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Effects of silver nanoparticles on soil microorganisms and maize biomass are linked in the rhizosphere

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ABSTRACT

Silver nanoparticles hold great promise as effective anti-microbial compounds in a myriad of applications but may also pose a threat to non-target bacteria and fungi in the environment. Because microorganisms are involved in extensive interactions with many other organisms, these partner species are also prone to indirect negative effects from silver nanoparticles.

Here, we focus on the effects of nanosilver exposure in the rhizosphere. Specifically, we evaluate the effect of 100 mg kg^{-1} silver nanoparticles on maize plants, as well as on the bacteria and fungi in the plant's rhizosphere and the surrounding bulk soil. Maize biomass measurements, microbial community fingerprints, an indicator of microbial enzymatic activity, and carbon use diversity profiles are used. Hereby, it is shown that 100 mg kg^{-1} silver nanoparticles in soil increases maize biomass, and that this effect coincides with significant alterations of the bacterial communities in the rhizosphere. The bacterial community in nanosilver exposed rhizosphere shows less enzymatic activity and significantly altered carbon use and community composition profiles. Fungal communities are less affected by silver nanoparticles, as their composition is only slightly modified by nanosilver exposure. In addition, the microbial changes noted in the rhizosphere were significantly different from those noted in the bulk soil, indicated by different nanosilver-induced alterations of carbon use and community composition profiles in bulk and rhizosphere soil.

Overall, microorganisms in the rhizosphere seem to play an important role when evaluating the fate and effects of silver nanoparticle exposure in soil, and not only is the nanosilver response different for bacteria and fungi, but also for bulk and rhizosphere soil. Consequently, assessment of microbial populations should be considered an essential parameter when investigating the impacts of nanoparticle exposure.

1. Introduction

Microorganisms form significant interactions with a wide array of biota in terrestrial ecosystems, often with important consequences for all species present. Terrestrial plant species are colonized by a large number of microorganisms in several morphological regions, including interior tissues (endophytes), on their leaves (epiphytes) and in their rhizosphere. In the rhizosphere, the microbial genes greatly outnumber the plant genes (Mendes et al., 2013), resulting in a microbiome that has been referred to as the plant's second genome. Just like the human gut microbiome, the plant's rhizosphere microbiome has been implicated in many functions that support its host's well-being. For example, rhizosphere microorganisms can increase nutrient uptake, protect against pathogens, enhance abiotic stress tolerance and promote the development of the plant (Berendsen et al., 2012). However, some rhizosphere organisms are also capable of causing plant disease or impeding plant growth. Alternatively, plants also influence the microbial community within their rhizosphere, largely mediated through root structure and exudates that determine the physical and chemical conditions within the plant root zone. As root structure and exudates change during the plant's life cycle, the conditions in the rhizosphere are altered, likely selecting for a

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different microbial community. Other factors such as xenobiotics can disturb the rhizosphere conditions, having both direct and indirect consequences for the plant and its microbial community.

The dramatic increase in the use of silver nanoparticles (AgNP) has resulted many beneficial applications but also raised some concern. On the one hand, nanotechnology has begun to make efficient use of the well-known anti-microbial properties of silver. On the other hand, the increasing quantities in which AgNP are produced and applied, as well as the unique characteristics resulting from the nanoscale size, have led to concern over the toxicological implications of exposure for nontarget species. Results from studies with several plant species are largely in agreement over the phytotoxic effects of AgNP in artificial conditions such as hydroponic systems. AgNP have been demonstrated in hydroponics to impair root elongation, seed germination and plant biomass production of plants like zucchini, cucumber, onion, rye grass and rice (Stampoulis et al., 2009; Anjum et al., 2013; Dimkpa, 2014; Gardea-Torresdey et al., 2014). However, in the much scarcer soil-based studies, the concentrations at which a negative effect of AgNP on plants are observed are often higher than in hydroponics studies, and the proportion of reported neutral AgNP effects on plant growth increases (Dimkpa, 2014). As mentioned, the interest in AgNP mainly originates from its well-known potency as a broad spectrum antimicrobial agent effective against diverse species of Gram-positive and Gram-negative bacteria (Morones et al., 2005; Kim et al., 2007), as well as against several fungal species, although reports of this anti-fungal activity are considerably less numerous (Panacek et al., 2009). As both bacteria and fungi are present in the rhizosphere of plants, the antimicrobial effects from AgNP exposure, may have significant implications for the host plant health. Notably, the amount of research investigating the implications on rhizosphere-based nanoparticle exposure on both the microbes present and the supporting host plant is scarce.

This study investigates the effects of AgNP exposure on maize plants and the bacteria and fungi in the plant rhizosphere. The strongly microorganism-oriented toxicity of AgNP in soil will be used to increase our understanding on the plant–microbe interaction in the rhizosphere, in particular under abiotic stress such as from NP exposure. Maize is chosen as the experimental crop because of its importance in global agriculture, and because of the likelihood of significant AgNP-exposure as AgNP may enter the agro-ecosystem through several pathways: during manufacturing, from use of nano-enabled agrichemical products, and from the application of NP-containing biosolids. Specifically, maize plants are grown for 75 days in soil containing 100 mg kg^{-1} AgNP, and the effects of this exposure on biomass production and on the activity and community structure of its associated bacteria and fungi are monitored over time. This concentration of 100 mg kg^{-1} AgNP is high when compared to data of studies that examine the fate of unintentionally released AgNP from non-agricultural related products (Gottschalk et al., 2009; Simonin and Richaume, 2015). However, because agricultural maize fields accumulate also AgNP from biosolids and nano-enabled agrichemical sources, AgNP concentrations are higher in these systems than in those that just suffer from unintentional release. Using a concentration like the one we applied will provide heretofore unavailable data of the effects of a new contaminant at a concentration on the dose–response curve where an effect is anticipated.

2. Material and methods

2.1. Material and experimental set-up

Uncoated silver nanoparticles (99.99% purity, 20 nm diameter) were acquired in solid form from US Research Nanomaterials, Inc., Houston, Texas, USA. The experimental soil was collected from the top 30 cm of an agricultural corn field in Diepenbeek, in the eastern part of Belgium (50°56′05.3″N 5°24′41.2″E), and was characterized as sandy loam (55% sand, 30% silt, 15% clay) with a pH of 6.98, an electrical conductivity (EC) of 335 μS cm^{-1} and an effective cation-exchange capacity (CEC) of 20.7 meq/100 g. After collection, the soil was 6 mm sieved and homogenized. *Zea mays* variety LG 30.223 seeds were obtained from LimaGrain Belgium.

In order to evaluate the effects of AgNP-exposure on plants and microbial communities both in bulk soil and rhizosphere over time, the following experimental design was applied. A total of 120 pots with 1 kg of soil each was used, with half of them being amended with 100 mg kg^{-1} AgNP by mechanical mixing during 5 min. As mentioned above, we recognize that this exposure concentration is high (Simonin and Richaume, 2015), although such concentrations could be achieved in the future on maize fields due to the fact that they accumulate nanoparticles from multiple sources (biosolids, nano-enabled agrichemicals). Moreover, we used this high level to establish a baseline response in the system. The other half of the pots, without AgNP, were used as controls. Half of both control and AgNP pots were planted with a maize seed; the other half of the pots remained unplanted. Before planting, maize seeds were soaked in tap water overnight. All pots were randomly placed in a greenhouse under the following conditions: photoperiod of 14 h daylight, a temperature cycle of 22°C/18 °C and a relative humidity of 60%. After 16, 25, 39, 53 and 75 days, plants were harvested and samples were taken from the rhizosphere, here defined as soil that remained attached to the root after light shaking, and the bulk soil for microbial community analysis. Specifically, at each harvesting point, 6 pots with maize plants (3 control and 3 AgNP-exposed), and 6 pots with bare soil (3 control and 3 AgNP-exposed) were randomly selected; the former 6 being used for plant measurements and rhizosphere samples, and the latter 6 for bulk soil samples. The total leaf length of each harvested plant was determined, and harvested shoots and roots were oven dried at 60 °C and subsequently weighed. Bulk soil and rhizosphere samples were used for fluorescein diacetate (FDA) hydrolysis analysis, the Biolog EcoPlate™ assay and bacterial and fungal automated ribosomal intergenic spacer analysis (ARISA) fingerprinting.

2.2. Biolog EcoPlate™ analyses

Samples from rhizosphere and bulk soils were used immediately after harvest for community-level physiological profiling (CLPP) with Biolog EcoPlates™ (Biolog, California, USA). For each sample, 1 g of soil was dissolved in 10 ml 0.01 M phosphate buffer (pH = 7.2), and after thorough shaking, the solution was further diluted 1:20. After settling of remaining particles, 120 μl was dispensed into each well of all 31 carbon sources (3 replicates per plate). The plates were incubated in the dark at 25 °C, and absorbance at 590 nm of all wells per plate was measured twice every 24 h using a FLUOstar® Omega plate reader, with the final measurement taking place after 94 h. Absorbance data were used to calculate the average well color density (AWCD), richness (R) with threshold equal to 0.25, and Shannon-Weaver index (H) per treatment. Absorbance values per carbon source were also used separately in subsequent statistical analyses.

2.3. FDA assay

Immediately after harvesting, samples from bulk and rhizosphere soils were used for the FDA-hydrolysis assay (Schnurer and Rosswall, 1982; Adam and Duncan, 2001). Briefly, 1 g of soil (replicated twice) for each of the 3 samples per treatment were incubated for 3 h at 30 °C with 50 ml of 60 mM sodium phosphate

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