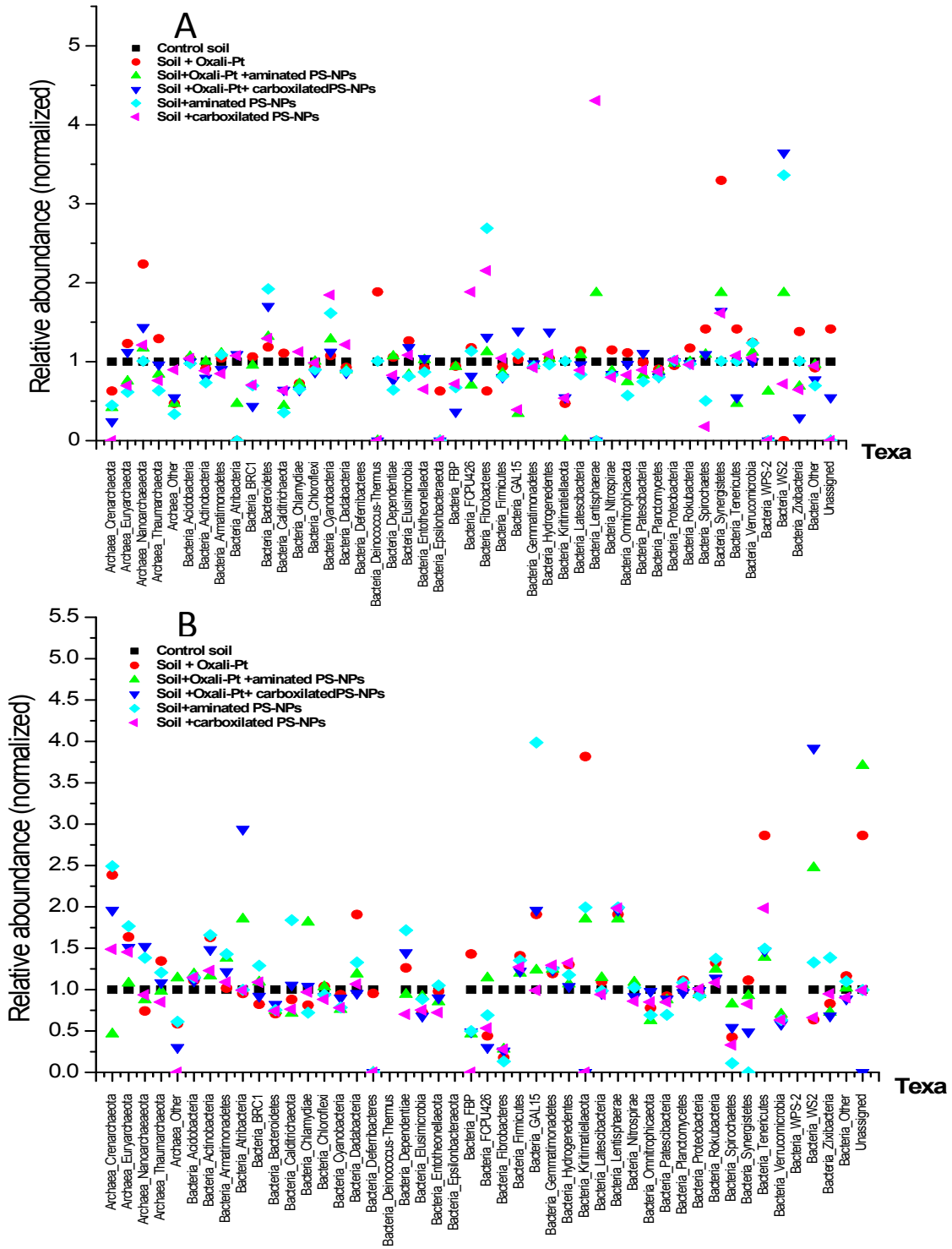


**Fig. S11.** The effect of oxaliplatin together with aminated and carboxylated PS-NPs on the native microbial communities (taxa) in Weizmann Institute campus soil. The x-axis shows the individual taxa. The y-axis shows the relative abundance of each taxon, normalized to the

abundance of each taxon in control soil. Panels (A) and (B) are two replicates of the experiment.

**7. Isolation of Bacterial DNA from soil**

The protocol supplied with the DNeasy power soil kit from Qiagen was followed for isolation of the DNA from soil, as follows:

- 0.25 g of soil samples were vortexed horizontally using vortex adapter with 60 µL of solution C1, in power bead tubes for 20 min followed by centrifugation at 10,000 g for 30 s.
- 500 µL of supernatant was transferred into a 2 mL clean collection tube.
- 250 µL of solution C2 was added, vortexed for 5 s and incubated at 4 ºC for 5 min followed by centrifugation at 10,000 g for 1 min.
- 600 µL of supernatant was transferred into a 2 mL clean collection tube.
- 200 µL of solution C3 was added, vortexed for 5 s and incubated at 4 ºC for 5 min followed by centrifugation at 10,000 g for 1 min.
- Avoiding pellet, 750 µL of supernatant was transferred into a 2 mL clean collection tube and 1200 µL of solution C4 was added, vortexed for 5 s.
- 675 µL of above solution was loaded in MB spin column, centrifugation at 10,000 g for 1 min and the flow through was discarded. This step was repeated twice.
- 500 µL of solution C5 was added to the MB spin column, followed by centrifugation at 10,000 g for 1 min and the flow through was discarded.
- MB spin column was placed carefully into a 2 mL clean collection tube and 50 µL of solution C6 (10 mM Tris-HCl, pH 8.5) was added to the centre of white filter membrane, followed by centrifugation at 10,000 g for 30 s. MB spin column was discarded. 50 µL of pure DNA was stored at -20 ºC.
- Concentration of the purified DNA was checked in Nanodrop spectrophotometer and the amplified with PCR (PCR conditions: 5 min denaturation at 95 °C, 28 cycles of: 95 °C at 30 s, 55 °C at 45 s, 72 °C at 30 s).
- After PCR the amplified DNA product was checked with DNA gel electrophoresis initially for 3 samples (soil-control, soil with aminated PS-NPs, soil with carboxylated PS-NPs). Fig. S11 shows the image of gel electrophoresis showing DNA bands (amplicons) of size ~300 bp, which is ideally within the desired range for the used PCR primer.

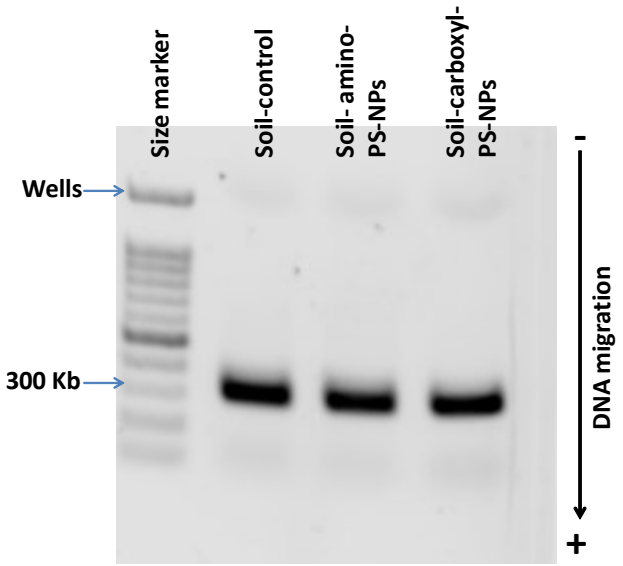


**Fig. S12.** DNA gel electrophoresis of PCR amplicon for samples marked over the gel-lane. Left lane shows the DNA marker.

## References


1. C. Loos, T. Syrovets, A. Musyanovych, V. Mailänder, K. Landfester, G.U. Nienhaus, and T. Simmet, Functionalized polystyrene nanoparticles as a platform for studying bio–nano interactions, Beilstein J. Nanotechnol. 2014, **5**, 2403–2412.
2. N. Goykhman, I. Dror, and B. Berkowitz, Transport of platinum-based pharmaceuticals in water-saturated sand and natural soil: Carboplatin and cisplatin species, Chemosphere 2019, **219**, 390-399.
3. R. Gavlak, D. Horneck, and R. O. Miller, Soil, plant and water reference methods for the western region, 3rd Edition. Western Coordinating Committee on Nutrient Management, USA, 2005.