Additional file 1

Impact of silver nanoparticle (AgNP) exposure on the soil microbial community depending on functionalization, concentration, exposure time, and soil texture

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I Synthesis of AgNP

Two differently functionalized AgNP were used, Ag10-COOH functionalized with carboxy groups and Ag10-NH2 functionalized with amino groups.

The AgNP were synthesized by a ligand exchange starting from hydrophobic silver particles (Ag-HPB). Ag-HPB was synthesized by adding oleylamine to silver acetate solution in toluene. This solution was boiled for 24 h to get a hydrophobic colloidal silver solution. Later methanol was added to precipitate the AgNP. The precipitate was washed several times with MeOH to clean it from excess of oleylamine and others residues to get solid Ag-HPB nanoparticles. For Ag10-COOH or Ag10-NH2, 50 mg Ag-HPB were solved in 1 mL toluene and 50 mg mercaptopropionic acid (MPA, HS–CH_2–CH_2–COOH) in 0.3 mL of MeOH or 50 mg cysteamine hydrochloride (HS–CH_2–CH_2–NH_3Cl) in 0.3 mL MeOH were added respectively. These ligands bind to the silver particles surface through interactions of the surface with the HS-group of the ligand. The mixtures of Ag-HPB/MPA or Ag-HPB/cysteamine were stirred for 1 hr, then centrifuged and the solid parts were washed several times with MeOH. Afterwards, water was added and the pH of the nanoparticles solutions was adjusted to pH 3-4 with HCl (Ag10-NH2) or to pH 8-9 with NaOH (Ag10-COOH), then centrifuged (15 min, 17 400 g) to remove the aggregated and partially modified particles to receive the final Ag10-COOH or Ag10-NH2 colloidal solutions. The concentration of the stock solutions were 180 mg/l for Ag10-COOH and 21 mg/l for Ag10-NH2.

I.I Transmission electron microscopy of AgNP

To investigate the shape and size of Ag10-COOH and Ag10-NH2 microscopically, the stock suspensions were treated in an ultrasonic bath at a power of 42 W/L for 15 min. Afterwards they were diluted to a concentration of 10 µg/mL by water (HiPerSolv CHROMANORM® for HPLC). 2 µL of these suspensions were applied to a single slot copper grid, laminated with polyvinyl butyral. The samples were air-dried for 12 h and analyzed in a Philips CM 12 transmission electron microscope working at an acceleration voltage of 80 kV. To determine the size of the NP, the diameter of 100 particles was measured and the average determined.

I.II ζ-potential and size of AgNP

The nanoparticle surface charge (ζ-potential) was measured by Laser-Doppler-microelectrophoresis (Malvern Zetasizer Nano-ZS, Malvern Instruments Ltd., UK). Samples of the stock solution were prepared by diluting the native Ag10-COOH and Ag10-NH2 suspension, respectively, 1:10 in ultrapure water (UPW). For ζ-potential measurements the diluted samples were analyzed in a Zeta cell DTS 1060C via Zetasizer Nano-ZS. All measurements were carried out at a temperature of 25 °C. The ζ-potential reported herein was obtained as the average of three independent measurements (100 replicates per measurement) performed on each sample.

The particles size was determined by dynamic light scattering (DLS) measurements in batch-mode using a Zetasizer Nano S (Malvern Instruments Ltd., UK). The native AgNP suspension was diluted in UPW (Millipore Milli-Q Integral, Billerica, USA) to a final concentration of 0.5 mg/mL. After 30 s mixing by thorough shaking (IKA Vibrax VXR, Staufen, Germany), the size of the AgNP was measured in acidic, alkaline, and neutral environment to prove the independence of the respective pH values on AgNP characteristics. Six independent DLS measurements were carried out and the obtained values were averaged.

Additionally to the analysis of the stock solution, ζ-potential and hydrodynamic diameter of the AgNP were determined at different pH values (pH 4, pH 7.4 and pH 10). Prior to the measurements the stock solution was diluted in pure water (Millipore) with the pH-values 4, 7.4 or 10 to a concentration of 10 µg/mL, vortexed for 10 sec, incubated for 1 h or 24 h under permanent rotation (100 rpm at 37°C) and vortexed for 30 sec prior to analysis via Zetasizer Nano-ZS.

I.III Asymmetrical flow field-flow fractionation (AF4) of AgNP

Both nanoparticulate silver samples were prepared and diluted prior to AF4 fractionation. The stock solution was sonicated at 42 W/L for 15 min (Sonorex Digital 10 P, Bandelin Electronic, Berlin, Germany) and diluted to the final concentration with UPW followed by thorough manual shaking. After preparation the samples were fractionated by an Asymmetrical Flow Field-Flow Fractionation

(AF4) system from Postnova Analytics (AF2000 MT, Postnova Analytics GmbH [1] including an autosampler (PN 5300) and channel thermostat (PN 4020), which was equipped with an analytical channel. A Mylar spacer of 350 µm height was placed in the analytical channel that had a tip-to-tip length of 277 mm. The temperature of the thermostat was set to 25 °C. The AF4 system was hyphenated to a UV/Vis detector (PN3211) and a Zetasizer Nano-S detector from Malvern Panalytical Ltd. (Malvern, United Kingdom). The wavelength of the UV/Vis detector was set to 420 nm.

For the Dynamic Light Scattering (DLS) measurements the instrument was equipped with a Zetasizer flow cell (type ZEN0023). The measurements were carried out at a detection angle of 173 ° with no attenuation, the measurement position of the flow cell was fixed at 4.2 mm and the temperature was set to 25 °C. For optimum results, measurement duration of 3 s was selected. The instrument control of the AF4 and the data analysis were performed by the NovaFFF AF2000 Control software (version, 2.1.0.4, Postnova Analytics, Landsberg, Germany). DLS data were evaluated with the General Purpose mode within the Malvern Zetasizer software (version 7.12). All subsequent hydrodynamic diameters from DLS measurements are presented as z-average values and averages from a triplicate measurement set.

For the Ag10-COOH fractionation by AF4 the pH of the carrier liquid was adjusted to pH = 9.4 with 0.1 M NaOH (C. Roth GmbH &Co. KG, Karlsruhe, Germany). A regenerated cellulose membrane with a molecular weight cut-off of 10 kDa was placed inside the separation channel. Ag10-COOH was used at a final concentration of 36 mg/L. The injection volume was set to 250 µL. A detector flow rate of 0.5 mL/min was applied. The injection and focusing step were performed with an injection flow rate of 0.2 mL/min, an initial cross flow rate of 1.1 mL/min and an injection and focusing time of 7 minutes. After a 30 s transition time the elution was started with a 5 min long constant cross flow followed by a linear cross flow decay finally reaching a 0.1 mL/min cross flow rate after 20 min. This cross flow rate was kept constant for 10 min. Afterwards a rinse step of 13 min was used to ensure reproducible results by flushing the system and removing potential larger aggregates. The recovery of the separation system for Ag10-COOH was above 90%.

The carrier liquid for the fractionation of Ag10-NH2 was adjusted to pH = 4.0 with HNO_3 (65%, Avantor Perf. Materials Poland S.A.) and the ultrafiltration membrane was changed to PVDF with a molecular weight cut-off of 30 kDa to achieve a meaningful recovery above 75% throughout the fractionation. Ag10-NH2 was diluted as described above to a final concentration of 21 mg/L. After injecting 250 µL of the sample it was focused within the separation channel with an injection flow rate of 0.2 mL/min and an initial cross flow rate of 1.0 mL/min for 7 min. The elution step started after a 30 second long transition time with an constant cross flow rate of 5 min continuing with a 25 minute long linear cross flow rate decay down to 0 mL/min. A rinse step followed the elution step and a detector flow rate of 0.5 mL/min was used.

II Impact of the main factors on the biological parameters in a loamy soil

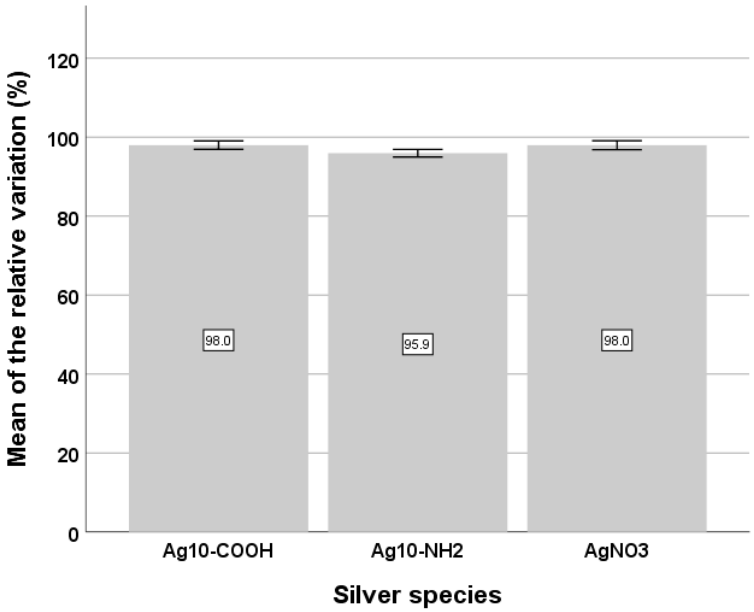

Figure S1: Impact of the main factor *silver species* on the entirety of the relative variation of the biological parameters in a loamy soil. The error bars represent the 95% confidence interval. N = 4320.

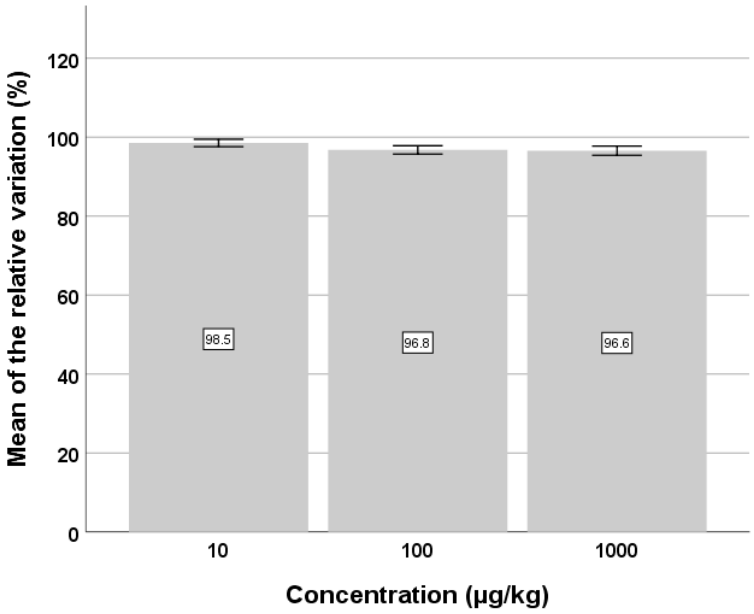

Figure S2: Impact of the main factor *concentration* on the entirety of the relative variation of the biological parameters in a loamy soil. The error bars represent the 95% confidence interval. N = 4320.

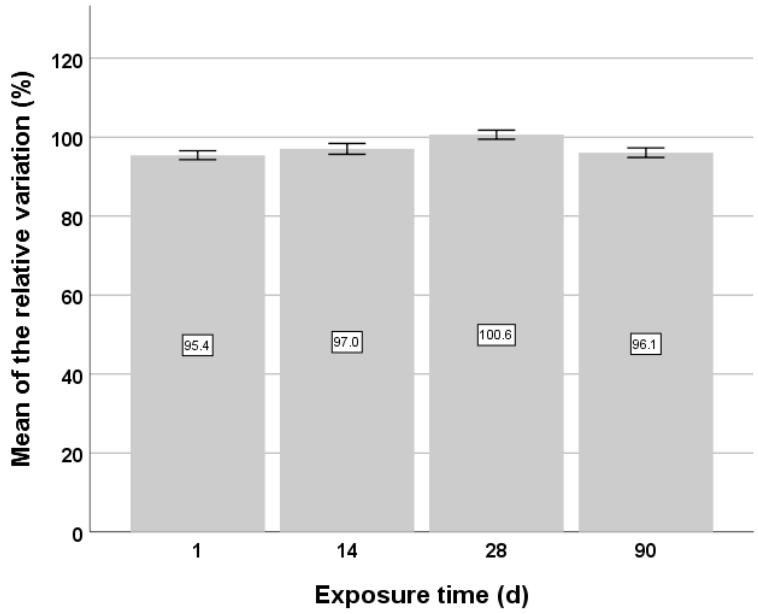

Figure S3: Impact of the main factor *exposure time* on the entirety of the relative variation of the biological parameters in a loamy soil. The error bars represent the 95% confidence interval. N = 4320.

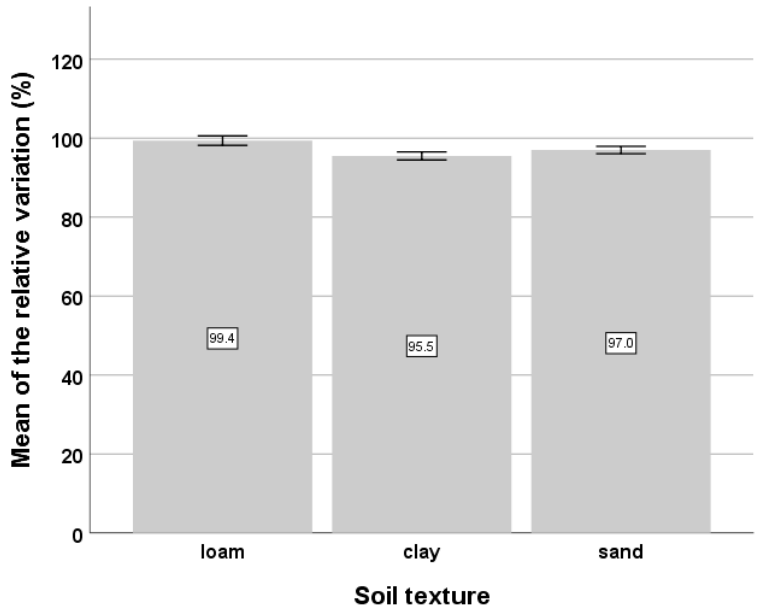

Figure S4: Impact of the main factor *soil texture* on the entirety of the relative variation of the biological parameters in a loamy soil. The error bars represent the 95% confidence interval. N = 4320.

III References

1. Agnieszka W, Zofia S, Aleksandra B, Artur B (2012) *Evaluation of factors influencing the biomass of soil microorganisms and DNA content.* 2:64.