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

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Abstract

Background: Increasing exposure to engineered inorganic nanoparticles takes actually place in both terrestric and aquatic ecosystems worldwide. Although we already know harmful effects of AgNP on the soil bacterial community, information about the impact of the factors functionalization, concentration, exposure time, and soil texture on the AgNP effect expression are still rare. Hence, in this study, three soils of different grain size were exposed for up to 90 days to bare and functionalized AgNP in concentrations ranging from 0.01 to 1.00 mg/kg soil dry weight. Effects on soil microbial community were quantified by various biological parameters, including 16S rRNA gene, photometric, and fluorescence analyses.

Results: Multivariate data analysis revealed significant effects of AgNP exposure for all factors and factor combinations investigated. Analysis of individual factors (silver species, concentration, exposure time, soil texture) in the unifactorial ANOVA explained the largest part of the variance compared to the error variance. In depth analysis of factor combinations revealed even better explanation of variance. For the biological parameters assessed in this study, the matching of soil texture and silver species, and the matching of soil texture and exposure time were the two most relevant factor combinations. The factor AgNP concentration contributed to a lower extent to the effect expression compared to silver species, exposure time and physico–chemical composition of soil.

Conclusions: The factors functionalization, concentration, exposure time, and soil texture significantly impacted the effect expression of AgNP on the soil microbial community. Especially long-term exposure scenarios are strongly needed for the reliable environmental impact assessment of AgNP exposure in various soil types.

Keywords: Silver nanoparticles, Soil, Soil texture, Exposure time, Functionalization, Soil microbial community, LAP, Enzymes, Functional diversity

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Background

The production volume and the application fields of silver nanoparticles (AgNP) increased continuously in the last decade given by their unique properties such as high surface-to-volume ratio, high chemical reactivity, and specific optical properties [1–3]. Apart from the initial medical utilization, AgNP are actually used in households, industry and agriculture such as for water purification, plant growth promotion and textiles cleaning [4, 5]. In consequence, their emission into the environment during all stages of the life cycle, including production, product use, disposal and weathering is unavoidable [6]. The exact extent of the release is unknown due to missing reliable and robust analytical methods for detecting trace concentrations of AgNP in complex matrices [7]. Thus, several scientists modeled the fate and concentrations of AgNP in the environment and named the soil compartment as one of the main sink of AgNP released into the environment [2, 6, 8–14]. For Europe, an annual AgNP increase of 0.6 t and 2.09 t was calculated for soils and sediments, respectively [8].

Today, information about the impact of AgNP on the soil microbiome are still rare, although microbial communities are important and sensitive targets for determining the environmental hazards of AgNP [15]. Recently, we registered significant negative effects on soil microbial biomass (− 38.0%), bacterial ammonia oxidizers (− 17.0%), and the *beta-Proteobacteria* population (− 14.2%) after 1-year exposure to 0.01 mg AgNP/kg in a loamy soil, while *Acidobacteria* (44.0%), *Actinobacteria* (21.1%) and *Bacteroidetes* (14.6%) were significantly stimulated [16, 17]. Therefore, a detrimental disturbance on soil ecosystem functions, such as nitrification, organic carbon transformation and chitin degradation could be assumed.

Numerous studies documented the differing physico–chemical and concomitant toxicological behavior of AgNP in dependence of the soil type. As a function of pH, ionic strength, temperature, amount of dissolved ions and of natural organic matter, oxygen concentration, grain size distribution and others [18–21], AgNP could undergo various physico–chemical transformations such as reduction, oxidation, aggregation, dissolution, complexation and further secondary reactions [21–24]. Consequently, these transformations in turn affect the toxicity mechanism of AgNP as well as their bioavailability. For example, in comparative studies with different soil types, Schlich and Hund-Rinke [19] as well as Rahmatpour et al. [25] showed that AgNP caused lower toxicity in soils with higher clay content due to the AgNP immobilization by heteroaggregation with clay particles [19, 23, 25].

In addition, the AgNP species itself may significantly impact on its environmental behavior. Their size, shape, surface-coating agent, charge and stability are only a few of the properties by which AgNP can differ [1]. Today, extensive functionalization strategies are available to modify the surface chemistry of a variety of engineered nanoparticles (NP) [26]. Those coatings are used to stabilize NP against aggregation when stable suspensions are required for product functionality or for improved delivery of the product. The coating may also provide other functionalities, such as biocompatibility or targeting of specific cells in biomedical applications [27]. For example, AgNP can be coated by citrate or polyvinylpyrrolidine to increase their stability [28], modified with ATP to act as a selective antibiotic [29] or equipped with COOH– and NH_2-groups to affect their surface charge in terms of their function in imaging and drug delivery [30]. Once released into the environment, the surface functionalization of AgNP significantly determines its physico–chemical fate, its bioavailability and its toxicity [22, 26]. Exemplary, Wu et al. [31] observed in a nanocosm experiment that polyethylene glycol AgNP had the highest overall toxicity, followed by silica AgNP, and lastly aminated silica-coated AgNP due to there different dissolution rates und thus stability.

Further to the soil type and the AgNP functionalization, several studies documented a significant impact of the exposure time on the toxicity of AgNP in soils [16, 32–34]. By a statistically significant regression and correlation analysis between silver toxicity and exposure time we recently confirmed loamy soils as a sink for silver nanoparticles and their concomitant silver ions due to ageing processes of the silver species and their slow return to the biological soil system [17].

Considering the predicted increase of AgNP into the soil environment, the known toxicity of AgNP to the soil microbial community as well as the variable fate of AgNP in the soil compartment, the aim of this study was to give a more holistic view of the impact of AgNP exposure on the soil microbial community in dependence of the factors AgNP functionalization, AgNP concentration, exposure time, and soil texture. The study was conducted with a long-term incubation period of 90 days using three soil textures (loam, clay, sand) and two different charged AgNP at concentrations in a range of 0.01–1.00 mg AgNP/kg soil. We quantified the effects on several biological parameter: the microbial biomass, the abundance of bacteria, the enzymatic activity as well as marker genes for selected processes of the inorganic nitrogen cycle and for selected higher bacterial taxa. Furthermore, we used $AgNO_3$ as control to determine the effect of Ag^+ ions on the AgNP results. Based on our preceding observation that the nitrate content of 36.5% in the $AgNO_3$ compound might also have effects on the microbial community [16], we used NO_3 as a further control. This experiment was

restricted to the loamy soil. Here, we analysed the impact charged and uncharged AgNP as well as the effects of $AgNO_3$ and NO_3 on the microbial community.

Materials and methods

Silver nanoparticles and controls

Two differently functionalized AgNP were used, Ag10-COOH functionalized with carboxy groups and Ag10-NH_2 functionalized with amino groups. The AgNP were synthesized by a ligand exchange starting from hydrophobic silver particles (Ag-HPB) and the addition of toluene and mercaptopropionic acid or cysteamine hydrochloride in MeOH to receive the final Ag10-COOH or Ag10-NH_2 colloidal solutions. The concentration of the stock solutions were 180 mg/L for Ag10-COOH and 21 mg/L for Ag10-NH_2. Size, shape and nanoparticle surface charge (ζ-potential) of AgNP were analysed by transmission electron microscopy (Philips CM 12, Netherlands), dynamic light scattering (DLS, Zetasizer Nano S, Malvern Instruments Ltd., UK), asymmetrical flow field-flow fractionation (AF4, AF2000 MT, Postnova Analytics GmbH, Germany) and Laser-Doppler-microelectrophoresis (Malvern Zetasizer Nano-ZS, Malvern Instruments Ltd., UK).

Methods of synthesis and particle characterization can be found in detail in Additional file 1.

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 s, incubated for 1 h or 24 h under permanent rotation (100 rpm at 37 °C) and vortexed for 30 s prior to analysis via Zetasizer Nano-ZS.

Silver nitrate ($AgNO_3$) was used as a positive control. Silver concentrations in the $AgNO_3$ controls were the same as those in the AgNP treatments. As a further control, NO_3^- was used in form of KNO_3. The nitrate concentration in the KNO_3 controls were the same as those in the $AgNO_3$ treatments.

Test soils

Three soil textures were selected: a silty sand, a loamy clay and a silty loam. Approximately 20 kg of each soil was sampled from the A-horizon (0–30 cm depth) in spring 2015 next to Trier, Germany. Land-use was forest for the sandy soil and arable field for the clayey and the loamy soil. The soil had a clay content of 0–5% for the sandy, 45–65% for the clayey, and 25–35% for the loamy soil, respectively. After sampling, the soils were thoroughly sieved to < 2 mm and stored at 6 °C until further use. Characteristic soil parameters are listed in Table 1.

Table 1 Characterization of the test soils

Parameter	Properties		
Location site	Wolsfeld	Trierweiler	Helenenberg
Soil type (WRB)	Podzol	Stagno-Cambisol	Stagno-Luvisol
Soil texture	Silty sand	Loamy clay	Silty loam
Clay content (%)	0.0–5.0	45.0–65.0	25.0–35.0
Sand content (%)	70.0–90.0	5.0–40.0	25.0–45.0
pH (0.01 mol/L CaC_{l2})	3.2	7.4	7.2
TOC (%)	1.8	5.2	4.7
TN (%)	0.1	0.3	0.2
CEC (mmol/kg dry matter)	9.1	199.4	193.3
Ca^{2+} (mmol/kg dry matter)	4.7	136.0	123.5
Mg^{2+} (mmol/kg dry matter)	2.4	47.6	55.8
K^+ (mmol/kg dry matter)	0.4	14.5	12.8
Na^+ (mmol/kg dry matter)	0.3	1.1	0.9
Fe^{2+} (mmol/kg dry matter)	1.1	0.0	0.0
Mn^{2+} (mmol/kg dry matter)	0.2	0.3	0.4

WRB world reference base for soil resources

Experimental design

Before starting the experiment, the soil was moistened and incubated at 18 °C for 7 days. The application of the test materials was performed in petri dishes, each filled with soil equivalent to 25 g dry weight.

AgNP test solutions were prepared immediately before use: AgNP stock solutions were sonicated at 42 W/L for 15 min and gradually diluted with UPW. Then, 1 mL of the *agent species* Ag10-COOH, Ag10-NH_2, $AgNO_3$ or KNO_3 solutions, at different concentrations, was added in small drops onto the soil surface to obtain final concentrations of 0.01, 0.10 and 1.00 mg/kg dry weight. Negative controls only received an application of UPW. Soil water content after the addition of the test solutions were average 22.0% (sand), 19.4% (clay) and 18.5% (loam), which was equivalent to 42.6%, 41.4%, and 37.4% WHC_{max}, respectively. For each soil texture, agent species, concentration, and day, separate samples in different soil dishes were prepared as 4 replicates (e.g. 4×0.01 mg Ag10-COOH kg^{-1} sand for day 1). Subsequently, soils were extensively mixed by stirring with a spoon, and then they were transferred to plastic containers (Centrifuge Tubes, 50 mL, VWR, Darmstadt, Germany) and sealed by Parafilm®. They were incubated at 15.1 (± 1.8 °C) in the dark for 1, 14, 28, and 90 days. Water evaporation was determined gravimetrically and then compensated with the addition of UPW. Samples were finally stored at − 20 °C. For analyses, samples were defrosted by incubation overnight at 6 °C. Each replicate was analysed on the

effect expressions of the target variables leucine aminopeptidase activity, microbial biomass as well as of functional and taxonomic genes.

Furthermore, results of our previous studies [16, 17] of the effect assessment of AgPure, with an average size of 20 nm and polyacrylate stabilization, were used to calculate the impact of the five *agent species* AgPure, Ag10-COOH, Ag10-NH_2, $AgNO_3$, and KNO_3 at concentrations of 0.01; 0.1; and 1.0 mg Ag/kg soil after exposure of 1, 14, 28, and 90 days in the silty loam soil on the same target variables.

Analysis of biological parameters

Leucine aminopeptidase activity

Leucine aminopeptidase (EC 3.4.1.1; LAP) was investigated according to Marx et al. [35], with modifications [36]. Briefly, 1 mol/L L-leucin-7-AMC was used as substrate for LAP, and 7-amino-4-methylcoumarin [37] was used as a standard. Incubation of the soil slurry with the substrate was performed at 30 °C. Measurements of fluorescence were performed after 0 and 2 h using a Victor Multilabel Plate Reader (Perkin Elmer, Germany; excitation wavelength: 355 nm, emission wavelength: 460 nm). LAP activity was calculated as substrate turnover per g dry soil h.

The potential contribution of AgNP to the total fluorescence signal was measured for each AgNP concentration. Then, AgNP was added to autoclaved soil, and the same procedure was conducted. The resulting fluorescence signal was compared to the fluorescence intensity of the pure autoclaved soil. At this, the used AgNP did not exhibit autofluorescence (data not shown).

DNA extraction and microbial biomass measurements

DNA extraction and purification were performed using the Genomic DNA from soil kit (Macherey–Nagel, Düren, Germany) according to the manufacturer's instructions and stored at − 20 °C. For the measurement of microbial biomass, 10 µL of DNA was transferred into the well of a 96-well microplate and shaken for 5 s before absorbance was measured at 260 nm [38] using Victor Multilabel Plate Reader (Perkin Elmer, Germany).

Quantitative detection of functional and taxonomic genes

16S rRNA genes were used as a proxy to quantify the abundance of bacteria, as described by Bach et al. [39]. The abundance of bacteria harboring the *nifH* gene was used as a marker for the potential to fix nitrogen and measured according to Rösch et al. [40]. To analyze the effects of the different silver materials on the ammonia-oxidizing bacteria, the *amoA* primer system described by Rotthauwe et al. [41] was used. To quantify the abundance of taxon-specific 16S rRNA gene copy numbers, qPCR assays for *Acidobacteria* [42, 43], *Actinobacteria* [43, 44], *alpha-Proteobacteria* [45], *Bacteroidetes* [43, 46], and *beta-Proteobacteria* [47, 48] were performed. Detailed descriptions of the used assays are given by Grün and Emmerling [17] and Grün et al. [16].

All qPCR reactions were conducted on a thermal cycler equipped with an optical module (Analytik Jena, Jena, Germany). All samples were run in triplicate wells. Single qPCR reactions were prepared in a total volume of 20 µL. The InnuMix SYBR-Green qPCR Master-Mix was purchased from Analytik Jena (Jena, Germany). Primer concentrations were 10 pmol/µL, and amplification specificity was assessed by melting curve analysis and gel electrophoresis on a 1.5% agarose gel after qPCR. Standard curves were based on cloned PCR products from the respective genes [43].

Statistical analyses

All data were processed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, USA). The obtained biological values of qPCR (copy gene number per kg dry soil), leucine aminopeptidase activity (substrate turnover per g dry soil h) and measurement of microbial biomass (ng DNA per g dry soil) of negative controls (0.00 mg Ag/kg) of the 4 sample replicates were averaged for each biological parameter and day. Subsequent, the relative variation of each silver treated sample of one concentration and one sampling date were calculated as follow:

$$\text{Relative variation (\%)} = \frac{\text{biological value of treated sample}}{\text{averaged biological value of untreated samples}} \times 100$$

In the following, the relative variation was set as target variables. *Exposure time* (1, 14, 28, 90 days), *concentration* (0.01; 0.1; 1.0 mg Ag/kg soil), *soil texture* (loam, sand, clay) and *silver species* (Ag10-COOH, Ag10-NH_2, $AgNO_3$) were set as factors with different factor levels (e.g. Ag10-COOH, Ag10-NH_2, $AgNO_3$).

For the effect assessment of a factor level on the target variable, the mean of a target variable (e.g. leucine aminopeptidase) at a distinct factor level (e.g. Ag10-COOH) was calculated. Within a factor, the target variable was also pre-evaluated for a normal distribution by the Shapiro–Wilk test and variance homogeneity by the Levene test.

To test the influence of the four factors as well as the influence of the factor combination on the target variable, multi-factorial ANOVA was performed. Here, tests of between-subject effects provided information about

significant relationships. By means of the Bonferroni post hoc test the significance of group mean differences within a factor were calculated.

Finally, a multivariate analysis of variance was performed to simultaneously mitigate the influence of the four factors on the 10 dependent target variables. The factors were set as independent variables, whereas the relative variations of the biological parameters were set as dependent variables. The test statistic was computed by Pillai's trace.

Results

Characterization of the AgNP suspensions prior to application into soils

Characteristics of the Ag10-COOH stock solution in water were as follows: hydrodynamic diameter (DLS, z-average): 85.3 ± 2.9 nm; polydispersity index (PDI) (DLS) = 0.234 ± 0.031; ζ-potential: −41.4 ± 1.1 mV (in UPW). AF4-UV-DLS measurement revealed an average hydrodynamic diameter of 18.1 ± 0.5 nm at the UV peak maximum. Over the main UV peak the hydrodynamic diameter ranged from 18 nm to around 28 nm. Larger particle sizes with a hydrodynamic diameter up to around 118 nm were detected as well but larger particle fractions were low in concentration based on the corresponding UV signal (Fig. 1a).

Characteristics of the Ag10-NH_2 stock solution in water were as follows: hydrodynamic diameter (DLS, z-average): 62.1 ± 3.1 nm; polydispersity index (PDI) (DLS) = 0.379 ± 0.072; ζ-potential: +39.2 ± 0.2 mV (in UPW). Average hydrodynamic diameter at the UV peak maximum obtained from AF4-UV-DLS measurement: 82.3 nm ± 3.1 nm with a size distribution from around 8 nm to 142 nm (Fig. 1b).

The analysis of the ζ-potential and hydrodynamic diameter of the AgNP under different pH values resulted in pH-dependent characteristics of the NP (Fig. 2). The hydrodynamic diameter was 197 ± 13.9 nm after 1 h incubation in a pH 4 solution and 169 ± 9.2 nm in a pH 7.4 solution for Ag10-NH_2 (Fig. 2a). There was no difference in the results after 1 h and 24 h incubation. Under alkaline conditions (pH 10), the hydrodynamic diameter increased to 601 ± 85 nm (1 h) or 1911 ± 475 nm (24 h). Starting from the stock solution, with a positive surface charge of +39.2 ± 0.2 mV, the surface charge decreased with increasing pH value (Fig. 2b). After 1 h incubation of Ag10-NH_2 in aqueous pH 4 solution, the ζ-potential was +36.9 ± 2.5 mV and decreased to −7.4 ± 1.5 mV after 1 h incubation in a matrix with pH 10. But there was no significant difference between the results after 1 h and 24 h incubation.

Ag10-COOH behaved contrarily. After 1 h incubation, the hydrodynamic diameter of Ag-COOH at pH 4, 7.4 and 10 was comparable. After 24 h the size significantly increased at pH 4, but not at pH 7.4 and 10 (Fig. 2c). The ζ-potential mainly did not vary significantly between the different pH values. After incubation for 24 h at pH 10 there was a significant increase in the surface charge to negative (−54.1 ± 2.9 mV) (Fig. 2d).

Transmission electron microscopy revealed an averaged diameter of 5.7 ± 2.3 nm for Ag10-COOH (Fig. 3a). Their distribution among the grid was not homogeneous but rather arranged in clusters. Between the dark grey and black single particles there was a lighter colored layer with very small objects in it. This could be a residue from

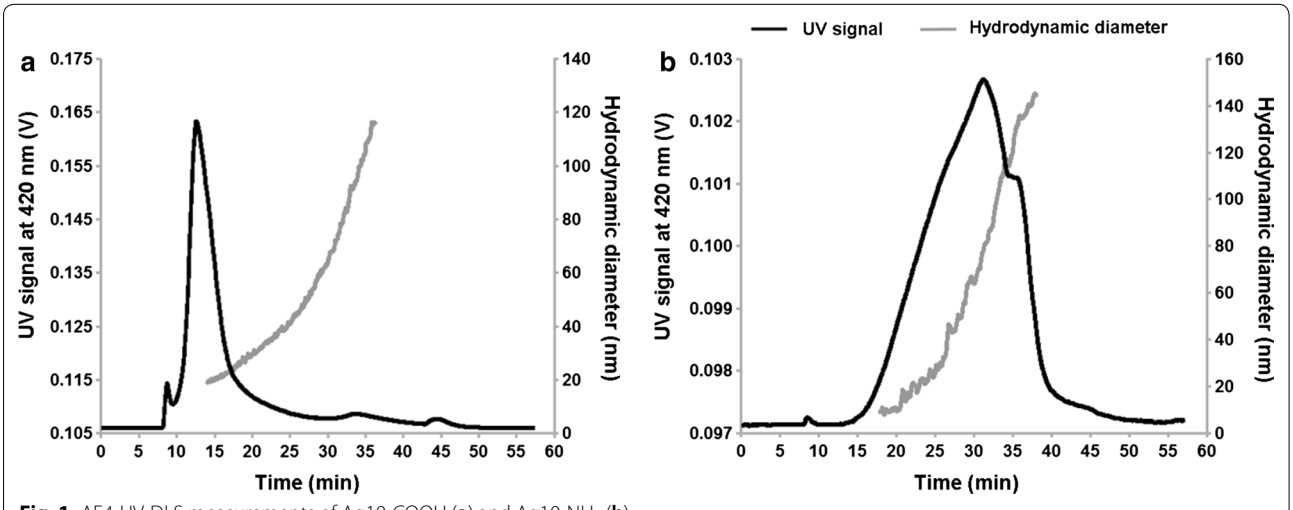

Fig. 1 AF4-UV-DLS measurements of Ag10-COOH (**a**) and Ag10-NH_2 (**b**)

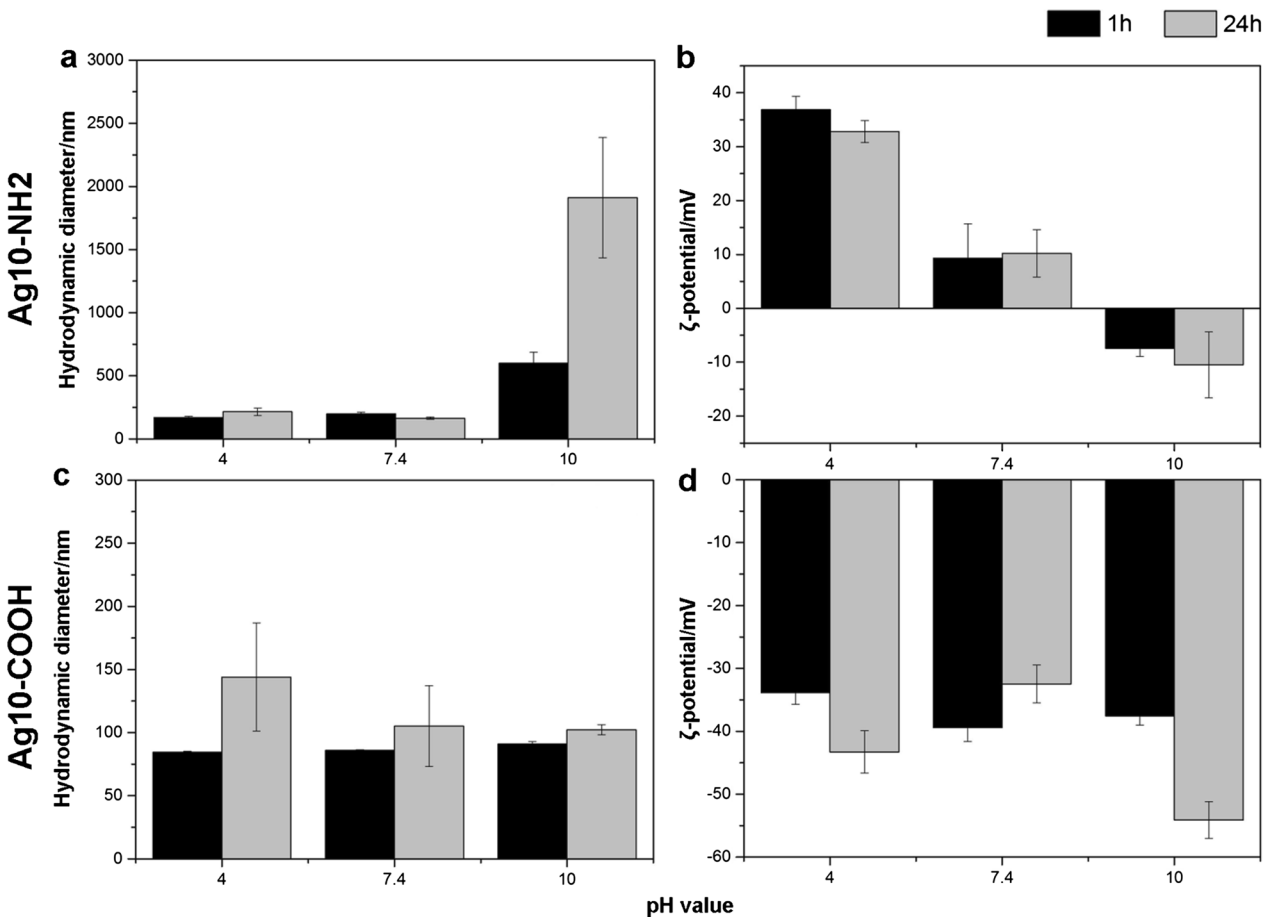

Fig. 2 PH-dependent hydrodynamic diameter and ζ-potential of Ag10-COOH (**a**, **b**) and Ag10-NH_2 (**c**, **d**). $N = 6$

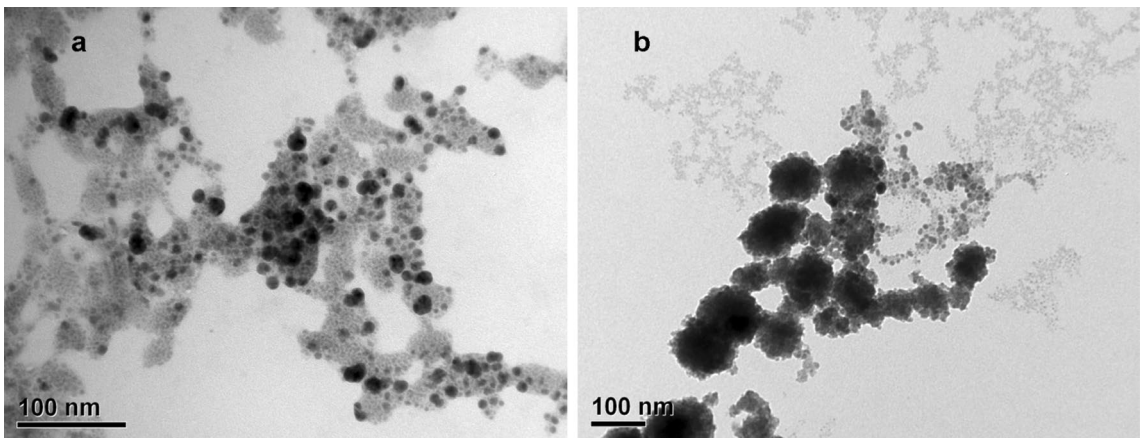

Fig. 3 Transmission electron microscope (TEM) image of Ag10-COOH (**a**) and Ag10-NH_2 (**b**) in water

the production process. The single particles were shaped roundish to oval. The Ag10-NH_2-NP showed an average diameter of 6.2 ± 3.4 nm (Fig. 3b). They were not distributed homogeneously among the grid and agglomerate to secondary particles with a diameter of over 100 nm. Beside these particles there were areas with single nanoparticles which had a roundish to oval shape and a plain surface.

Impact of silver species, exposure time, concentration, soil texture and their combinations on biological parameters

Results of multi-factorial ANOVA revealed significant main effects of the factors *silver species*, *concentration*, *exposure time* and *soil texture* on the relative variation of the bacterial phyla *Actinobacteria*, *Acidobacteria*, *alpha-Proteobacteria*, *Bacteroidetes* and *beta-Proteobacteria* (Table 2). Comparable significant results were observed for the leucine aminopeptidase (LAP) activity, the microbial biomass, the abundance of all bacteria (*16S rRNA*), as well as for the ammonia-oxidizing bacteria (amoA) (Table 2). In case of the relative variation of the free-living nitrogen-fixing bacteria (*nifH*) only the factors and factor levels of *exposure time* and *soil texture* caused significant effects (Table 2). *Silver species* and *concentration* provoked no significant impacts on *nifH* (Table 2). The interaction effects of the factor combinations were predominantly significant for all variables (Table 2).

The considered main factors as well as their interactions could explain at least 81.5% ($R^2 = 0.815$) of the variance of the respective target variables; in the case of *beta-Proteobacteria* even 93.9% ($R^2 = 0.939$, Table 2).

Results of multivariate analysis revealed significant main effects for all factors and factor combinations (Table 2). MANOVA scored the factor combination *soil texture* × *silver species* as the strongest option ($F = 53.2$), followed by the combination of *soil texture* × *exposure time* ($F = 46.8$) (Table 2). Nevertheless, also the main factors *silver species* ($F = 44.2$), *exposure time* ($F = 43.9$) and *soil texture* ($F = 46.5$) could broadly explain the variance compared to the error variance itself (Table 2). The main factor *concentration* could elucidate the least variance ($F = 12.7$) (Table 2).

In Fig. 4 the influence of the highest scored factor combinations *silver species* × *soil texture* and *exposure time* × *soil texture* on the relative variation of the biological parameters are highlighted.

The silver species Ag10-COOH caused similar effects in the loamy (101.2%) and the clayey (99.4%) soil, whereas Ag10-COOH diminished the relative variation of the biological parameters significantly in pairwise comparison to the sandy soil (93.3%; $p = 0.000$) (Fig. 4a). The effects of Ag10-NH_2 were also very similar between the loamy (95.8%) and the clayey soil (98.0%), causing no significant differences. The pairwise comparison of clayey and sandy (94.0%) soil exhibited significant differences on the biological parameters ($p = 0.003$), while between sandy and loamy soil no differences could be detected. $AgNO_3$ control slightly stimulated the entirety of the relative variation of the biological parameters in the loamy (101.2%) and sandy (103.6%) soil. In contrast $AgNO_3$ caused a significant decrease of the relative variation (89.1%, $p = 0.000$) in the clayey soil. The order of increasing toxicity were for the loam Ag10-COOH = $AgNO_3$ < Ag10-NH_2, for the clayey soil Ag10-COOH = Ag10-NH_2 < $AgNO_3$ and for the sandy soil $AgNO_3$ < Ag10-NH_2 = Ag10-COOH.

As shown in Fig. 4b, a 1-day exposure to the silver species at different concentration led to a clear distinction between the effect in the sandy soil relative to the clayey ($p = 0.029$) as well as the loamy soil ($p = 0.001$). Here, the sandy soil exhibited the lowest toxicity. During the mid-term exposure of 14 and 28 days, this trend reversed and the sandy soil proved to be more toxic in response to treatment with silver compared to the loamy soil ($p \leq 0.05$). However, after 90 days of silver exposure, an increase in the relative deviation of the biological variables from their untreated controls from the sandy to the clayey to the loamy soil could be observed (Fig. 4b). At this, there were only significant pairwise differences between the sandy and the loamy soil ($p = 0.000$), as well as between the sandy and the clayey soil ($p = 0.001$).

Impact of agent species, exposure time, concentration and their combinations on biological parameters in a loamy soil

Results of multi-factorial ANOVA revealed significant main effects of the factors *agent species* and *exposure time* on the relative variation of all biological target variables in the loamy soil (Table 3). In the case of the main factor *concentration*, only LAP activity, microbial biomass, *amoA*, *Actinobacteria*, *Acidobacteria*, and *beta-Proteobacteria* were significantly affected by the factor levels. The main factor *exposure time* was able to explain the largest part of the variance compared to the error variance of all target variable except for *Acidobacteria* and *beta-Proteobacteria* (Table 3). For these, the highest *F*-values were created by the main factor *agent species*. The main factor *concentration* could elucidate the least variance.

The majority of significant mean differences in a factor were found for Ag10-COOH and AgPure, 0.01 and 0.10 mg/kg silver as well as 0.01 and 1.00 mg/kg silver, and 1 day and 14 days, 14 days and 90 days as well as 28 days and 90 days (Table 3). No factor combination achieved higher variance explanations than the single main factors (Table 3).

Based on the results of the MANOVA, all factors and factor combinations revealed significant main effects (Table 3). The main factor *exposure time* clarified the largest amount of variance by a *F*-value of 56.0, followed by the main factor *agent species* ($F = 18.8$) (Table 3). The main factor *concentration* could elucidate the least variance again. The means of the highest scored individual factor levels of *exposure time* and *agent species* are visualized in Fig. 5.

Table 2 Results of the (M)ANOVA including the main factors *silver species*, *concentration*, *time*, and *soil texture*, *N* = 4320

Factor	Factor level	MANOVA			ANOVA																	
		df	*F* (Pillai's trace)	*p*	LAP activity		Microbial biomass		16S rRNA		nifH		amoA		Actinobacteria		Acidobacteria		Alpha-Proteobacteria		Bacteroidetes		Beta-Proteobacteria	
					Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp
Silver species		2	44.2	***	8.9	***	13.0	***	90.5	***	2.2	ns	149.8	***	58.8	***	56.2	***	4.7	**	20.2	***	15.4	***
	Ag10-COOH				95.9	a	97.3	ab	105.0	ab	104.0	ns	102.3	ab	101.4	ab	88.0	a	93.2	a	95.9	a	97.0	ab
	Ag10-NH_2				96.5	b	102.6	a	95.7	a	103.1	ns	92.6	ac	96.1	ac	89.9	b	95.0		93.5	ab	94.1	a
	$AgNO_3$				100.1	ab	100.3	b	96.5	b	102.6	ns	104.6	bc	90.8	bc	97.6	ab	96.0	a	97.0	b	94.0	b
Concentration (mg/kg)		2	12.7	***	18.4	***	7.7	**	13.4	***	2.1	ns	5.3	***	10.8	***	13.4	***	4.0	*	24.4	***	14.5	***
	0.01				101.2	ab	97.8	ab	100.6	a	103.1	ns	100.5	a	97.9	a	94.7	ab	96.2	a	97.7	ab	95.5	a
	0.10				94.9	a	100.6	a	99.7	b	102.6	ns	100.6	b	93.0	ab	90.7	a	94.4		94.7	a	96.2	b
	1.00				96.4	b	101.8	b	96.9	ab	104.0	ns	98.5	ab	97.0	b	90.2	b	93.6	a	94.0	b	93.3	ab
Exposure time (days)		3	43.9	***	51.7	***	101.8	***	50.4	***	42.8	***	96.2	***	10.0	***	5.3	**	41.3	***	89.5	***	64.6	***
	1				89.0	abc	91.8	abc	93.4	abc	99.6	ab	107.6	abc	93.2	ab	89.6	ab	94.9	ab	101.7	abc	93.3	ab
	14				102.5	ad	97.0	ad	103.0	ad	105.7	ac	99.8	ad	94.2	cd	91.1		92.4	c	93.5	a	91.2	acd
	28				102.3	be	112.1	bde	97.9	bde	106.8	bd	98.6	be	98.1	ac	93.4	a	101.5	acd	94.9	bd	100.5	bce
	90				96.3	cde	99.4	ce	102.1	ce	100.7	cd	93.4	cde	98.4	bd	93.2	b	90.2	bd	91.8	cd	95.1	de
Soil texture		2	46.5	***	233.6	***	20.4	***	79.7	***	99.2	***	162.2	***	22.2	***	45.0	***	47.0	***	18.4	***	46.3	***
	Loam				101.7	ab	103.1	a	101.7	a	102.7	ab	101.1	ab	99.3	ab	90.7	ab	98.0	a	97.4	ab	98.0	ab
	Sand				84.2	ac	100.7	b	102.0	b	98.7	ac	105.7	ac	92.9	ac	96.8	ac	96.7	b	94.2	a	95.0	ac
	Clay				106.6	bc	96.5	ab	93.5	ab	108.2	bc	92.7	bc	95.8	bc	88.0	bc	89.6	ab	94.8	b	92.1	bc
Factor combination																								
Soil × silver		4	53.2	***	16.3	***	82.9	***	34.1	***	113.0	***	70.6	***	56.5	***	144.6	***	40.0	***	165.8	***	103.2	***
Soil × concentration		4	6.7	***	17.7	***	8.0	***	4.1	***	5.8	***	9.7	***	3.1	*	2.7	*	1.4	ns	12.5	***	3.2	*
Soil × time		6	46.8	***	157.7	***	101.4	***	43.2	***	136.1	***	226.7	***	38.1	***	69.4	***	46.0	***	106.8	***	235.1	***
Silver × concentration		4	5.6	***	6.7	***	4.3	**	18.1	***	4.3	**	2.1	ns	8.6	***	5.7	***	8.3	***	9.8	***	5.6	***
Silver × time		6	25.8	***	26.3	***	112.8	***	82.2	***	44.7	***	72.3	***	21.9	***	25.1	***	7.2	***	29.9	***	93.6	***
Concentration × time		6	12.1	***	4.0	***	13.9	***	17.2	***	13.1	***	12.5	***	16.8	***	16.6	***	7.3	***	22.5	***	43.3	***
Soil × silver × concentration		8	11.4	***	17.8	***	13.5	***	27.8	***	11.1	***	12.8	***	25.7	***	9.0	***	2.0	*	24.3	***	14.1	***
Soil × silver × time		12	24.7	***	30.3	***	46.3	***	54.9	***	133.4	***	49.0	***	36.0	***	46.4	***	20.2	***	74.3	***	55.4	***
Soil × concentration × time		12	9.1	***	6.6	***	18.8	***	10.6	***	12.1	***	14.8	***	10.0	***	6.5	***	6.2	***	16.2	***	21.5	***
Silver × concentration × time		12	9.1	***	14.8	***	7.4	***	11.6	***	13.4	***	10.0	***	13.2	***	12.7	***	8.8	***	16.3	***	24.4	***

Table 2 (continued)

Factor	Factor level	MANOVA			ANOVA																	
		df	*F* (Pillai's trace)	*p*	LAP activity		Microbial biomass		16S rRNA		nifH		amoA		Actinobacteria		Acidobacteria		Alpha-Proteobacteria		Bacteroidetes		Beta-Proteobacteria	
					Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp
Soil × silver × concentration × time		22	8.1	***	4.3	***	22.7	***	10.0	***	13.4	***	11.4	***	10.7	***	12.0	***	8.1	***	22.5	***	27.0	***
R^2					0.897		0.919		0.902		0.930		0.931		0.871		0.892		0.815		0.927		0.939	

The relative variations (%) were set as dependent variable. The factors were set as independent variable

sp significant pairs of multiple comparisons within a factor (post-hoc test), *ns* not significant

Underlined values = *F*- and *p*-value of the ANOVA

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

a, b, c, d = pairs of significant group mean differences

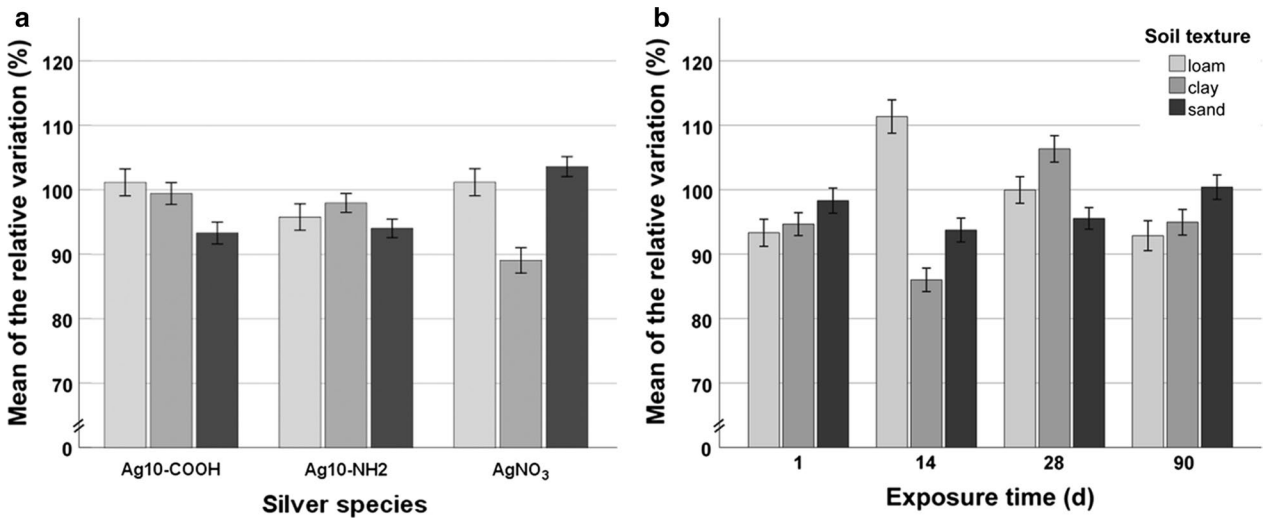

Fig. 4 Impact of factor combinations on the entirety of the relative variation of the biological parameters. **a** *Silver species* × *soil texture*, **b** *exposure time* × *soil texture*. The error bars represent the 95% confidence interval. $N = 4320$. The relative variations (%) were set as dependent variable. The factors were set as independent variable. To calculate the impact of the factor combination *silver species* × *soil texture* on the relative variation of the biological parameters, the relative variations were unconnected to the factors *concentration* and *exposure* time (**a**). To calculate the impact of the factor combination *exposure time* × *soil texture* on the relative variation of the biological parameters, the relative variations were unconnected to the factors *concentration* and *silver species* (**b**)

The relative variation of the biological parameter in the presence of the different silver and nitrate concentrations changed significantly with the exposure time ($p = 0.000$) (Fig. 5a). After a decrease of the relative variation on day 1, the relative variation increased by 14.5% on day 14 to 109.4% ($p = 0.000$). Within the mid-term exposure, a similar, but negatively change of 10.8% could be observed at day 28 ($p = 0.000$). At the end of the experiment, a further, but not significant decline of the relative variation of the biological parameter could be observed (Fig. 5a).

By means of the entirety of the relative variation of the biological parameters the means of AgPure (96.4%) and Ag10-NH_2 (95.8%) caused no significant pairwise disparity in their effect characteristic (Fig. 5b). This could also be stated for the means of Ag10-COOH (101.2%), $AgNO_3$ (101.2%) and KNO_3 (104.2%) (Fig. 5b). In contrast, AgPure and Ag10-NH_2 each differed significantly from Ag10-COOH, $AgNO_3$ and KNO_3 in their pairwise comparisons ($p \leq 0.007$).

The results of the factor combinations of the multivariate multi-factorial analysis revealed lower *F*-values than for the main factors *agent species* and *exposure time* individually. However, the factor combination *agent species* × *exposure time* was scored as the strongest option ($F = 14.5$) within the factor combination possibilities (Table 3). At this, 1 day exposure demonstrated Ag10-NH_2 (89.8%) as the agent species with the highest influence on the relative variation of the biological parameter in the loamy soil, followed by Ag10-COOH (92.3%) (Fig. 6). AgPure and $AgNO_3$ showed only small influences on the relative variation. Between $AgNO_3$ and KNO_3 no significant pairwise difference could be observed. At day 14, all agent species provoked an increase of the relative variation. The biological parameter were stimulated by 1.8% (AgPure) to 16.0% ($AgNO_3$) ($p = 0.000$). Again, between $AgNO_3$ and KNO_3 no significant pairwise difference could be observed. After 4 weeks of exposure, the stimulating effect of all agent species weakened (Fig. 6). In the case of Ag10-COOH and KNO_3 a decrease of the relative variation of 7.3% and 5.8%, respectively, could be observed. The strongest effects on the relative variation of the biological parameters due to the different agent species were created at day 90 (Fig. 6). The agent species Ag10-COOH and KNO_3 provoked significant stimulations of the biological parameters of 12.5% and 15.1%, respectively (Table 4). In contrast, AgPure, $AgNO_3$ and Ag10-NH_2 significantly diminished the relative variation by 14.8, 16.3 and 17.8%, respectively (Table 4).

Discussion

The soil microbial community is responsible for several soil ecosystem services, like the recycling of organic matter, the soil fertility and structure, the biogeochemical nutrient cycles, the toxin degradation and the pathogen control [49–53]. Especially bacteria are the main performer of functional processes, which are integral for maintenance of healthy soil environments [54].

Table 3 Results of the (M)ANOVA including the main factors *agent species*, *concentration*, and *time*, *N* = 2400

Factor	Factor level	MANOVA			ANOVA																	
		df	*F* (Pillai's trace)	*p*	LAP activitiy		Microbial biomass		16S rRNA		nifH		amoA		Actinobacteria		Acidobacteria		Alpha-Proteobacteria		Bacteroidetes		Beta-Proteobacteria	
					Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp	Mean	Sp
Agent species		4	18.8	***	15.6	***	29.3	***	38.3	***	40.7	***	35.3	***	16.0	***	41.5	***	5.6	***	26.1	***	112.5	***
	AgPure				96.5	ab	93.9	ab	96.9	abc	96.2	abc	107.4	ab	91.9	ab	100.6	ab	96.1	a	96.5	abc	87.7	abcd
	Ag10-COOH				106.6	acd	103.6	acde	108.1	ad	108.3	ad	102.1	acde	105.7	acd	88.5	acde	94.8	b	92.0	adef	102.4	aefg
	Ag10-NH_2				95.1	ce	111.9	bcfg	92.0	def	93.5	def	92.9	bcfg	104.2	be	78.7	bcfg	95.8	c	96.6	dgh	97.2	beh
	$AgNO_3$				103.5	f	93.7	*df*	105.0	beg	106.4	beg	108.4	df	88.7	cef	105.0	df	103.4	abcd	103.5	begi	94.3	cfi
	KNO_3				116.3	bdef	92.2	eg	111.7	cfg	112.0	cfg	107.8	eg	98.2	df	100.5	eg	94.6	d	100.0	cfhi	108.7	dghi
Concentration (mg/kg)		2	6.7	***	4.9	**	16.2	***	1.7	ns	1.3	ns	13.5	***	6.4	**	6.2	**	0.9	ns	1.9	ns	6.7	**
	0.01				107.5	a	94.3	ab	103.7		102.8		104.1	a	102.9	ab	97.1	a	96.2		97.3		96.5	a
	0.10				100.1	a	98.9	ac	102.8		102.2		106.5	b	94.3	a	91.5	a	96.6		97.3		97.4	b
	1.00				103.2		104.1	bc	101.4		104.3		100.3	ab	96.0	b	95.2		98.1		98.4		99.8	ab
Exposure time (days)		3	56.0	***	139.5	***	72.7	***	47.5	***	98.0	***	59.0	***	17.8	***	31.3	***	47.8	***	176.7	***	68.0	***
	1				86.9	ab	84.5	abc	95.1	ab	91.2	abc	99.9	a	94.9	ab	93.3	ab	100.0	ab	110.4	abc	92.4	ab
	14				137.3	acd	113.2	ade	113.0	acd	108.5	ade	114.4	abc	105.5	ac	104.9	acd	96.3	cd	97.9	ad	103.3	ac
	28				96.5	bc	102.3	bdf	97.1	ce	96.8	bdf	103.5	bd	88.4	bcd	95.7	ce	108.0	ace	95.5	be	101.6	bd
	90				93.6	d	96.4	cef	104.9	bde	115.6	cef	96.8	cd	100.3	d	84.4	bde	84.2	bde	86.8	cde	94.5	cd
Factor combination																								
Agent × concentration		8	5.7	***	8.3	***	3.9	***	8.5	***	4.2	***	3.3	**	3.1	**	6.3	***	1.9	ns	8.5	***	17.5	***
Agent × time		12	14.5	***	13.5	***	54.1	***	23.9	***	75.0	***	40.1	***	24.0	***	20.6	***	13.4	***	54.8	***	86.0	***
Concentration × time		6	8.0	***	2.3	*	19.3	***	2.8	*	3.7	**	6.7	***	7.8	***	6.0	***	1.5	ns	23.8	***	47.6	***
Agent × concentration × time		22	4.8	***	4.2	***	7.5	***	3.1	***	7.2	***	5.1	***	6.5	***	4.3	***	4.2	***	6.1	***	19.9	***
R^2					0.823		0.882		0.809		0.900		0.850		0.789		0.800		0.722		0.903		0.936	

The relative variations (%) were set as dependent variable. The factors were set as independent variable

Sp significant pairs of multiple comparisons within a factor (post-hoc test), *ns* not significant

Underlined values = *F*- and *p*-value of the ANOVA

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

a, b, c, d = pairs of significant group mean differences

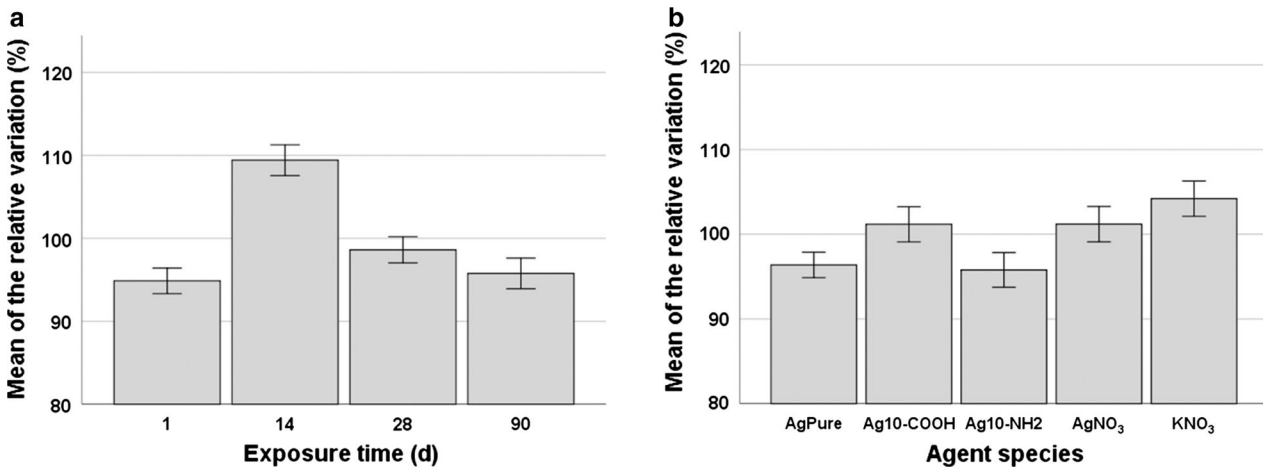

Fig. 5 Impact of the factors *exposure time* (**a**) and agent *species* (**b**) in a loamy soil. The error bars represent the 95% confidence interval. $N = 2400$. The relative variations (%) were set as dependent variable. The factors were set as independent variable. To calculate the effect of the *exposure time* on the relative variation of the biological parameters, the relative variations were calculated unconnected to the factors *concentration* and *agent species* (**a**). For the calculation of the effect expression of the factor *agent species*, the relative variations were unconnected to the factors *concentration* and *exposure time* (**b**)

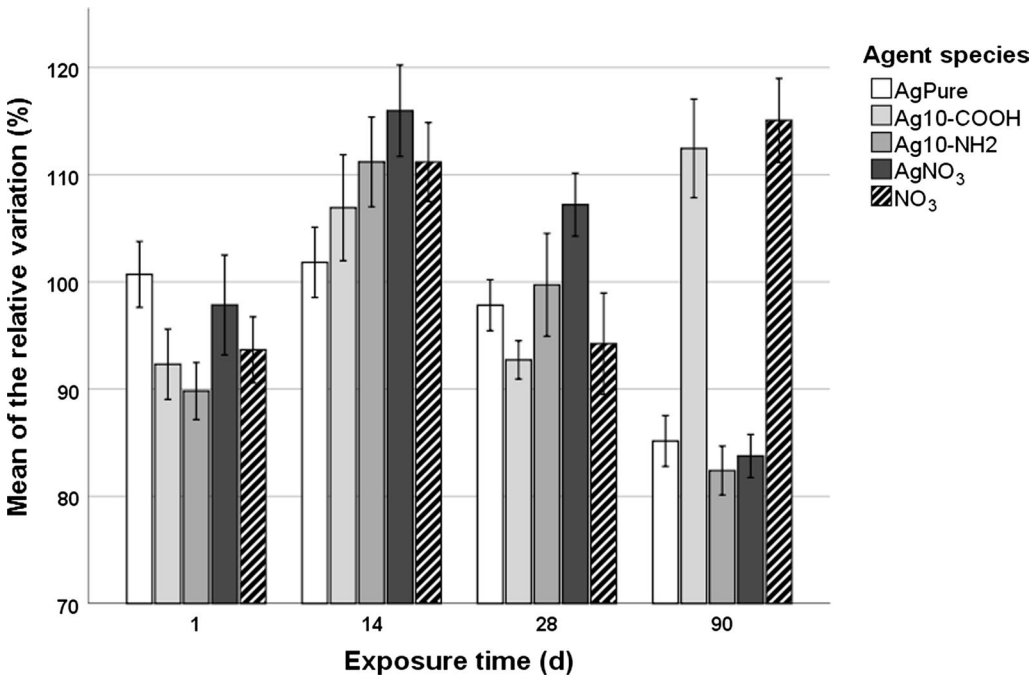

Fig. 6 Impact of the factor combinations *exposure time* × *agent species* in a loamy soil. The error bars represent the 95% confidence interval. $N = 240$. The relative variations (%) were set as dependent variable. The factors were set as independent variable. To calculate the impact of the factor combination *exposure time* × *agent species* on the relative variation of the biological parameters, the relative variations were unconnected to the factor *concentration*

In this study, we used the DNA content of soil samples as a proxy for the microbial biomass and the abundance of 16S rRNA genes as an indicator of bacterial abundance. Furthermore, we measured LAP activity as well as the gene abundance of *nifH* and *amoA* as proxies for the nitrogen cycle, which drives many ecosystem activities in soils, including plant production [40, 41, 55]. Finally, we documented the response of

Table 4 Pairwise comparisons (*p*) of the post-hoc test, *N* = 2400

	AgPure	Ag10-COOH	Ag10-NH_2	$AgNO_3$	KNO_3
1 days					
AgPure		0.007	0.000	ns	0.042
Ag10-COOH	0.007		ns	ns	ns
Ag10-NH_2	0.000	0.000		0.011	ns
$AgNO_3$	ns	ns	0.011		ns
KNO_3	0.042	ns	ns	ns	
14 days					
AgPure	ns	ns	0.015	0.000	0.015
Ag10-COOH	ns		ns	0.021	ns
Ag10-NH_2	0.015	ns		ns	ns
$AgNO_3$	0.000	0.021	ns		ns
KNO_3	0.015	ns	ns	ns	ns
28 days					
AgPure		ns	ns	0.001	ns
Ag10-COOH	ns		0.037	0.000	ns
Ag10-NH_2	ns	0.037		0.018	ns
$AgNO_3$	0.001	0.000	0.018		0.000
KNO_3	ns	ns	ns	0.000	
90 days					
AgPure		0.000	ns	ns	0.000
Ag10-COOH	0.000		0.000	0.000	ns
Ag10-NH_2	ns	0.000		ns	0.000
$AgNO_3$	ns	0.000	ns		0.000
KNO_3	0.000	ns	0.000	0.000	ns

Comparisons of the relative variation of the biological parameter to untreated control in dependence of the factors *exposure time* and *agent* species in the loamy soil. Only significant pairs were stated by their *p* value. In case of "ns" no significant comparison could be observed

Acidobacteria, *Actinobacteria*, *alpha-Proteobacteria*, *Bacteroidetes* and *beta-Proteobacteria* as representatives of the main soil bacterial phyla. Despite the phylogenetic diversity [56], not cultivability [57] and functional redundancy [58] still make it difficult to link members of bacterial phyla in soils with their function, some specific soil functions could be assigned to specific soil bacteria [41, 59–63].

The influence of freezing and defrosting is known to influence the structure and function of microbial communities [64, 65]. Nevertheless, this procedure was executed considering the high amount of samples to be processed. Since this method was applied to all samples, the results were comparable and give statistical inferences about the influence of the factors *silver/agent species*, *concentration*, *exposure time* and *soil texture*.

Silver/agent species

The main determining factor *silver species* caused almost significant effects on the investigated biological parameters of the three test soils (Table 2), whereby the main factor *agent species* caused significant effects on all investigated biological parameters in the loamy soil (Table 3).

In a simultaneous consideration of all biological parameters by the MANOVAs, an increase in the toxicity of KNO_3 (*104.2%*) = Ag10-COOH (98.0%/*101.2%*) = $AgNO_3$ (97.95%/*101.2%*) < AgPure (*96.4%*) = Ag10-NH_2 (95.9%/*95.8%*) could be observed, whereby between Ag10-COOH and $AgNO_3$ no significant pairwise difference could be calculated (Additional file 1: Fig. S1). Values of the main factor *agent species* are shown in italic.

The coating of NP is crucial for their reactivity and physico–chemical transformations, such as the dissolution rate, the availability of surface areas, the surface charge, the aggregation rate and the long-term stability [27, 66, 67]. Analysis of the ζ-potential of the AgNP under different pH values (Fig. 2b, d), indicated no significant impact of pH on ζ-potential of the Ag10-COOH particles. The high negative ζ-potentials of -33.9 ± 1.8 mV at pH 4 and -39.4 ± 1.5 mV at pH 7.4 of the Ag10-COOH indicate high stability of these nanoparticles. The COOH-coating could minimize Ag^+ ion release as well as direct contact of the AgNP with microorganisms or soil compartments such as clay particles or natural organic matter due to their function as a physical barrier [27, 31, 66]. The investigation of Long et al. [68] confirmed this hypothesis. They examined the Ag^+ release and toxicity of different coated AgNP which were very similar to our Ag10-COOH particles and observed only a slightly release of Ag^+ and a lower associated toxicity to *Escherichia coli*. In addition, the negative surface charge could led to an attachment of soil cations such as Ca^{2+}, Mg^{2+} or K^+ on the Ag10-COOH particles and an increase of their physico–chemical barrier.

Furthermore, the coating of AgNP plays a crucial role in determining their cellular uptake mechanism [26], where the negative surface charge of Ag10-COOH indicates a low affinity to negatively charged membranes of microorganisms. Nevertheless, Ag10-COOH induced both adverse and advantageous effects in view of the investigated individual biological parameters. Here, individual defense strategies [28, 69] as well as species dependent toxicological susceptibility [17, 52, 70] of the biological parameters might be the underlying reasons.

In contrast, the ζ-potential of Ag10-NH_2 decreased with increasing pH in our experiment (Fig. 2b) and consequently a decrease of stability could be deduced in the test soils. As a consequence of the missing physico–chemical barrier a high availability of AgNP surface area as well a high release rate of Ag^+ ions is expectable. Both, the mean of the relative variation of all biological

parameters (95.9%/*95.8%*) as well as the mean of LAP activity (96.5%/*95.1%*), *16S rRNA* (95.7%/*92.0%*), *amoA* (92.6%/*92.9%*), *Acidobacteria* (89.9%/*78.7%*) and *Bacteroidetes* (93.5%/*96.6%*) (Table 2, 3) underlined the toxic effects of Ag10-NH_2. Moreover, the positive surface charge of Ag10-NH_2 could have led to a strong association with the negatively charged membranes of microorganisms [71, 72] and affect the surface tension of the membrane resulting in an increase of pore formations [26].

AgPure showed similar harmful effects such as Ag10-NH_2, which could be also attributed to release of Ag^+ ions. Their low particle concentration, their polyacrylate stabilization and the high pH value of soil could have prevented initial aggregation and agglomeration of AgPure [16, 17, 73]. In addition, the high concentration of divalent cations, such as Ca^{2+} und Mg^{2+} in the loamy soil (Table 1) could promote AgPure and Ag10-NH_2 dissolution, resulting in the displacement of Ag^+ ions from the nanoparticle surface and thus toxicity [74].

Surprisingly, the $AgNO_3$ control documented low toxicity by average 97.95% and *101.2%* of the relative variation. The use of silver nitrate is ubiquitously as control in toxicological studies with AgNP to estimate the influence of dissolved Ag^+ ions from AgNP [1, 4, 75, 76]. Here, the toxicity order of the MANOVAs suggest a high release of Ag^+ ions due to Ag10-COOH (98.0%/*101.2%*) relative to Ag10-NH_2 (95.9%/*95.8%*) and AgPure (*96.4%*) and thus a high direct toxic impact of Ag10-NH_2 and AgPure NP itself. However, an individual view on the biological parameters with regard to the ANOVAs resulted in a different manner. The bacterial abundance (*S16 rRNA*) and the abundance of *Actinobacteria* were stimulated by Ag10-COOH exposure and diminished by $AgNO_3$, whereas LAP activity, microbial biomass and *Acidobacteria* were stimulated or not influenced by $AgNO_3$ and diminished by Ag10-COOH (Table 2). These individual responses of the biological parameters explain the majority of significant mean differences found between Ag10-COOH and $AgNO_3$ within the main factors *silver species*. By averaging MANOVA, these important observations are lost and could have led to deceptive conclusions. Based on the individual ANOVAs (Tables 2, 3), as well as the particle and soil characterizations, the hypothesis of a high release of Ag^+ by Ag10-COOH should be excluded and could be related rather to Ag10-NH_2 and AgPure.

In detail, the presented results in Tables 2 and 3 revealed no or stimulation effects due to $AgNO_3$ in case of LAP, *amoA*, *Acidobacteria* and *Bacteroidetes*, whereas the used AgNP caused predominantly lower relative variations of these biological parameters. Similar observations were documented in short-term studies dealing with the effect of low concentrations of AgNP and $AgNO_3$ on organisms related to nitrogen cycle, where $AgNO_3$ might cause lower or even stimulating effects relative to the AgNP. Quite recently, we observed stimulating effects by $AgNO_3$ to ammonia-oxidizing and nitrogen fixing bacteria after short-term exposure, whereby AgNP led to their decrease [16]. Schlich et al. [77] documented a significant stimulation of the nitrite production after 7 day exposure to 0.19 mg/kg $AgNO_3$ up to 19.4%, whereby AgNP caused an inhibition. Yang et al. [78] found that 2.5 µg/L of Ag^+ as $AgNO_3$ upregulated AMOA genes *amoA1* and *amoC2* by 2.1 by 3.3-fold. Furthermore, Choi et al. [79], Masrahi et al. [24] and Liang et al. [80] observed lower effects on microbial nitrification due to $AgNO_3$ relative to AgNP after short-term exposure. Here, in view of the very similar effect expressions of $AgNO_3$ (101.2%) and KNO_3 (104.2%) we suspect, that the nitrate contained in the $AgNO_3$ might also impact the biological parameter such as the silver itself in the $AgNO_3$ control. LAP activity and *amoA* are proxies for the nitrogen cycle. Because of the spatial structure imposed by soil particles resulting in local variations in oxygen availability over small distance [81], both aerobic and anaerobic conditions can be found in the same soil sample. Thus, $AgNO_3$ control could act as substrate for nitrate reducing bacteria, such as *gamma-Proteobacteria* or *Acidobacteria* [63, 82, 83], which were less sensitive to AgNP or Ag^+ in form of $AgNO_3$ [78, 84, 85] and promoted their increase due to nitrate utilization via denitrification and/or dissimilatory nitrate reduction [81, 83] resulting in new nitrogen sources for the ammonia-oxidizing and nitrogen fixing bacteria.

The increase of the abundance of *Bacteroidetes* might be the result of harboring silver resistance genes [69] and simultaneously increased availability of carbon through decreased silver-sensitive microbes, which promoted the as r-strategist know *Bacteroidetes* [56]. Nevertheless, the average factor expression of $AgNO_3$ in case of *Actinobacteria* (90.8%/*88.7%*) and *beta-Proteobacteria* (94.0%/*94.3%*) (Tables 2, 3) indicated still harmful effects. Consequently, there could be an interplay of stimulating and detrimental effects, which might underlay in the agent itself, but also in consequential shifts of the microbial community and nutrient availability.

The *F*-values of the biological parameters in both MANOVAs indicated the main factors *silver species* and *agent species* as strong options to explain large parts of the variances compared to the error variances (Tables 2, 3). With respect to the three different used AgNP, our results confirmed a distinctive impact of the AgNP functionalization on their fate and toxicity in the soil environment. The general applicability of $AgNO_3$ as suitable positive control should be subject of further investigations.

Concentration

The main determining factor *concentration* caused as well almost significant effects on the investigated biological parameters in the three test soils (Table 2). The majority of significant mean differences in the factor were found for 0.01 and 1.00 mg/kg silver. Here, the toxicity of the silver species increased predominantly with increasing concentrations (Additional file 1: Fig. S2), which was already observed in a variety of recent soil studies [5, 24, 86, 87].

The concentration of AgNP strongly influences their physico–chemical transformation. Once released into the environment, AgNP concentration is crucial for dissolution and aggregation mechanisms [28]. Merrifield et al. [63] documented AgNP homoaggregation as insignificant at realistic environmental concentrations, whereby dissolution and also heteroaggregation processes were more likely. Based on the low test concentrations of 0.01–1.00 mg/kg silver, dissolution seemed to be the most probable transformation type in our study. Dissolution hypothesis was supported by the average high pH value of soils that could have prevented initial aggregation and agglomeration of AgNP [73] as well as the average high concentration of divalent cations, such as Ca^{2+} und Mg^{2+} (Table 1), which promoted AgNP dissolution and resulted in the displacement of Ag^{+} ions from the nanoparticle surface [74]. Furthermore, the high ζ-potentials of +36.8 and +39.2 mV for Ag10-NH_2 as well as of −33.9 and −39.4 mV for Ag10-COOH at pH 4 and 7.4, respectively, indicated high interparticular repulsive forces, which diminished the aggregation probability due to high stability [88]. However, according to Klitzke et al. [14] a decrease of ζ-potential after AgNP exposure to soil solution has to be assumed.

A direct interaction of the microbes with the differently charged AgNP is also conceivable. However, the relative variation of the biological parameters differed only by 2.0% between 0.01 and 1.00 mg/kg silver. This low effect expression can be attributed to the low bioavailability of the silver species as well as to bacterial resistance mechanisms. The released silver ions could bind to clay particles [19, 33] or organic material [20, 89, 90] and thus be less bioavailable. In addition, the likelihood of a microbe to encounter a silver particle or ion is generally low concerning the applied low test concentrations. In the event of a coincidence, bacteria possess various commonly defense mechanisms to escape the toxic influence of silver, such as the thickness of their peptidoglycan-membrane as the first line of defense [91], efflux systems to extrude heavy metal ions [4, 92, 93] and the production of extracellular proteins and exopolysaccharide [94] to neutralize small amounts of toxic compounds [95, 96]. Furthermore, some bacteria obtain specific silver resistance genes, which encode periplasmic silver-specific binding protein (SilE), silver efflux pumps (P-type ATPase), and membrane potential-dependent polypeptide cations/proton antiporter (SilCBA) [4].

Nevertheless, compared to the *F*-values of the other main factors, the factor *concentration* could elucidate the least variance of the mean values considering all variables (Tables 2, 3). Based on our results, the main factor *concentration* at environmentally relevant levels has, therefore, only a very weak influence on the AgNP effect characteristics respecting the microbial community in soils.

Exposure time

The main determining factor *exposure time* caused consistently significant effects on the investigated biological parameters in the three test soils after silver exposure (Table 2), with the majority of significant mean differences between the factor expressions 1 day and 28 d. With the exception of the relative variation of the *Bacteroidetes* and the ammonia-oxidizing bacteria (*amoA*), all biological parameter were negatively affected at day 1 (Table 2). This observation was rather unusual, because several studies measured high and fast sensitivity of ammonia-oxidizing bacteria with AgNP and $AgNO_3$ [24, 77, 78]. Apart from that, we recently recognized a tolerance of *amoA* harboring bacteria after short-term exposure to AgNP [16]. Furthermore, Schlich et al. [77] and Samarajeewa et al. [86] observed stimulatory effects with ionic and nanoparticulate silver to ammonia-oxidizing bacteria, which could be ascribed to hormone-like responses to low silver concentrations. By contrast, the stimulation of *Bacteroidetes* due to silver addition was in agreement with previous observations [52, 69, 70, 97, 98]. They exhibit silver resistance genes [78].

As the exposure time increased until day 28, the injury on the microbial community decreased (Additional file 1: Fig. S3). It is likely that the short-term effects after 1 day were observed as a result of the initial release of bioavailable Ag^{+} by AgNP and $AgNO_3$, causing toxic effects on the microbial community. Dissolution experiments [99, 100] verified fast solubility of AgNP in soils. With increasing exposure time, the silver species became less bioavailable due to interactions with organic matter, clay minerals or pedogenic oxides [19, 33]. Furthermore, upcoming mechanisms such as self-protection [4, 91–93, 95, 96], resistance [4], resilience [58] and/or cryptic growth [101], might also be possible explanations for the limited effects on the microbial community in the soils.

Results of the ANOVA using data of the loamy soil only documented a similar trend (Table 3). Here, all biological parameters were also negatively affected at day 1,

with the exception of the relative variation of the *Bacteroidetes and* the ammonia-oxidizing bacteria (*amoA*). In virtue of the high clay content (approximately 30%) of the loamy soil and its high content of organic carbon (2.9%) (Table 1), the retention of AgNP and Ag^+ ions arose earlier and the toxicity of the agents decreased even at day 14 (Table 3) [14, 20, 102, 103].

On day 90, an impairment of the microbial community could be observed relative to day 28 for both ANOVAs (Tables 2, 3). Similar trends were observed in our previous studies [16, 17]. As AgNP and Ag^+ ions gradually aged, they slowly returned to the biological soil system as a continuously sink of silver agents. Diez-Ortiz et al. [33] also documented a progressive increase in AgNP toxicity with time and assumed a time-dependent enlargement of silver in soil pore water due to slow dissolution.

The *F*-values of the biological parameters in both MANOVA indicated the main factor *exposure time* as a strong option to explain a large part of the variances compared to the error variances (Tables 2, 3). Especially in case of the exclusion of the factor *soil texture* from the MANOVA, the factor *exposure time* yielded the maximum *F*-value (Table 3). These results strongly underline the significance of the exposure time for AgNP ecotoxicity investigations, attributable to the changes of silver bioavailability and its specification status during long-term experiments in soils.

Soil texture

Also the main factor *soil texture* caused significant effects on all investigated biological parameters in the three test soils (Table 2). The majority of significant mean differences were found between the loamy soil and the clayey soil. This was noticeable, because their investigated soil characteristics such as pH, TOC, TN and CEC were very similar. The most distinct difference resulted from their grain size distributions of clay (45–65% vs. 25–35%) and sand (5–40% vs. 25–45%) (Table 1). Actually, recent studies have shown a positive correlation between the clay content of soils and the toxicity of AgNP: lower grain size of soils resulted in lower toxicity of AgNP [19, 23, 25]. Indeed, this was appropriate for the sandy soil with a mean of 97.0% and the loamy soil with a mean of 99.4% relative abundance of all biological parameters (Additional file 1: Fig. S4). However, the mean of the relative abundance of the clayey soil (95.5%) illustrated the opposed situation: lower grain size of the clayey soil resulted in higher toxicity of silver (Additional file 1: Fig. S4). In fact, not only the particle size distribution can be responsible for the silver toxicity. Schlich and Hund-Rinke [19] documented that the highest AgNP toxicity was associated with more acidic soils, whereas the lowest toxicity was associated with more alkaline soils. They supposed that the soil pH value influences AgNP dissolution and the release of ions [19]. Similarly, this could be observed for the loamy soil (pH = 7.2) and the sandy soil (pH = 3.2) in this study (Table 2). At the same time, the results of the clayey soil with a pH value of 7.4 seemed to disagree again with the hypothesis.

The results of the factor combination *silver species* × *soil texture* (Fig. 4a) might elucidate the crux of the observed mean silver toxicity order loamy soil < sandy soil < clayey soil. In case of Ag10-NH_2 and Ag10-COOH, the loamy and the clayey soil exhibited lower toxicity compared to the sandy soil (Fig. 4a). This confirmed the positive correlation between the grain size distribution of soils and the toxicity of AgNP as well as the association of high AgNP toxicity by more acidic soils [19, 23, 25]. In contrast, $AgNO_3$ caused a strong decrease of the entirety of the relative variation of the biological parameters in the clayey soil (Fig. 4a), which caused an overwhelmed influence on the average formation in the MANOVA of the silver species. This indicated a completely different fate of $AgNO_3$ compared to AgNP in the test soils.

Soil texture × silver species

The factor combination *soil texture* × *silver species* yielded the highest *F*-value in the MANOVA considering the entirety of the relative variation of the biological parameters (Table 2).

With regard to the investigated AgNP it could be observed that their toxicity was lower in the loamy and clayey soil than in the sandy soil (Fig. 4a). In case of Ag10-NH_2 the high availability of soil cations in the loamy and the clayey soil (Table 1) might have promoted the dissolution of the lower stable AgNP [14], resulting in the release of Ag^+ ions. These were bound to clay particles and became less bioavailable [33]. The absent effect of the $AgNO_3$ control as measure for Ag^+ impact in the loamy soil might confirm this hypothesis. Furthermore, the high content of natural organic matter, derived by the land-uses of the loam and clay locations, could have been reduced the toxicological effects of the Ag10-NH_2 due to inhibition of oxidation [104]. At this, positive charged Ag10-NH_2 were adsorbed by negatively charged organic matter, which dominated now the surface properties leading to higher steric stability and thus also lower bioavailability [18].

In contrast, the sandy soil exhibited contrary soil properties and thus toxicity (Table 1). Although the low amount of soil cations reduced the dissolution affinity of AgNP, the low pH of 3.2 and the concomitant higher amount of OH^- ions might decreased the Ag10-NH_2 stability due to the deprotonation of the amino groups. Under aerobe conditions, AgNP oxidation followed and Ag^+ ions were released [104]. The small clay content of

the sandy soil (0.0–5.0%) did not provide enough capacity for Ag^+ retention resulting in direct harmful interactions of Ag^+ with the microorganisms, such as interactions with enzymes of the respiratory chain reaction, increase of DNA mutation frequencies or morphological changes of cell wall membrane [105]. However, the stimulating effects of the $AgNO_3$ control on the biological parameters in the sandy soil disagreed with the Ag^+ dissolution theory (Fig. 4a). Therefore, it might be that the Ag10-NH_2 particle itself caused the negative effects in the sandy soil. Positively charged NP were observed to strongly associate with membranes, which leads to a higher cellular uptake [26].

The lower toxicity of the Ag10-COOH relative to the Ag10-NH_2 particles based in general on their higher stability and their surface barrier, which reduced Ag^+ dissolution. Furthermore, their negative surface charge promoted the attachment of soil cations of the loamy and the clayey soil and could have prevented direct interactions with negatively charged membranes of microorganisms [26, 27, 31, 66, 68]. Analogously to the Ag10-NH_2, Ag10-COOH showed strongest toxicity in the sandy soil (Fig. 4a). Here, interactions with soil cations as well as natural organic material seemed to be less probable considering the soil and AgNP characteristics (Table 1). In addition to a low oxidation of Ag10-COOH and the concomitant lower Ag^+ release, a direct interaction of these AgNP with microorganisms can be assumed. An internalization of negatively charged nanoparticles could be occured through nonspecific binding and clustering of the particles on cationic sites on the plasma membrane and subsequent endocytosis or by direct diffusion through the cell membrane [106]. Here, Ag10-COOH could act as a trojan horse: metabolization processes in food vacuoles and lysosomes allow the uncoating of AgNP and enable the direct release of Ag^+ ions into the cytoplasm causing intracellular damages [32, 107].

However, there is always a challenge to accurately differentiate what proportion of the toxicity is from the ionic form and what proportion of the nanoform [76]. For resolving this question, $AgNO_3$ was used as measure for Ag^+ impact in our study. Results of silver nitrate exposure in the loamy soil confirmed their low contribution to the AgNP toxicity (Fig. 4a). Ag^+ ions seemed to be bound to clay particles or other soil compartments and became less bioavailable. Conspicuously, $AgNO_3$ caused the highest toxicity in the clayey soil and a low stimulation in the sandy soil. In fact, Schlich and Hund-Rinke [19] observed also a lower toxicity of $AgNO_3$ compared to AgNP in a sandy soil (RefeSol 04A) investigating the potential ammonium oxidation, but there was no promotion. The high toxicity of $AgNO_3$ in the clayey soil resulted by a decrease of the bacterial taxa, whereas LAP activity, *nifH* and *amoA* were hardly influenced or even stimulated (data not shown). In case of the proxies for nitrogen cycle, we suspect a significant portion of the nitrate compound of the $AgNO_3$ control in the results. However, reasons for the detrimental effect on the bacterial structure were intricate to find. Probably, interactions with existing soil contaminants could be a reason for the high $AgNO_3$ toxicity. The clay location was farmed and treated with herbicides and pesticides. With this, $AgNO_3$ might have been bound to such contaminants, thus creating a new toxic agent. For example, Uwizeyimana et al. [108] indicated that pesticide and metal mixtures negatively affected earthworms. However, detailed studies on the impact of nanoparticle-pesticide combinations on microbial community in soils are currently lacking, but necessary to perform a comprehensive risk assessment of AgNP in soils.

Soil texture × exposure time

The factor combination *soil texture* × *exposure time* revealed the second highest *F*-value of 46.8 in the MANOVA (Table 2). With regard to the results in Fig. 4b, it could be clearly observed, that the spans of the effect characteristics of the individual soils differ significantly from each other during the four examination dates. While the effect levels in the sandy soil on days 1, 14, 28 and 90 extended over an amount of 6.3% (lowest value of 93.7% on day 14, highest value of 100.0% on day 90), the amounts for the loamy and the clayey soil were 21.5% and 20.3%, respectively. This indicated the loamy and the clayey soil as more complex soil systems considering the interplay of physico–chemical and biological interactions and transformations of the silver species contrary to the sandy soil. Whereas the effect expressions of the biological parameters in the sandy soil were indicative for consistent conditions, the effect expressions of the loamy and the clayey soil suggested a variety of temporal changes in the complex soil-time framework.

In case of the sandy soil it can be assumed, that there were continuously barely interactions of the silver species with the soil type characteristics, causing throughout similar effect expressions. In addition, the indigenous microbial community might show no observable adaption ability over time.

In contrast, the relative variations of the biological parameters in the loamy and the clayey soil differed not only in dependence on the exposure time, but also in their effect strength from each other, although their soil characteristics were very similar (Table 1). For example, on day 14, it could be assumed for the loamy soil, that the silver species were transformed, might be bound to the clay particles or capped by organic matter [33, 104], resulting in a decrease of toxicity and a stimulation of the

biological parameters due to their defense arsenal [4, 91–93, 95, 96] (Fig. 4b). Based on the similar soil characteristics, this should be also noted for the clayey soil. However, the biological parameter was diminished by 14.0% in the clayey soil at day 14 suggesting divergent indigenous microbial communities with different capacities to resist and adapt on silver emission. Also interactions with herbicides and pesticides of the clayey soil might have led to the creation of a new toxicant, which provoked the harmful effect. At day 28, the effective expressions of the loamy and the clayey soil reproached and none or stimulating effects were documented (Fig. 4b). At this, ageing of the silver species and their return to the biological soil system could have reduced the stimulating effects on the biological parameters in the loamy soil. Contrary, the ageing in the clayey soil of the silver species or the new toxicants as well as the now developed defense mechanisms of the microbial community after 4 weeks exposure caused stimulation by 6.3% (Fig. 4b).

Based on our data, however, we can only speculate about these events. More detailed investigations are necessary to reveal the time-dependent fate of the silver bioavailability in different soils, as well as the time-dependent responses of the microbial community to these silver species. For this purpose, the development of reliable and robust analytical methods for detecting specific silver species at trace concentrations in complex matrices is a fundamental requirement. Next, batch experiments can reveal time-dependent silver transformations and the concomitant effects on the microbial community. Furthermore, it would be useful to further deepen the soil characterization to evaluate possible effects of anthropogenic residues from e.g. herbicides or pesticides on the ecotoxicological potential of silver nanoparticles.

Agent species × exposure time

Regarding the MANOVA with the main factors *agent species*, *concentration* and *exposure time* in the loamy soil, the factor *exposure time* was able to explain the largest part of variance. The factor combinations achieved lower *F*-values. Nevertheless, the factor combination *agent species* × *exposure time* still achieved the highest *F*-value of 14.5 for the factor combinations. Furthermore, the results of the pairwise comparisons of the post hoc test (Table 4) as well as the ambiguous role of $AgNO_3$ control as a measure of Ag^+ dissolution gave reason to pay attention to this interaction.

Short-term exposure led only to a small difference between the investigated agents AgPure, Ag10-COOH, Ag10-NH_2, $AgNO_3$ and KNO_3 (Fig. 6). Ag10-COOH diminished the relative variations of the biological parameters by 7.7%. Based on their higher stability and the short exposure time, physico–chemical transformation could be excluded and led to the assumption of direct harmful interaction of these AgNP with microorganisms. In contrast, Ag10-NH_2 was reported as strong instable AgNP due to their surface functionalization. At this, higher dissolution of Ag^+ ions as toxicological agent might be a probable explanation for the decrease of the biological parameters by 10.2% (Fig. 6). However, relating to the low effects of the $AgNO_3$ control (97.95%), the Ag^+ ion dissolution theory seemed less likely. Therefore, a direct interaction could be assumed as well. AgPure caused on average no effects (100.7%) on day 1 and thus proved to be inert to physico–chemical transformations after short-term exposure. The KNO_3 control diminished the biological parameters on average by 6.3%, but without significant difference to $AgNO_3$ (Fig. 6, Table 4). This slightly adverse effect might be the result of NO_2^- accumulation, due to nitrate reduction [109, 110].

After 14 days exposure, the differences between the effect characteristics of the agent species changed. Ag10-NH_2 (111.2%), $AgNO_3$ (116.0%) as well as KNO_3 (112.0%) stimulated the biological parameter significantly, whereas AgPure (101.8%) and Ag10-COOH (107.0%) caused only low stimulatory effects. In case of the silver species, their bioavailability as well as those of the released Ag^+ ions could be reduced at this time point due to interactions with organic matter, clay minerals or pedogenic oxides [14, 20, 102, 103]. Furthermore, emerging self-protection mechanisms, such as the production of extracellular proteins or polysaccharides of the soil microbiome could neutralize toxic ions or cap AgNP [95, 96]. In addition, resilience mechanisms [58, 78, 101] might be possible explanations for the limited effects on the microbial community in the soil at day 14. The increase of the biological parameter due to $AgNO_3$ and KNO_3 exposure might result by an increase of nitrate reduction and the concomitant increase of nitrogen for the microbiome [81, 83]. Due to the synergy of microbial silver resistance mechanisms as well as the nitrogen substrate source, $AgNO_3$ increased the relative variation of the biological parameters. As already mentioned, several authors documented stimulation or minor negative effects on microorganisms related to nitrogen cycle after exposure of low concentrations of $AgNO_3$ in short-term exposure experiments [16, 24, 77–80].

Starting by day 28, ageing of the silver species and their slow return to the biological soil system presented a continuous sink of bioavailable silver, reducing the stimulating effects on the biological parameters (Fig. 6). An increase in AgNP toxicity with time can be linked to time-dependent enlargement of silver in soil pore water due to dissolution [33, 111]. However, the defense arsenal of bacteria was sufficient to resist silver toxicity at day 28. After 3 months exposure, there seemed to be a shock load of silver in case of AgPure (85.2%), Ag10-NH_2 (82.4%)

and $AgNO_3$ (83.8%) to which the bacterial community was not immediately prepared. Small-scale bioavailability, chemical alterations and possible transformations (oxidation, reduction, dissolution, sulfidation) of AgNP and Ag^+ [23, 24, 33, 90, 112, 113] in the loamy soil are possible physicochemical causes for the abrupt toxicity. Furthermore, it might be assumed that after short- and mid-term adaption to the silver contamination as well as the positioning of the silver species in the soil system, the bacterial population might have lost members with silver tolerance and were unanticipatedly shocked by the return of silver toxicant at day 90 resulting in strong reductions of the biological parameters [17].

In contrast, Ag10-COOH caused a significant stimulation of the investigated parameters by 12.5%, confirming their high stability. With prolonged exposure time, the likelihood of bounds between the negative charged Ag10-COOH and soil cations increased and with that their physico–chemical barrier, resulting in lower bioavailability. Together with the presumably low number of free Ag^+ ions in case of Ag10-COOH, no toxicity could be observed.

Based on the missing significant differences of $AgNO_3$ and KNO_3 at days 1 and 14, it could not be determined, if the effects were caused only by the Ag^+ of the $AgNO_3$ control, but maybe due to the NO_3^- of the KNO_3 control. First at the exposure times of 28 days and 90 days, significantly different effect characteristics of $AgNO_3$ and KNO_3 by 13.0% ($p = 0.000$) and 31.5% ($p = 0.000$) (Fig. 6, Table 4), respectively, could be observed. This caused us to suspect, that initial at this late time points the nitrate contained in the $AgNO_3$ control did not further exceedingly influence the results of the $AgNO_3$ control. In consequence, especially in case of biological parameters related to nitrogen cycle, such as LAP activity or the abundance von *amoA* harboring bacteria, the usage of $AgNO_3$ as proxy for Ag^+ release in AgNP short-term effect assessments could be deceptive. There is an urgent need for further research into the suitability of $AgNO_3$ as a measure of Ag^+ release. For example, batch experiments investigating all steps within the soil nitrogen cycle (in particular nitrogen fixation, nitrification, denitrification, dissimilatory nitrate reduction to ammonium) after short- and long-term exposure of $AgNO_3$ and NO_3^- will be helpful to resolve in detail, at which step the nitrate released from the $AgNO_3$ control presents an advantage for the microbial community and at which step the harmful influence of the silver predominates.

Conclusions

Impacts of the factors *silver species*, *agent species*, *concentration*, *exposure time* and *soil texture* on the relative variation of 10 biological parameters were analysed by *16S rRNA* qPCR as well as fluorometric and photometric techniques. The used AgNP were characterized in detail by electron microscopy, dynamic light scattering and asymmetrical flow field-flow fractionation. Analyses of variance revealed the factors *silver species*, *exposure time* and *soil texture* as the most relevant determinants for the effect expressions of the biological parameters. Furthermore, the factor combinations *soil texture* × *silver species* as well as *soil texture* × *exposure time* facilitates even larger explanations of the variance of the biological parameter.

Overall, the presented results demonstrate the importance of considering several factors in the effect assessment of AgNP. Based on our study, the relationship of the *soil texture* × *silver species* is the most significant factor combination for the environmental fate and toxicities of AgNP in soils.

Additional files

Additional file 1. Characterization of AgNP.

Additional file 2. Raw data.

Abbreviations

AgNP: silver nanoparticle(s); AF4: asymmetrical flow field-flow fractionation; Ag-HPB: hydrophobic silver particle(s); DLS: dynamic light scattering; MPA: mercaptopropionic acid; NP: nanoparticle(s); PDI: polydispersity index; UPW: ultrapure water.

Authors' contributions

JC designed and manufactured the Ag10-NH_2 and Ag10-COOH nanoparticles. SS performed and evaluated the transmission electron microscopy of AgNP. KY, MF, and DR performed the ζ-potential and size measurements of AgNP as well as the asymmetrical flow field-flow fractionation (AF4) of AgNP. GAL and EC designed and performed the soil experiments. GAL, EC and MW analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All datasets on which the conclusions of the paper rely are presented in the main manuscript (Additional files 1 and 2).

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