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Long-term effects of silver nanoparticles on the abundance and activity of soil microbiome

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Silver nanoparticles (AgNPs) are among the most extensively used engineered nanomaterials because of their well-established antimicrobial and unique physicochemical properties (Yin et al., 2015). Applications of AgNPs have now been expanded beyond their initial use in medicine to industry, agriculture, and households. Examples of AgNP-containing products include paints, clothes and textiles, personal care products, food packages, water filters, and appliances. It has been estimated that the current global consumption of AgNPs is more than 300 tons per year (McGillicuddy et al., 2017). AgNPs can be released from various consumer products into the environment throughout their lifecycle, from synthesis, distribution, application, to end-of-life disposal. Sun et al. (2014) predicted that the soil concentration of AgNPs could be increased by 1.2 to 2.3 ng/kg each year in Europe. Therefore, it is imperative to study the ecological impact of AgNPs.

After release into the environment, AgNPs can disperse, undergo aggregation or agglomeration, dissolve, or interact with other species in the medium (Yin et al., 2015; Zheng et al., 2015). Although AgNPs are directly associated with inflammatory, oxidative, and genotoxic effects, the dissolution or release of silver ion (Ag^+) that further performs as a biocide is

likely the primary mechanism of antimicrobial and toxic effects. Consequently, when present in soil, AgNPs can impact natural microbial communities. Soil microbiome is crucial for maintaining soil functions, such as supporting plant growth and degrading xenobiotics. Thus, it is of great importance to understand adverse effects of AgNPs on the soil ecosystems. Several studies have revealed that exposure of AgNPs at environmentally relevant concentrations can decrease the abundance and activity of soil microorganisms. However, these studies mainly focused on short or medium-term effects, and long-term effects remain unclear.

Grün et al. (2018) designed a one-year laboratory experiment to elucidate the long-term effects of AgNPs at environmentally relevant concentrations on the abundance and activity of the soil microbiome. Aliquots of a loamy soil were incubated with AgNPs or AgNO_3 at three concentrations (0.01, 0.10, and 1.00 mg/kg). Soil aliquots treated with AgNO_3 were used as positive controls; negative controls were those incubated with ultrapure water. Microbial biomass, abundance of bacteria, and enzymatic activity of soil samples were then measured at different time points (day 1, 7, 14, 28, 90, 180, and 365).

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Microbial biomass of the soil refers to the living fraction of soil organic matter, consisting of bacteria, fungi, archaea, and protozoan. The DNA content of soil samples was determined to indicate the microbial biomass. A significant decrease in microbial biomass was observed for positive controls treated with AgNO₃, but not for soils treated with AgNPs for one day. However, no significant difference was found for both AgNO₃ and AgNP-treated soils at later time points up to 90 days, implicating that soils can overcome the initial effects of Ag⁺ on microbial biomass. A concentration-dependent decrease in microbial biomass was observed for soil samples treated with either AgNO₃ or AgNP at day 180. The significant decrease remained for both types of treated samples after one-year exposure.

The abundance of soil bacteria was determined by quantifying bacterial 16S rRNA gene in soil samples using quantitative PCR. No significant decrease in abundance of 16S rRNA gene was observed for AgNP-treated soils at the first four time points, whereas AgNO₃-treated samples showed a significant increase between day 7 and day 28. It is possible that the nitrate of AgNO₃ might have promoted the growth of denitrifying bacteria. Both AgNP and AgNO₃ treatments for 90 days led to a significant decrease in the abundance of 16S rRNA gene. Interestingly, the lowest concentration resulted in the largest decrease. On day 180, the significant decrease in abundance of 16S rRNA was correlated with the silver concentration for both the AgNP and AgNO₃ treatment groups. Similar effects were observed after one-year.

Soil microbes play an important role in nitrogen cycle by converting organically bound nitrogen into ammonia. Ammonia-oxidizing bacteria (AOB) can further oxidize ammonia into nitrate. Leucine aminopeptidases (LAPs) catalyze the hydrolysis of leucine residues at the N-terminus of peptides and proteins. Therefore, LAP activity of soils was measured as an indicator for the organic nitrogen cycle. LAP activity was reduced after one-day exposure of AgNP or AgNO₃; it recovered after one week for both AgNP and AgNO₃ treatments and became higher than that of negative controls for the AgNO₃-treated soils on day 14. On days 90 and 360, a significant decrease in LAP activity was observed for soil samples treated with 0.10 and 1.00 mg/kg of AgNP or AgNO₃.

The *nifH* and *amoA* genes encode the enzymes that mediate nitrogen transformation. The *nifH* gene was measured to indicate the potential of the soil microbiome for N₂

fixation; the *amoA* gene was used for quantifying AOB. Although short-term exposure of AgNP or AgNO₃ did not generate consistent effects on the abundance of *nifH* gene, the significant decrease was observed for both AgNP and AgNO₃ treatments on days 180 and 360. Similarly, the abundance of the *amoA* gene was significantly reduced after one-year exposure of AgNP or AgNO₃. Therefore, long-term exposure of AgNP or AgNO₃ can negatively impact the nitrogen cycle.

The results of the study by Grün et al. (2018) revealed that the long-term exposure of AgNPs at environmentally relevant concentrations significantly induced adverse effects on the soil microbiome although the short-term effects were generally minor. One-year exposure of 0.01 mg/kg AgNP significantly decreased the microbial biomass, abundance of total soil bacteria, and the soil microbes responsible for nitrogen cycling. These findings filled the knowledge gap regarding long-term effects of AgNPs on soil microbiome. The results are valuable for understanding long-term ecological risk of releasing AgNPs into the environment. Information on the fate and transformation of AgNPs in the soils could help understand the long-term ecological risk of AgNPs.

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