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Electronic supplementary information (ESI)

Distribution of engineered Ag nanoparticles in the aquatic-terrestrial transition zone: A long-term indoor floodplain mesocosm study

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1 Materials and Methods

1.1 Mesocosm setup

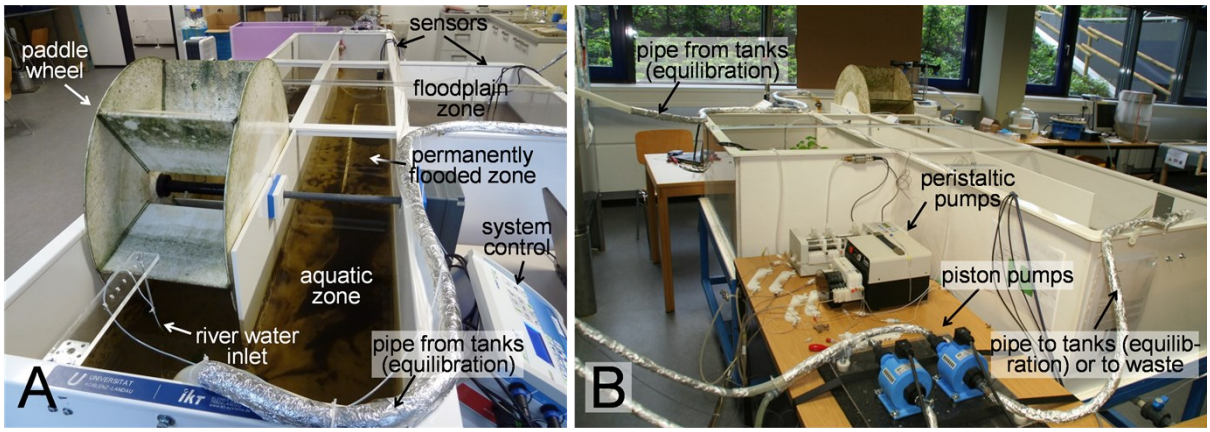

Figure S 1. Experimental setup of the mesocosm system. The view from the paddle wheel side (A). The view of the pumps (B).

1.2 Quartz sand

Table S1. Properties of applied quartz sand (manufacturer data).

Bulk density (g cm^{-3})	1.4
Density (g mL^{-1})	2.65
Mean grain size (mm)	0.2
Theoretical specific surface (cm^2 g^{-1})	116
Grain size distribution	
Grain classes (mm)	Mass weighted fraction (%)
> 0.355	1
0.250-0.355	13
0.180-.250	56
0.125-0.180	27
0.090-0.125	3
< 0.090	-
Chemical composition (weight %)	
SiO_2	99.5
Al_2O_3	0.25
Fe_2O_3	0.04
Ignition loss, 1000°C	0.2

1.3 Washing of quartz sand

In order to measure the release of ions, 15 g and 30 g of quartz sand were mixed with 30 mL ultrapure water (UPW, resistivity: 18.2 MΩ·cm, Direct-Q UV, Millipore, Molsheim, France) in 50 mL centrifuge tubes (VWR, Polypropylene, Darmstadt, Germany) and shaken for 24 h using a laboratory shaker (20 rpm, rotation angle: 360°, INTELLI-MIXER, NeoLab, Heidelberg, Germany). As a control 30 mL UPW without quartz sand was also shaken. After finishing of the first shaking, the supernatant was removed by decantation, quartz sand was redispersed again in fresh UPW and shaken again for 24 h. The water exchange and shaking was repeated again under same conditions. In supernatants from all washing steps, the electrical conductivity was measured (C863, Consort, Turnhout, Belgium). In the filtered (0.025 µm membrane, VSWP, Nitrocellulose, Millipore, Ireland) supernatants obtained after first shaking, the concentration of several elements was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES, 720, Agilent Technologies, Santa Clara, USA) after stabilization in 1% HNO_3 (sub-boiled).

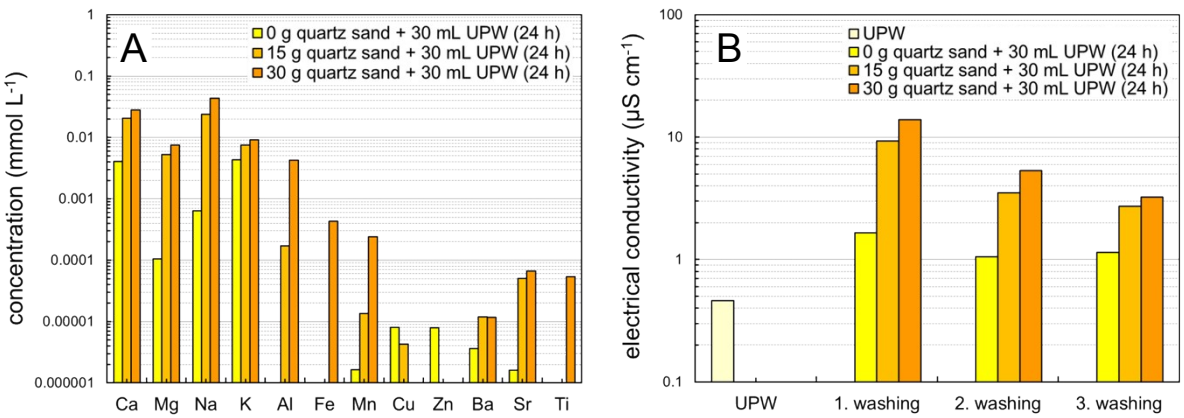

Figure S 2. Element concentrations (A) and electrical conductivity (B) in the washing waters after washing of quartz sand in ultrapure water (UPW). The element concentrations were measured in the first washing water after filtration with 0.025 µm membrane.

1.4 Water mixing tests

In order to measure the mixing conditions in mesocosm, the system containing first loading of gravel and quartz sand was filled up with deionized water (MINISTIL, P22 with electrical conductivity meter LKMA1.0, Orben Wasseraufbereitung, Wiesbaden, Germany) to the normal water level (23 cm) and later to the high water level (27 cm). After injection of the NaCl solution into the water phase, the electrical conductivity was measured at different locations, two depths (2.5 cm and 6.5 cm) and for normal and high water levels. The flow rate of water moved in the aquatic zone (AZ) by paddle wheel was in the range of 0.09-0.11 m s^{-1}. The measurement locations are shown in Figure S 3. In order to save time, the flow rate was slightly higher compared to the flow rate of 0.03 m s^{-1} applied later in the experiment.

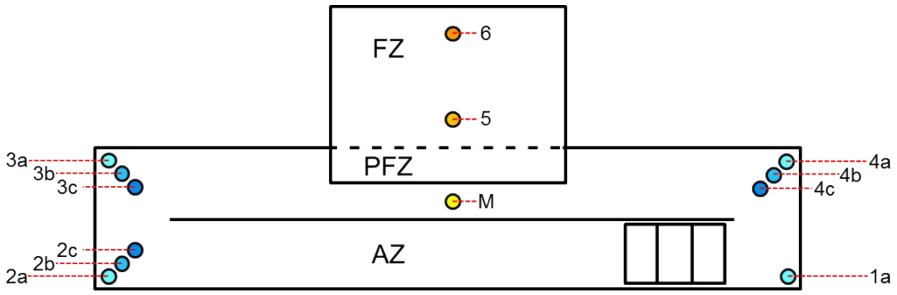

Figure S 3. Electrical conductivity measurement locations (circles) in mesocosm system for the determination of mixing conditions by injection of salt (NaCl) solution. The measurements were performed at two depths (2.5 and 6.5 cm) and for normal and high water level.

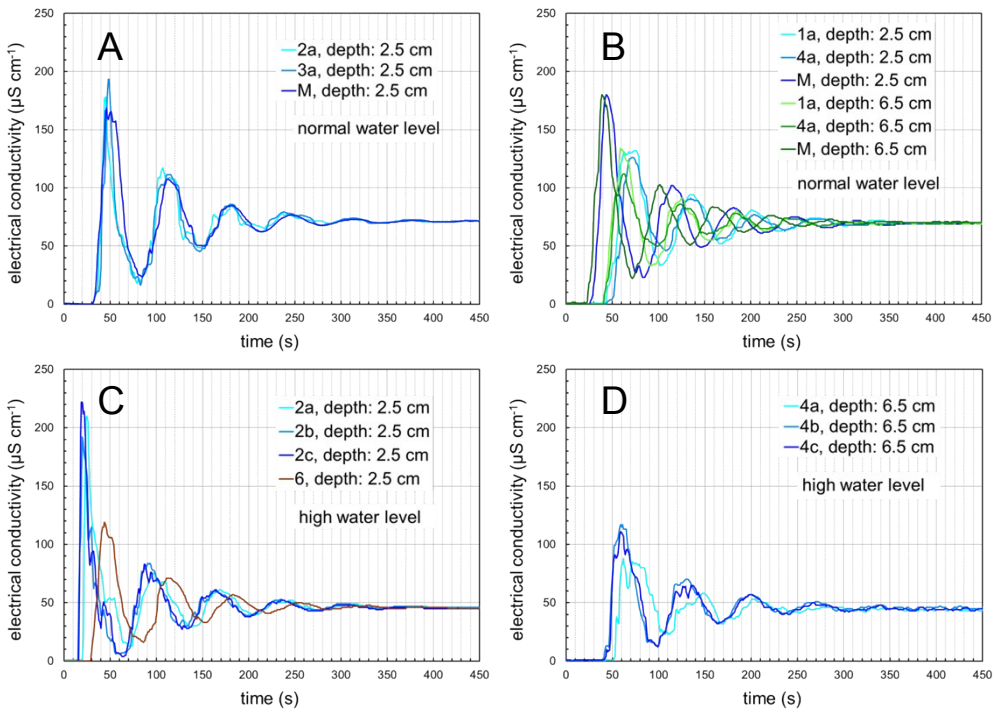

Figure S 4. Electrical conductivity values measured at selected locations and two depths (2.5 and 6.5 cm) in the water phase of the mesocosm system for normal (A, B) and high (C, D) water level after injection of salt (NaCl) solution. The background was subtracted from the measured electrical conductivity values.

1.5 River water and soil

For the mesocosm study, river water (ca. 3000 L) was collected on July 12, 2014 (9 days before the start of the experiment) from the river Rhine (Leimersheim, Germany) and stored in the water tanks in the laboratory at room temperature. The water level in the river during sampling was high and adjacent floodplain area was completely flooded. The temperature and pH of the water directly after sampling were 19.3°C and 7.9, respectively. In order to ensure high oxygen concentration in Rhine water, the tanks were continuously aerated using an air pump (AquaOxy 2000, Oase, Hörstel, Germany). The soil for the mesocosm experiment was collected on May 23,

2014 (ca. 2 months before the start of the experiment) from the floodplain area of the River Rhine (Karlskopf, Germany) near to the river water sampling location. Soil was sampled from 2-10 cm depth, homogenized, dried at room temperature, ground with a mortar and pestle, and sieved (< 2 mm). The German DIN ISO 11277/2002-08 standard[1] was applied for the determination of grain size distribution of soil. The total C, H, N, and S content was determined with a CHNS analyzer (elementar vario MICRO cube, Elementar Analysensystme Hanau, Germany). The pH for soil was determined in a solution of 0.01 mol L^{-1} $CaCl_2$ directly after sampling in the field.

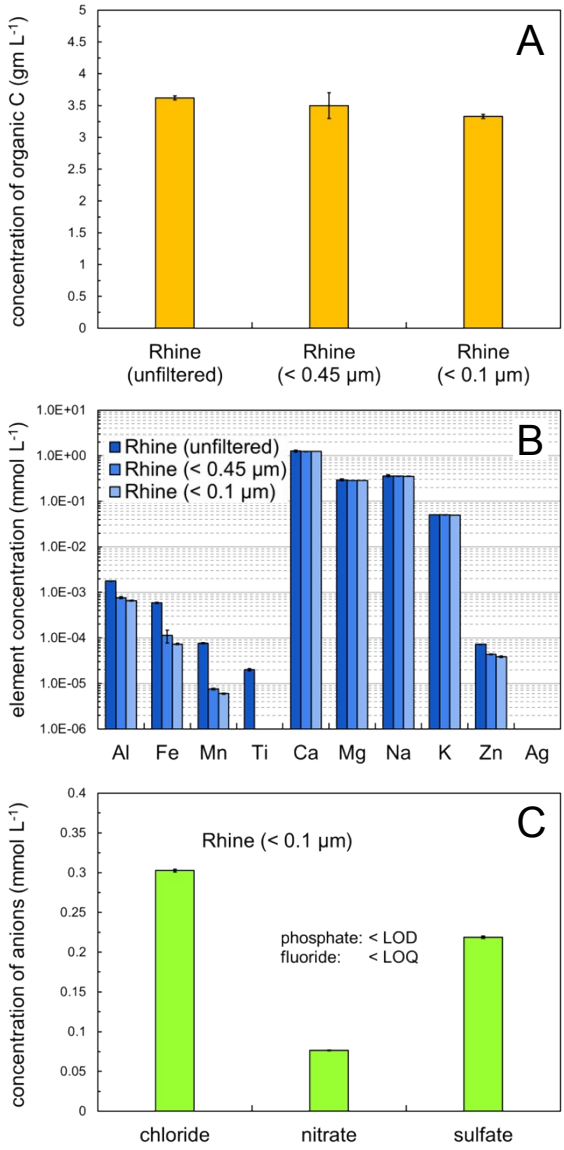

Figure S 5. Concentration of organic carbon (A) and elements (B) in Rhine water before and after filtration (< 0.45 µm, < 0.1 µm) and concentration of anions in filtered (< 0.1 µm) Rhine water (C). The columns and error bars represent the mean values and standard deviations determined from three replicate samples, respectively. The abbreviations "LOD" and "LOQ" mean limit of detection and limit of quantification, respectively ($LOD_{fluoride}$ = 0.01 mmol L^{-1}, $LOQ_{fluoride}$ = 0.03 mmol L^{-1}, $LOD_{phosphate}$ = 0.05 mmol L^{-1}, $LOQ_{phosphate}$ = 0.18 mmol L^{-1}).

Table S2. Mineral composition (n = 2), CHNS content (n = 3), and pH value (n = 3) of the soil (mean value ± standard deviation).

Mineral composition (weight %)	
Sand (0.060-2.000 mm)	10.4 ± 1.5
Slit (0.002-0.060 mm	65.8 ± 1.4
Clay (< 0.002 mm)	23.8 ± 1.2
Loss of the method	2.1 ± 1.9
CHNS content (%)	
C	6.30 ± 0.10
H	0.75 ± 0.03
N	0.41 ± 0.02
S	0.33 ± 0.01
pH	7 ± 0

1.6 Mesocosm experiment

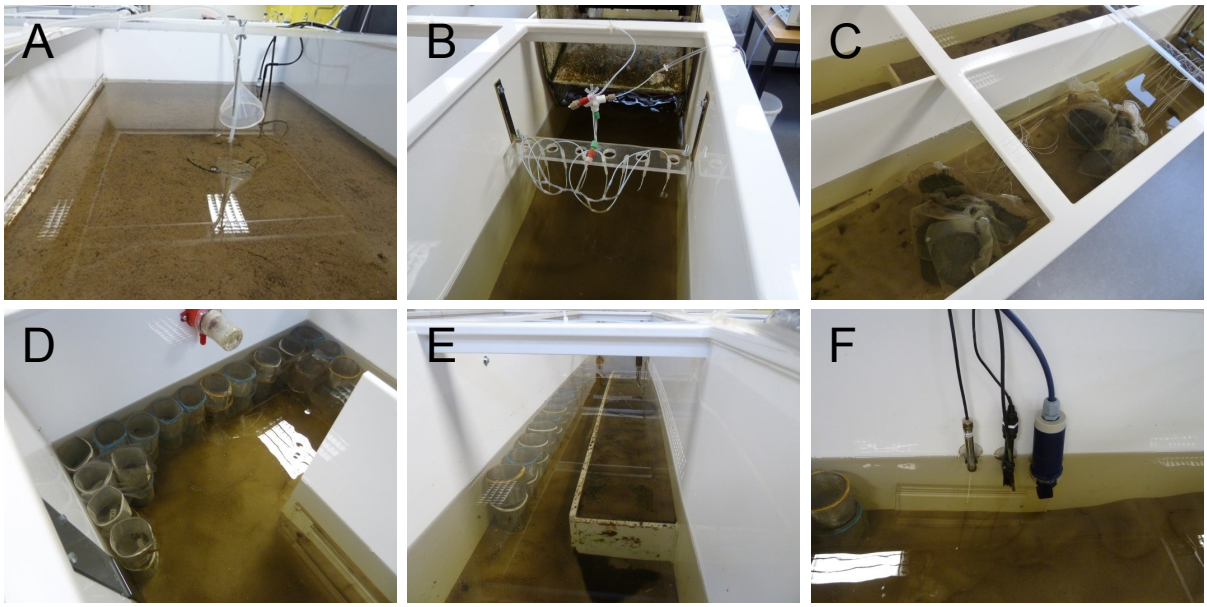

Figure S 6. Flooded floodplain zone (FZ) and sensors for the monitoring of redox potential and tensiometer pressure difference (A). Capillary tubes for the nanoparticle input into the water phase (B). The ends of the capillary tubes are submerged into the water. Leaf bags submerged into the water (C). The cages with *Gammarus fossarum* for low flow (D) and high flow (E) exposure. The sensors for the on-line monitoring of O_2 concentration, pH value, and electrical conductivity of the water phase and sampling location for the water samples (F).

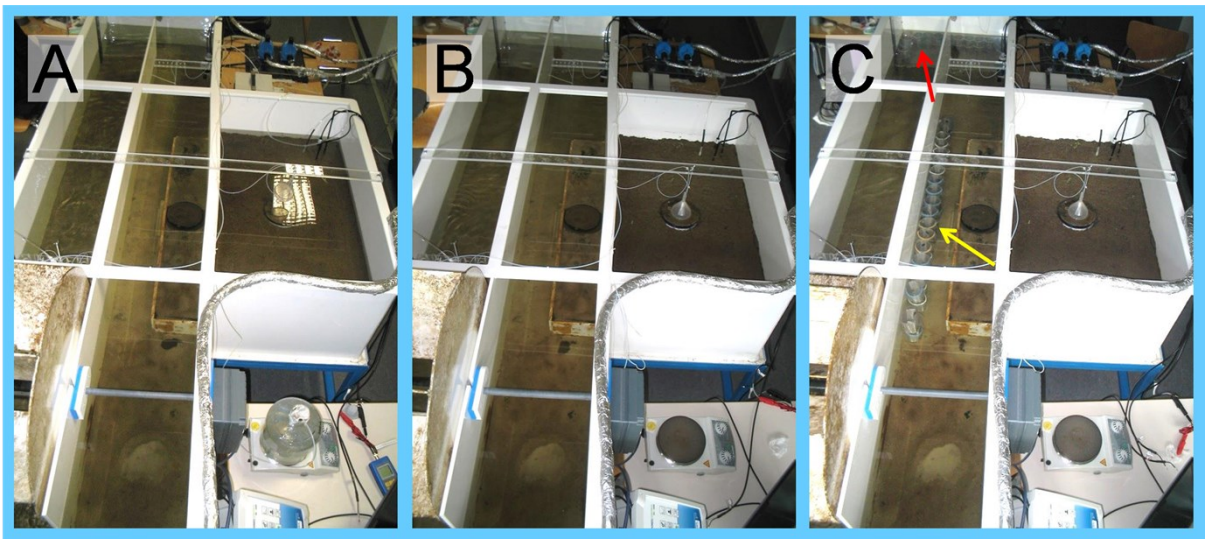

Figure S 7. High water level and flooding event (A). Low water level between flooding events (B). Application of *Gammarus fossarum* feeding assay (C). The red arrow shows the cages with animals for low flow exposure and yellow arrow for high flow exposure.

1.7 Synthesis and characterization of Ag-NPs

Silver nanoparticles (Ag-NPs) were synthesized by a citrate reduction method The details of the synthesis method are given in our earlier studies[2,3]. Three batches of Ag-NP dispersions were synthesized with a volume of 2 L each. All dispersions were pooled in one stock dispersion finally containing 1 mmol L^{-1} Ag and 5 mmol L^{-1} trisodium citrate. The prepared stock dispersion was stored in dark at 4°C. The stock dispersions of Ag-NPs were characterized by several methods. The hydrodynamic diameter of Ag-NPs was determined using dynamic light scattering (DLS) at a scattering angle of 165° (Delsa Nano C, laser wavelength: 658 nm, Beckman Coulter, Fullerton, USA). In order to evaluate the long-term colloidal stability of nanoparticles, the hydrodynamic diameter of Ag-NPs in stock dispersion was measured 4 and 5 years after preparation. For the determination of the zeta potential, an electrophoretic light scattering was applied using same devise. For further characterization of initial Ag-NPs, transmission electron microscopy (TEM, CM200 FEG/ST, 200 kV, PHILIPS, Eindhoven, Netherlands) and scanning electron microscope (SEM, ZEISS LEO 1530, 10 kV) were used. For TEM and SEM measurements, the samples were prepared by deposition of a drop of nanoparticle stock dispersion onto a 3 mm copper grid covered with a combined holey and ultrathin (about 3 nm) carbon film (Ted Pella, Inc., Redding, USA) or a commercially available silicon wafer, respectively with subsequent air-drying for ca. 1 h. For TEM measurements, the Ag-NP stock dispersion was diluted with UPW by a factor of 1:10. The sample for SEM was measured without dilution.

1.8 Experiments for the determination of interactions between nanoparticles and polypropylene material of mesocosm

The interactions between silver nanoparticles (Ag-NPs) and polypropylene (PP) material of mesocosm were evaluated in separate sorption experiments. The 200 mL Rhine water containing 100 µg L^{-1} Ag-NPs was shaken (3015, GFL, Burgwedel, Germany) in 250 mL glass bottles in the presence and absence of PP plates (length: 5 cm, width: 2 cm, and high: 1 cm). The PP plates were fixed in the glass bottles with a 20 cm nylon string (wire). Additionally, the same solution with wire but without PP plates was also shaken. The bottles were removed from the shaker at several time points. For each time point and application, three replicate samples were prepared. After shaking, 10 mL sample was removed and the Ag concentration was measured after microwave assisted acid digestion.

1.9 Sampling of leaves, biofilms, plants, sediment, and soil

Leaves were collected several times between weeks 10 (control phase) and 22. Until analyze, the leaves were stored at -20°C. After termination of the experiment, biofilms were sampled from the sediment top surface of the AZ and permanently flooded zone (PFZ) and scraped off of the paddle wheel and mesocosm wall surfaces. In biofilm samples, the organisms were identified. The residual sand was removed by sedimentation and washing of biofilm samples three times with the water from the AZ. Biofilms were removed from water by centrifugation at 4025 g for 10 min (UNIVERSAL 320, Hettich, Tuttlingen, Germany) and stored at 4°C. The dry mass of biofilm samples was determined by drying the materials in a drying oven (BINDER FD 53, Binder, Tuttlingen, Germany) at 105°C for 24 h. The plants from the FZ were cut above the roots to avoid disturbing the soil. Therefore, we did not analyze the Ag concentration in roots. To remove the soil adhered on the stems and leaves, the plant material was carefully washed with water from equilibration phase, dried at room temperature over night, and stored in plastic bags. To determine the dry mass of plants, they were dried in a drying oven at 60°C for 48 h and 105°C for 24 h. A PP tube (diameter: 12 cm) was used to collect the sediment of the AZ from different depths (0-0.5, 0.5-4 cm). The stainless steel (V2A) ground engravers were used to collect sediment layers of the PFZ (diameter: 1 cm; depths: 0-0.5, 0.5-1, 1-3, 3-5, 5-8, 8-12 cm) and soil profiles of the FZ (diameter: 5 cm, depths: 0-1, 1-3, 3-5, 5-10, 10-20 cm). The sampling locations for sediment (AZ and PFZ) and soil (FZ) samples are shown in Figure S 8. The transport columns were removed from the PFZ and FZ and were cut in different layers (Table S3). Each layer of the sediment and soil was homogenized and divided in several aliquots. One aliquot was dried at 105°C for 48 h to determine the water content.

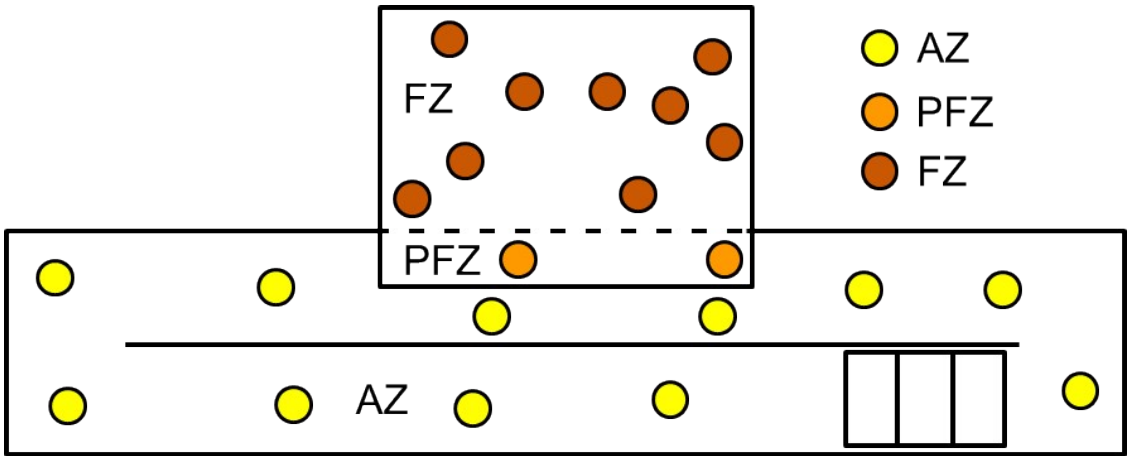

Figure S 8. Sampling locations for sediment (in the AZ and PFZ) and soil (in the FZ) samples.

Table S3. Layers of the PFZ and FZ soil columns.

	Soul column in the PFZ	Soil column in the FZ
Length of the soil column[1]	350 mm	450 mm
Layers[2]	0-350 mm in steps of 10 mm	0-40 mm in steps of 10 mm
		40-55 mm
		55-75 mm
		75-85 mm
		85-100 mm
		100-200 mm
		200-300 mm
		300-400 mm
		400-450 mm

[1] This length corresponds to the column section filled up by soil. The lowest 50 mm of each column was filled up by gravel. The total length (soil + gravel) of the column was 400 mm in the PFZ and 500 mm in the FZ.

[2] 0 mm corresponds to the top surface of the column.

1.10 Identification of Ag-NPs and their aggregates in mesocosm samples by electron microscope

To determine the Ag-NPs or their aggregates in biofilm, sediment, and soil, a SEM (FEI QUANTA FEG 650) equipped with energy-dispersive X-ray spectroscopy (EDXS, Bruker QUANTAX Esprit 1.9) was used at the Karlsruhe Institute of Technology (KIT, Karlsruhe, Germany). For SEM imaging and EDXS measurement, the biofilm samples were air dried on a silicon wafer and the dry sediment or soil samples were fixed on a carbon tape. Before imaging, the samples were coated with

a thin (5-10 nm) platinum layer using vacuum-sputtering (Leica EM MED020) to improve the imaging and to reduce the microscope beam damage, especially for the biofilm samples. The SEM was operated under high vacuum at 10 kV accelerating voltage with a 20000-50000 fold magnification. The distance between lens and the sample stage was between 10.3 and 10.9 mm. For each recording, the focus, contrast, and brightness were adjusted separately. Biofilm samples were additionally characterized in scanning transmission electron microscopy (STEM) mode. In STEM mode, the microscopy was operated at 30 kV accelerating voltage and the distance between lens and the sample stage was 8.3 mm.

1.11 Comparison of sample storage, digestion, and analysis methods

In order to compare the digestion methods, the water samples collected 13, 14, 16, and 17 weeks after start of experiment from the AZ were digested by crucible and microwave (details see below) digestion methods and analyzed by inductively coupled plasma mass spectrometry (ICP-MS, X Series II, ThermoFisher Scientific, Bremen, Germany). In order to evaluate the role of the freezing and analysis method, the water sample collected 22 weeks after start of experiment from the AZ was digested by crucible method directly after sampling and after 1 week freezing and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, 720, Agilent Technologies, Santa Clara, USA) and by ICP-MS. In order to evaluate the role of storage time and the effect of application of mechanical forces during sample pre-treatment, the water sample collected 20 weeks after start of experiment from the AZ was digested by crucible method immediately after sampling as well as after 6 month storage at 4°C in dark. The stored samples were treated by ultrasound for several durations before digestion. The samples were analyzed by ICP-MS. All samples were digested in triplicate.

The comparison between immediate digestion after sampling and digestion after 1 week freezing did not show any differences for the sample collected 22 weeks after start of experiment from the AZ (Figure S 9, A). The Ag analysis using ICP-OES and ICP-MS led also to the comparable results for mesocosm sample. Consequently, the aqueous samples collected from our mesocosm system can be digested and analyzed directly after sampling or after freezing storage.

The storage duration did not show any impact on the determination of the total Ag concentrations in aqueous samples. The digestion and analysis immediately after sampling as well as after 6 month storage at 4°C with and without pre-treatment by ultrasound led to the comparable total Ag concentrations in the sample (Figure S 9, B). The results also show that the loss of dissolved and particulate Ag due to the adsorption on the walls of the glass vials during sample storage was negligible and the treatment by ultrasound was not really necessary. This is important, since an immediate digestion and analysis of the samples were not always possible and often the samples were stored for several weeks prior to the analysis.

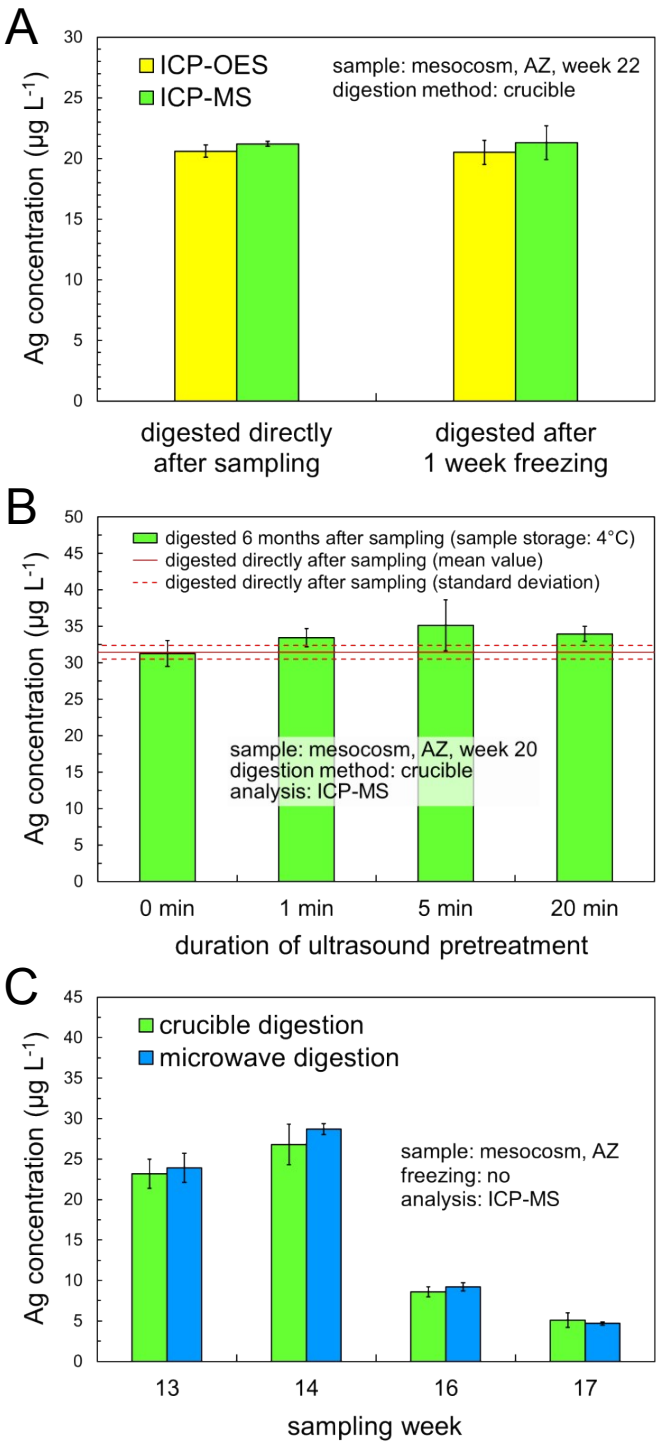

Figure S 9. Comparison of sample storage, digestion, and analysis methods. (A): Freezing versus not freezing and ICP-OES versus ICP-MS. The sample collected 22 weeks after start of experiment from the AZ was digested by crucible method directly after sampling and after 1 week freezing. (B): Immediate digestion after sampling versus digestion after 6 month storage at 4°C in dark. In order to redisperse particles, the stored samples were treated by ultrasound for several durations. The samples were digested by crucible digestion method and analyzed by ICP-MS. (C): Crucible digestion versus microwave digestion. Before digestion, the samples were kept at 4°C in dark. After digestion, the samples were measured by ICP-MS. The columns and error bars in (A), (B), and (C) represent the mean values and standard deviations determined from three replicate samples, respectively.

Furthermore, we compared the crucible acid digestion with microwave assisted acid digestion for several aqueous mesocosm samples. The advantage of the crucible digestion compared to the microwave digestion is in higher sensitivity since the analyte is not diluted during digestion. However, the crucible digestion is much more time consuming than the microwave digestion. Compared to the crucible digestion, the microwave digestion allows the treatment of much higher number of samples within the same time which is extremely relevant for complex long-term mesocosm experiments with high number of samples. The comparison between crucible and microwave digestion did not show any significant differences in determined total Ag concentrations for high (ca. 28 µg L^{-1}) as well as for low (ca. 5 µg L^{-1}) Ag concentrations (Figure S 9, C). Therefore, all aqueous mesocosm samples were analyzed after microwave digestion to save the time.

1.12 Crucible digestion of aqueous samples

In order to measure the concentration of total Ag, 10 mL of sample was completely dried overnight in a ceramic crucible at 60°C in a drying oven (BINDER FD 53, Binder, Tuttlingen, Germany). After drying, the crucible was filled up with 12 mL concentrated HNO_3 (65%, sub-boiled) and gently heated on a heating plate (RCT basic, IKA Labortechnik, Staufen, Germany) until the volume was reduced to ca. 500 µL. The residual volume was removed to 15 mL plastic tube. The crucible was washed with 1000 µL UPW and washing water was added to the tube. Finally, the tube was filled up with UPW to 10 mL. This solution was directly measured by ICP-MS or ICP-OES.

1.13 Microwave digestion of aqueous samples

In order to measure the total Ag concentration, the aqueous samples stored at 4°C in dark were digested by microwave assisted acid digestion. For the digestion, 10 mL sample was mixed with 4 mL acid in a Teflon microwave vessel. As an acid a mixture of hydrochloric acid (HCl, 35%, ROTIPURAN, Supra, Carl Roth, Karlsruhe, Germany) and nitric acid (HNO_3, sub-boiled) at the volume ratio of 1:1 was applied. The samples were digested in a microwave digestion system (MARS Xpress, CEM, Matthews, USA) applying a temperature ramp of 30 min from room temperature to 95°C and subsequent irradiation for 30 min at a constant temperature of 95°C. After digestion, 28 mL UPW was mixed to the sample. The diluted samples were measured by ICP-MS.

1.14 Determination of Ag content in leaf, biofilm, plant, sediment, and soil samples

The acid digestion method presented in our previous study[4] was modified and applied for biofilm samples. Briefly, biofilm samples (wet weights of 80-115 mg) were mixed with 0.5 mL UPW and 1.5 mL HNO_3 (65%, sub-boiled) in 15 mL plastic centrifuge tubes (Sarstetd). The centrifuge tubes were heated in a water bath at 60°C for 7 h. The temperature was controlled continuously using hotplate magnetic stirrer (RCT basic, IKA Labortechnik, Staufen, Germany). For the blanks, the samples were

prepared and treated similarly but without biofilm samples. After digestion, the samples were filled up to 10 mL with UPW and additionally diluted with UPW by a factor of 1:100. The digestions were performed in three replicates. The Ag concentration in diluted samples was measured by ICP-MS.

For the determination of Ag content of leaf or plant, 1 g dry material was digested with 1 mL HNO_3 (65%) at 80°C in a beaker glass fixed in a water bath for 4 days. Compared to the method applied for biofilms, we increased the digestion duration and temperature to ensure complete digestion of these solid materials. Silver was measured in triplicate or five times by ICP-MS.

To determine the total Ag content in different sediment and soil layers, a subsample was dried at 105°C for 48 h and ground with a mortar and pestle. The subsamples were digested in the microwave in triplicates. Briefly, 0.5 g sediment or soil was mixed with 4 mL of HNO_3 (65%) diluted by a factor of 1:2 with UPW and 10 mL HCl (35%) diluted by a factor of 1:5 with UPW. The digestion was performed using a two-step program with the temperature ramp of 60 min to 95°C (880 W) followed by an irradiation step of 30 min at 95°C (800 W) according to the EPA Method 200.9, revision 2.2[5] modified for microwave digestion. This method is recommended for the analysis of soil and sediment samples. The total Ag content was measured for each subsample in triplicates by ICP-OES. The total Ag content in unpolluted sediment was determined by ICP-MS.

The quality control for the digestion method was performed using a standard reference soil, ISE sample 989 (Wageningen University, Wageningen, Netherland). The Ag contents of reference material determined in these tests were slightly lower or in the range of the content of acid extractable Ag (Figure S 10) showing that the analysis of Ag in sediment and soil was suitable.

Certified calibration standards (ICP multielement standard solution, Carl Roth, Germany) were used for the calibration of ICP-OES. The quality of the ICP-OES measurements was controlled using certified reference materials NWTMDA 54.5 (Ag concentration: nominal value 13.2 ± 1.5 µg L^{-1}, actual value 14.7 µg L^{-1}, n = 16, LGC standards, Canada) or NWTMDA 53.3 (Ag concentration: nominal value 14.60 ± 1.75 µg L^{-1}, actual value 13.1 µg L^{-1}, n = 3, LGC standards, Canada).

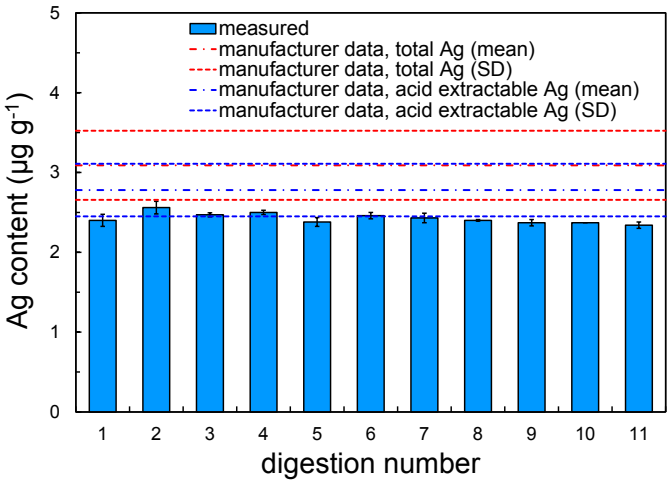

Figure S 10. Silver content of soil reference material (ISE 989): measured values and manufacturer data. The symbols and error bars represent the mean values and standard deviations (SD) determined from three replicate samples, respectively.

2 Results

2.1 Properties of initial Ag-NPs

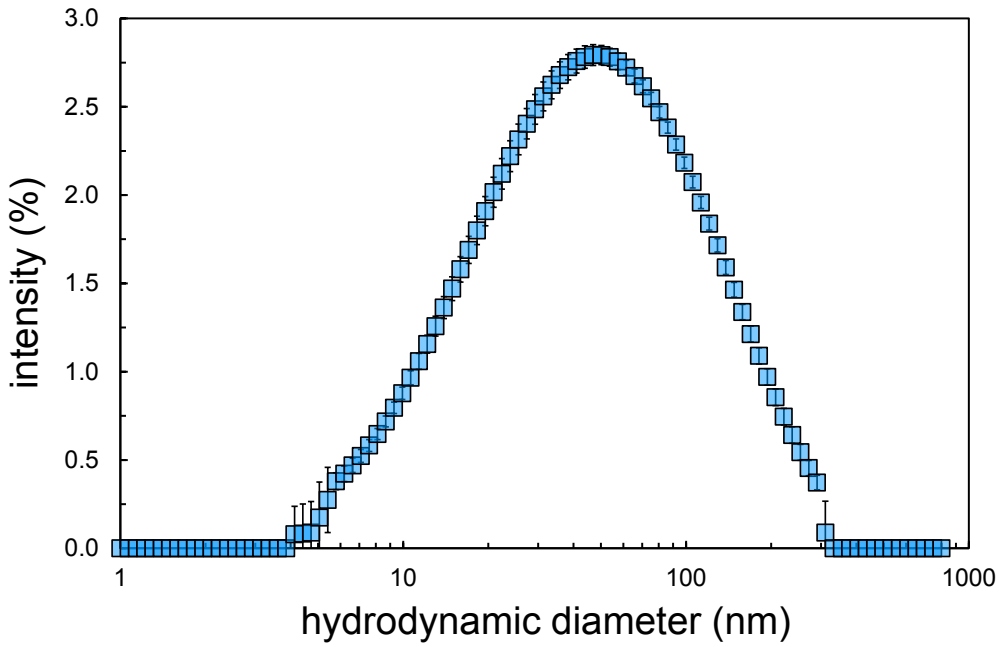

Figure S 11. Particle size distribution of Ag-NP stock dispersion determined from DLS measurements directly after preparation. The symbols and error bars represent the mean hydrodynamic diameters and the standard deviations determined from two replicate measurements of undiluted and two replicate measurements of diluted (1:3, with UPW) Ag-NP stock dispersions, respectively.

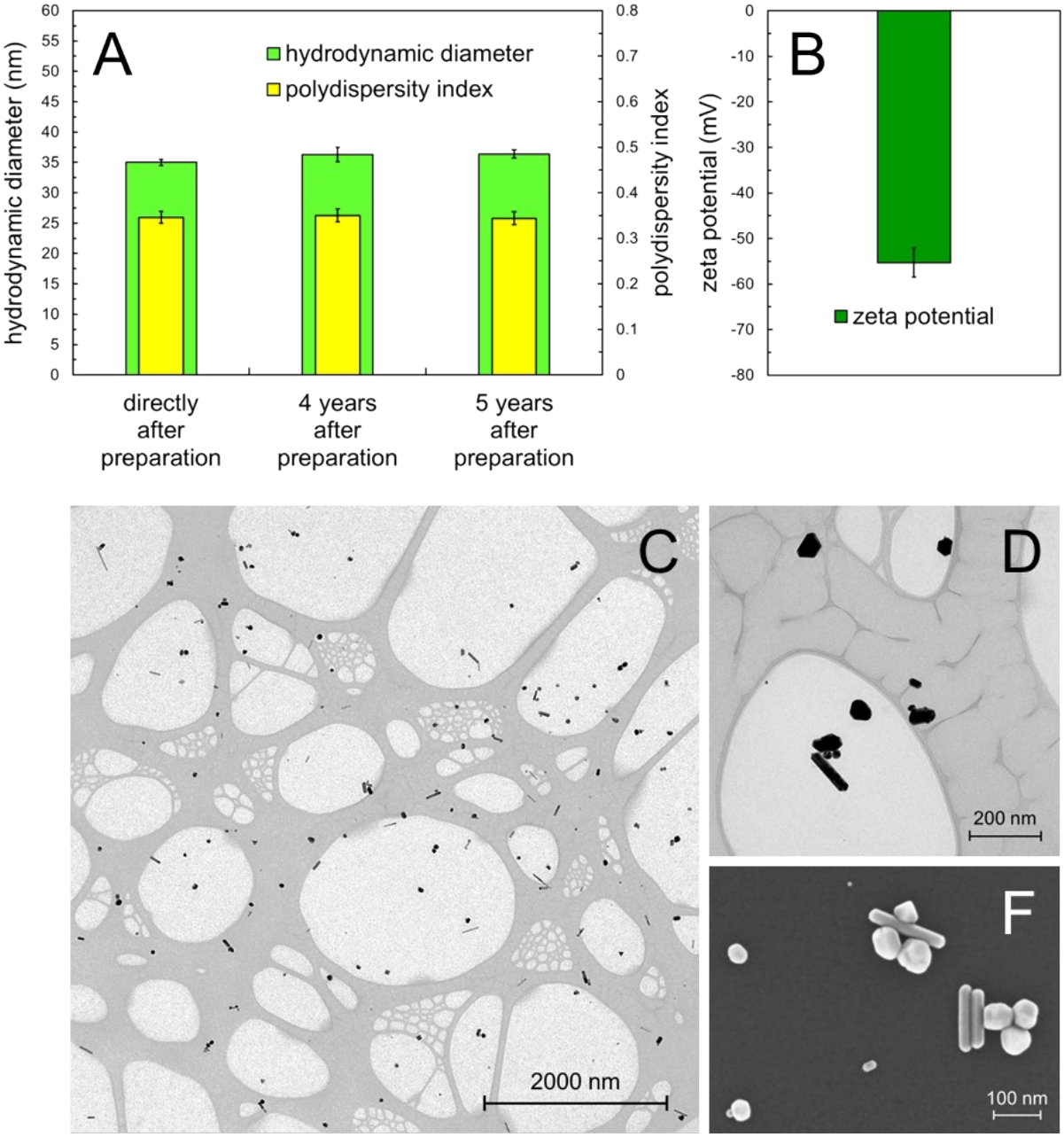

Figure S 12. Hydrodynamic diameter (A), polydispersity index (A), and zeta potential (B) of Ag-NP stock dispersion. TEM (C, D) and SEM (F) pictures of diluted (C, D) and undiluted (F) Ag-NP stock dispersions. The hydrodynamic diameter was determined from DLS measurements directly after preparation as well as 4 and 5 years later. The columns and error bars in (A) and (B) represent the mean values and standard deviations determined from 6-15 replicate measurements, respectively. The dilution factor for sample measured by TEM was 1:10.

2.2 Physico-chemical conditions in mesocosm

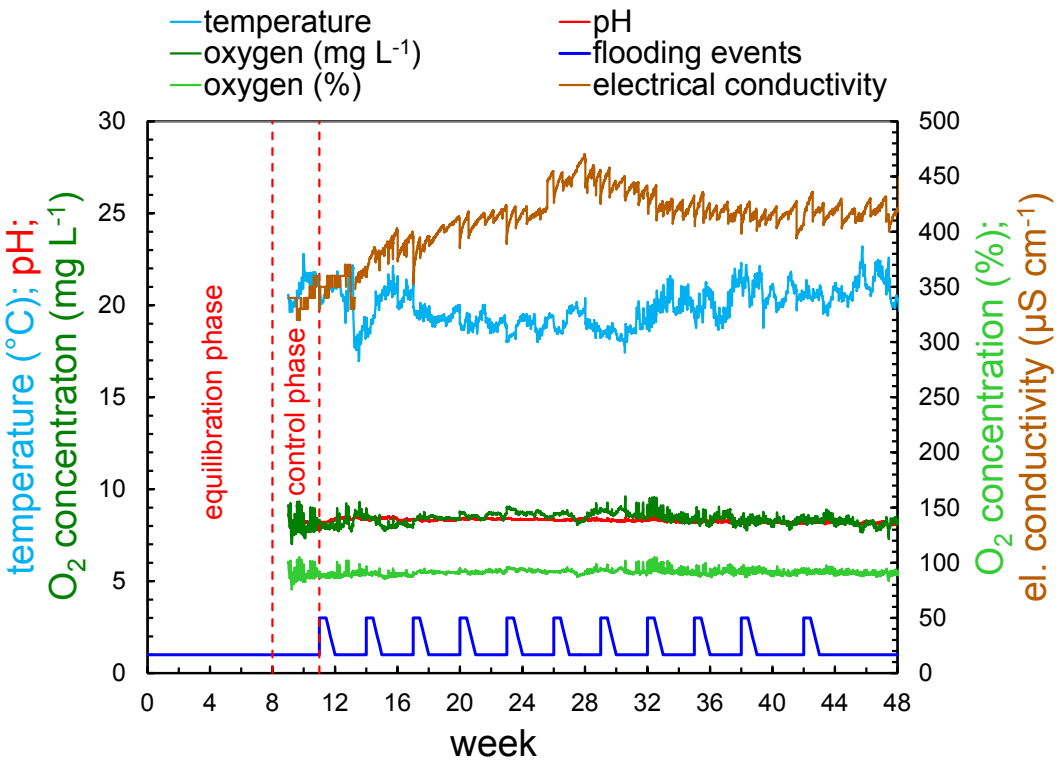

Figure S 13. The concentration of oxygen (in mg L^{-1} and in %), electrical conductivity, pH value, and temperature in the water phase of the AZ monitored on-line during mesocosm experiment.

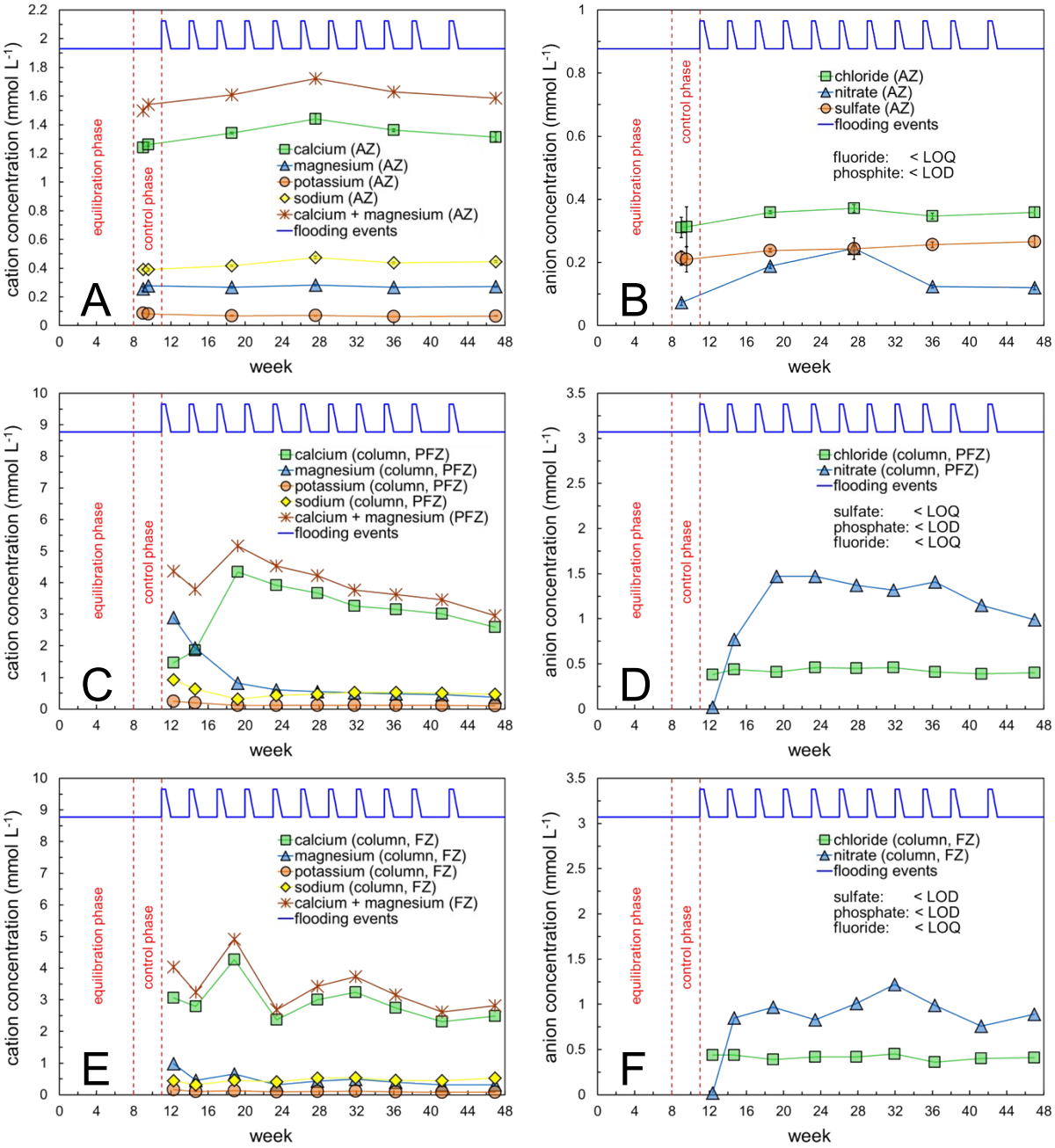

Figure S 14. The concentrations of cations (A, C, E) and anions (B, D, F) in the water phase of the AZ (A, B) and in column eluates of the PFZ (C, D) and FZ (E, F) at several time points after start of experiment. The symbols and error bars in (A) and (B) represent the mean values and standard deviations determined from three replicate samples, respectively. The abbreviations "LOD" and "LOQ" mean limit of detection and limit of quantification, respectively ($LOD_{fluoride}$ = 0.01 mmol L^{-1}, $LOQ_{fluoride}$ = 0.03 mmol L^{-1}, $LOD_{sulfate}$ = 0.03 mmol L^{-1}, $LOQ_{sulfate}$ = 0.1 mmol L^{-1}, $LOD_{phosphate}$ = 0.05 mmol L^{-1}, $LOQ_{phosphate}$ = 0.18 mmol L^{-1}).

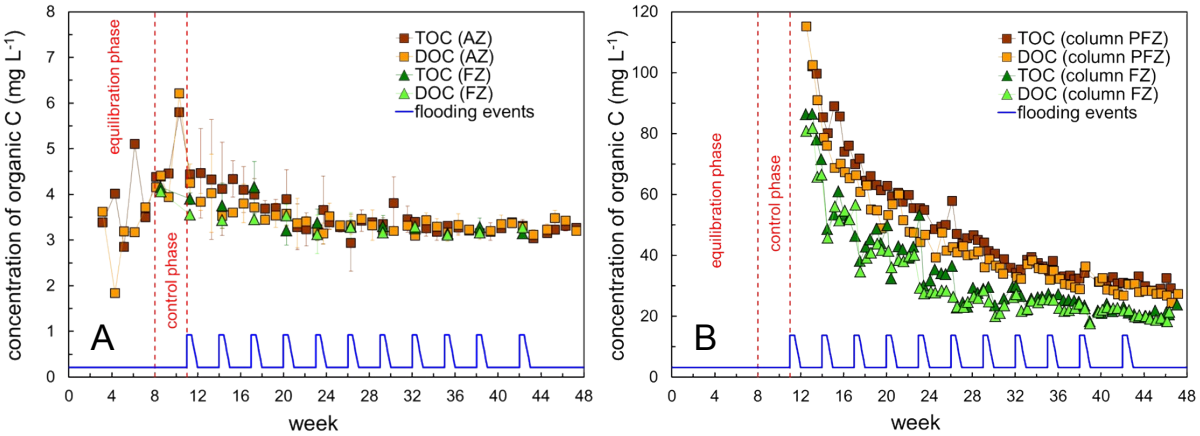

Figure S 15. The concentrations of organic carbon (TOC and DOC) in water phase of the AZ and FZ (A) and in column eluates of the PFZ and FZ (B) at several time points after start of experiment. The symbols and error bars in (A) represent the mean values and standard deviations determined from three replicate samples, respectively.

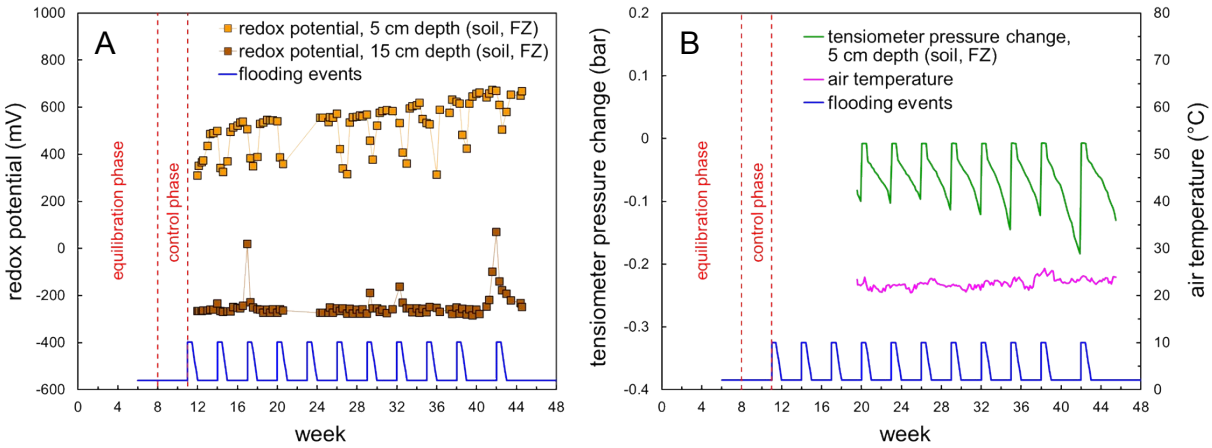

Figure S 16. The redox potential (A) and tensiometer pressure change (B) in the soil of the FZ and air temperature (B) at several time points after start of experiment. The redox potential was measured at 5 and 15 cm depths. The tensiometer pressure change was measured at the depth of 5 cm. Due to the problems with the sensor, the measurements of the tensiometer pressure change were started only after week 19.

2.3 Interactions between nanoparticles and polypropylene material of mesocosm

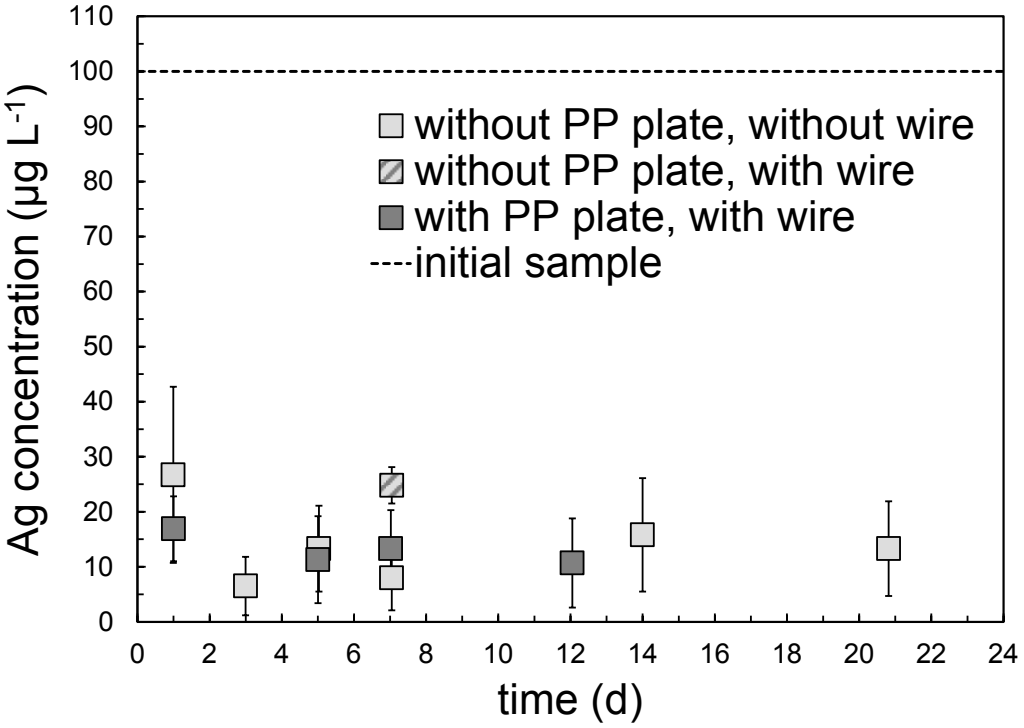

Figure S 17. The concentration of Ag in the water phase as a function of time. The data show the results of the sorption experiments for the evaluation of the interactions between nanoparticles and polypropylene material of the mesocosm system in water from River Rhine. Error bars correspond to standard deviations calculated from three replicate samples.

2.4 Ag enrichment in/on algae, leaves, and plants

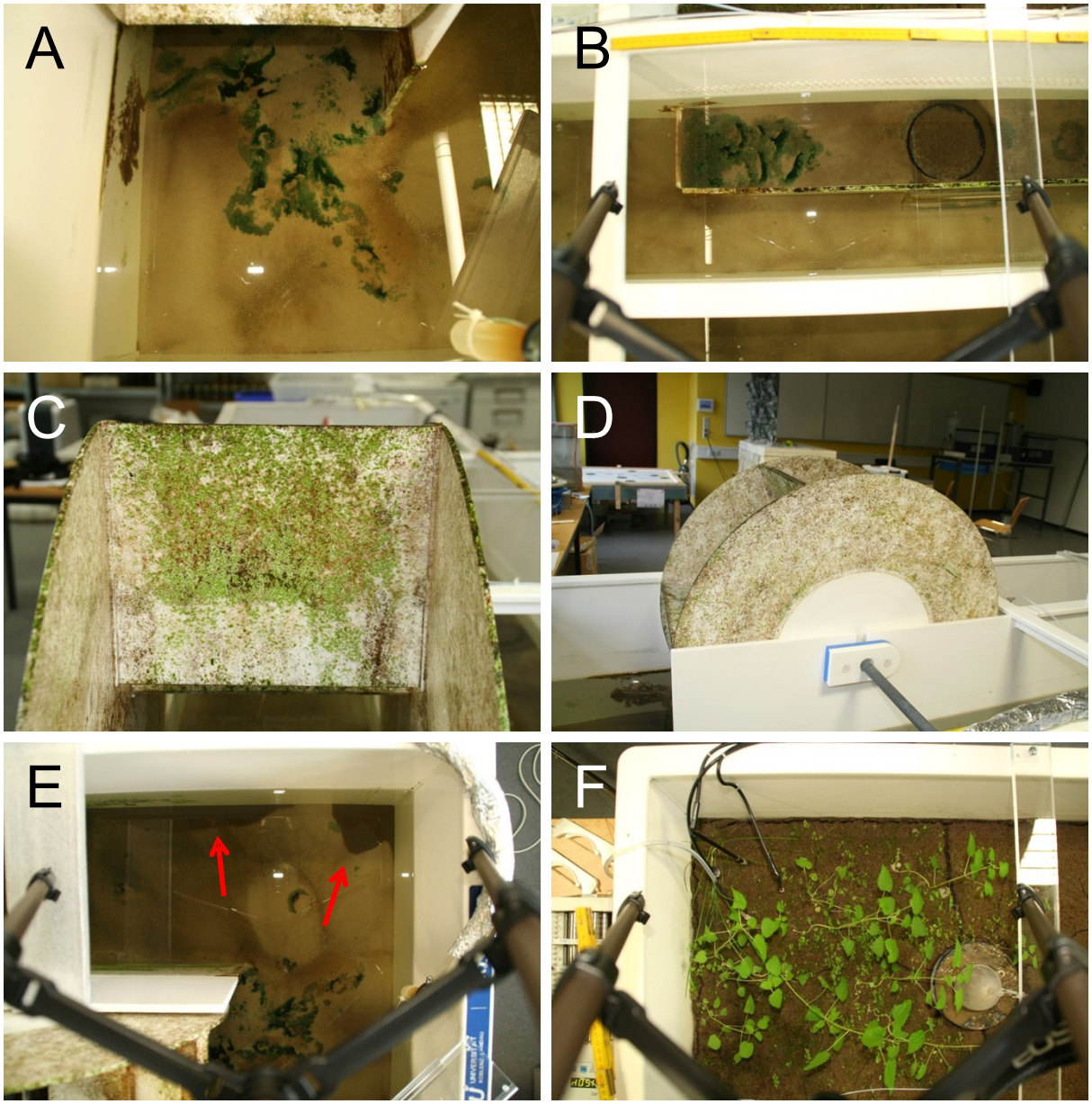

Figure S 18. Conditions at the end of the mesocosm experiment. Biofilms on the sediment surface in the AZ (A) and PFZ (B). Biofilms on the blades (C) and side faces (D) of paddle wheel. The brown precipitate (indicated by red arrows) on the sediment surface in the AZ (E). Plants in the FZ (F).

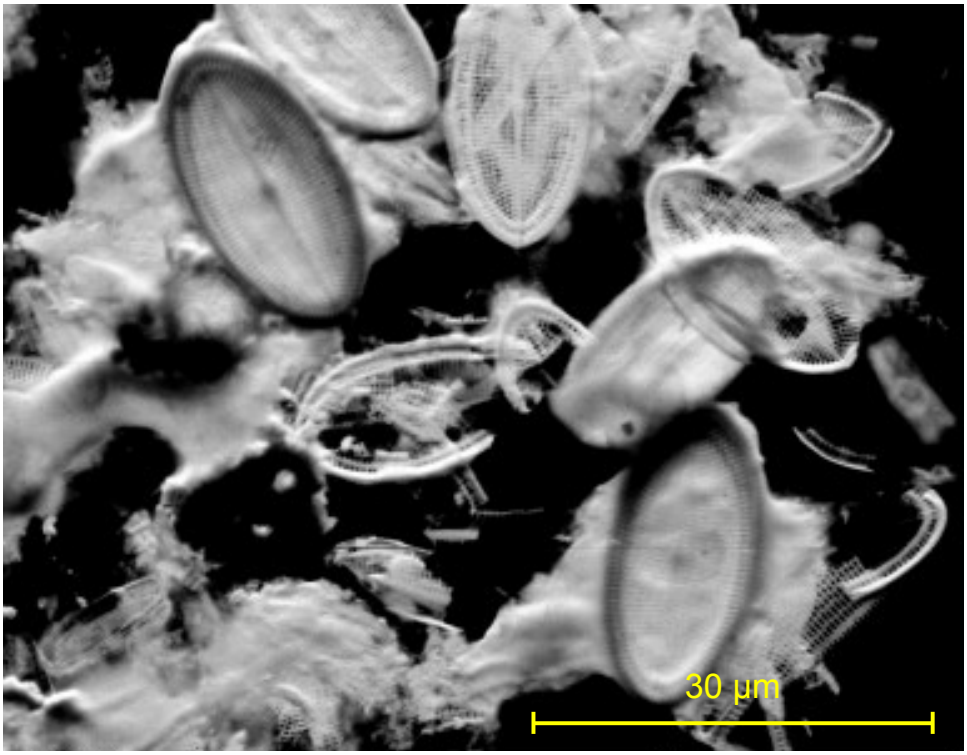

Figure S 19. The STEM picture of biofilm sample collected from the paddle wheel surface.

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