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Long-term effects of environmentally relevant concentrations of silver nanoparticles on microbial biomass, enzyme activity, and functional genes involved in the nitrogen cycle of loamy soil

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ABSTRACT

The increasing production and use of engineered silver nanoparticles (AgNPs) in industry and private households are leading to increased concentrations of AgNP in the environment. An ecological risk assessment of AgNP is needed, but it requires understanding the long term effects of environmentally relevant concentrations of AgNP on the soil microbiome. Hence, the aim of this study was to reveal the long-term effects of AgNP on soil microorganisms. The study was conducted as a laboratory incubation experiment over a period of one year using a loamy soil and AgNP concentrations ranging from 0.01 to 1 mg AgNP/kg soil. Soil treated with silver nitrate served as a positive control; the negative control was untreated soil. The short term effects of AgNP were, in general, limited. However, after one year of exposure to 0.01 mg AgNP/kg, there were significant negative effects on soil microbial biomass as quantified by extractable DNA ($p = 0.000$) and bacterial ammonia oxidizers ($p = 0.009$) were determined by quantification of gene copy numbers (*amoA*). Furthermore, the tested AgNP concentrations significantly decreased the soil microbial biomass, as quantified by extractable DNA ($p = 0.000$), the leucine aminopeptidase activity, as defined by substrate turnover ($p = 0.014$), and the abundance of nitrogen fixing microorganisms ($p = 0.001$) quantified by the gene copy number of *nifH*. The results of the positive control with $AgNO_3$ revealed predominantly stronger effects due to Ag^+ ion release. Thus, the increasing toxicity of AgNP during the test period may reflect the long-term release of Ag^+ ions. Nevertheless, even very low concentrations of AgNP caused disadvantages for the microbial soil community, especially for nitrogen cycling, and our results confirmed the risks of releasing AgNP into the environment.

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Introduction

Silver has been used for more than 100 years as a biocide to inhibit microbial growth (Nowack et al., 2011). While in early usage, mainly silver nitrate was applied, today silver nanoparticles (AgNPs) have become increasingly popular. The small size (1 to 100 nm) of the nanoparticles and the concomitant high surface to volume ratio, which results in a potentially higher chemical reactivity compared to bulk silver, has promoted the use of AgNP (Reidy et al., 2013). In addition to the large Ag^+ release, AgNP are expected to cause direct effects on both prokaryotes and eukaryotes, including impacts on cell walls and membranes, the production of reactive oxygen species (ROS), and adverse modifications of nucleic acids and proteins (Maillard and Hartemann, 2013; McShan et al., 2014; Reidy et al., 2013). Consequently, the utilization of AgNP has expanded to more and more fields of daily life. Apart from the initial medical uses, AgNP are actually used in households, industry and agriculture such as for water purification, plant growth promotion and textiles cleaning (Hänsch and Emmerling, 2010; Nowack, 2010; Reidy et al., 2013). AgNP are released into the environment during all stages of the life cycle, including production, product use, disposal and weathering (Gottschalk and Nowack, 2011), but the extent is still unclear. To date, there are only a few studies available that directly document the concentrations of nanoparticles in the environment (Benn and Westerhoff, 2008; Kägi et al., 2008). Recent studies have attempted to model and estimate the production volume and fate of nanoparticles and assess environmental concentrations and potential ecotoxicological risks for ecosystems. Nowack et al. (2011) estimated that approximately 320 tons/year of nanosilver is produced worldwide. In Europe, Sun et al. (2014) modelled a production of 32.4 tons/year nanosilver and predicted annual increases of AgNP in the range of 1.2 ng/(kg·year) to 2.3 μg/(kg·year) for sediments and soils.

As a consequence of the antimicrobial effects of AgNP, it is likely that AgNP also impact natural microbial communities in various ecosystems after nanoparticles have entered the environment. Recently, most studies have investigated the toxic effects of AgNP on single species in batch experiments, including *Escherichia coli* and *Bacillus subtilis* (Choi and Hu, 2008; Dhas et al., 2014; Gajjar et al., 2009; Morones et al., 2005). Although such results are fundamental for an understanding of the underlying toxicity mechanisms of AgNP, these data do not directly enable estimations of the ecotoxicological risk of AgNP in natural environments. For example, several studies indicated that the physicochemical characteristics of soils correlated to various effects by AgNP and thus toxicity, but they do not enable direct extrapolation of in vitro studies to the ecosystem scale (Rahmatpour et al., 2017; Schlich and Hund-Rinke, 2015). For example Schlich and Hund-Rinke (2015) revealed that AgNP toxicity decreased with increasing clay content and increasing pH in soils. Metreveli et al. (2016) observed aggregation of AgNP due to organic matter. Furthermore, the release of silver ions from AgNP was controlled by the presence of non-organic matter and organic constituents (Metreveli et al., 2016). Further studies documented interactions between organic soil matter and nanoparticles that affected deposition and toxicity behaviours (Chen and Elimelech, 2008; Dasari and Hwang, 2010; Shah and Belozerova, 2009).

Hänsch and Emmerling (2010) were one of the first teams to investigate the medium-term effects of environmentally relevant concentrations of AgNP in a range 3.2–320.0 μg AgNP/kg on soil microbes and activities. They observed a decrease in soil microbial biomass and an increase in the basal respiration and metabolic quotient with increasing AgNP concentrations in soil. Further studies revealed the effects of AgNP on bacterial nitrogen turnover and shifts in the bacterial community composition due to AgNP exposure (Asadishad et al., 2018; Colman et al., 2013; He et al., 2016; Peyrot et al., 2014; Samarajeewa et al., 2017; Schlich et al., 2016; Throbäck et al., 2007). For instance, He et al. (2016) noticed a significant decrease in soil nitrification potential and ammonia-oxidizing bacteria after the application of 0.1, 1.0 and 10.0 mg AgNP/kg sandy loam soil over a 30-day exposure period. A community survey of DNA in an arctic soil treated by 0.066% AgNP for 176 days demonstrated a clear decline in the soil's major orders Solirubrobacterales, Actinomycetales, Rhizobiales, Acidobacteriales and Clostridiales relative to the untreated control soil (Kumar et al., 2011). Moreover, Schlich and Hund-Rinke (2015) and Samarajeewa et al. (2017) found different effects of AgNP on soil microbes over a one- to two-month exposure period, respectively, which might be caused by physicochemical reactions, such as adsorption, agglomeration, aggregation and dissolution, in the surrounding environment of the applied nanoparticles (Pachapur et al., 2016). These studies highlighted the significance of long-term studies, which have been rare.

Despite these recent studies, knowledge about the impacts of AgNP on the activity and function of the soil bacteria community remains limited. Since there are numerous AgNP on the market, evaluating the potential ecotoxicological effects of all variations remains an essential challenge. The size, shape, surface-coating agent, charge and stability of these AgNP are some of the properties that can differ (Reidy et al., 2013), and the characteristics of soils are also very distinct.

Considering the significance of soil microbial communities for soil ecosystem function, such as plant growth carbon sequestration and degradation of xenobiotics (Emmerling et al., 2002) and the predicted increase of nanoparticle release into the environment, the aim of this study was to reveal the adverse long-term effects of AgNP on soil microorganisms and their functions. The study was conducted with an incubation period of one year using a loamy soil and environmentally relevant AgNP concentrations (0.01 to 1.00 mg AgNP/kg soil). We measured the effects on microbial biomass, abundance of bacteria, and enzymatic activity, and we quantified marker genes for selected processes of the inorganic nitrogen cycle using quantitative real-time PCR (qPCR). In addition, the fate of AgNP within the test soil was analysed by transmission electron microscopy (TEM).

1. Materials and methods

1.1. Experimental setup

To assess the effects of AgNP on soil microbes, 20 kg of a loamy soil was sampled from the Ap horizon (0–30 cm depth)

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