Applied Soil Ecology 119 (2017) 307–316

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Silver nanoparticles deteriorate the mutual interaction between maize (*Zea mays* L.) and arbuscular mycorrhizal fungi: a soil microcosm study

Jiling Cao[a,b,c], Youzhi Feng[a,b], Shiying He[d], Xiangui Lin[a,b,*]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, Jiangsu Province, China
[b] Joint Open Laboratory of Soil and the Environment, Hong Kong Baptist University and Institute of Soil Science, Chinese Academy of Sciences, Nanjing, Jiangsu Province China
[c] University of Chinese Academy of Sciences, Beijing, China
[d] Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province, China

ARTICLE INFO

Keywords:
Arbuscular mycorrhizal communities
Maize
Ag accumulation
Dissolved organic matter
Alkaline phosphatase activity

ABSTRACT

The effects of silver nanoparticles (AgNPs) on plants and soil microbial communities have been widely documented. However, the influence of AgNPs on plant growth and rhizospheric microbial communities, especially the important symbiotic microbes, such as arbuscular mycorrhizal (AM) fungi, remains under debate. In this study, a greenhouse pot experiment was established to investigate the responses of maize (*Zea mays* L.) growth and rhizospheric AM fungal assemblages to different application levels (0.025, 0.25 and 2.5 mg kg^{-1}) of AgNPs or bulk Ag. The results indicated that 2.5 mg kg^{-1} of AgNPs significantly decreased ($p < 0.05$) plant biomass and dissolved organic carbon (DOC) content in rhizospheric soils compared to the control and bulk Ag conditions. Growth inhibition was associated with increased Ag accumulation in plant tissues and increased antioxidant enzyme activity. A similar toxicity for the AM fungal community was observed as a significant decrease ($p < 0.05$) in their diversity and remarkable variations in their structure, which were closely correlated with plant root biomass, soil soluble Ag and DOC content. Consequently, AgNPs caused a reduction in AM fungal growth and ecological function, characterized by a significantly decreased ($p < 0.05$) root mycorrhizal colonization rate, soil alkaline phosphatase activity, available phosphorus (P) content and P nutrition in plants. These results suggest that high concentrations of AgNPs can deteriorate the mutual interaction between plants and AM fungi and negatively influence the rhizospheric soil P cycling, both of which go against plant growth and soil fertility.

1. Introduction

Silver (Ag) is well known for its antimicrobial activity (Silver, 2003). The rapid development of nanotechnology has increased the use of Ag in the form of nanoparticles (NPs) as antimicrobial additives in paints, detergents, plastics, washing machine liners, food supplements and textiles (Impellitteri et al., 2009). As a result of their high production and usage, AgNPs are increasingly entering the environment, and soil is predicted to be a major sink (Gottschalk et al., 2013). Therefore, AgNPs may have profound impacts on soil ecosystem due to their high reactivity (Anjum et al., 2013). AgNPs have been documented to show an inhibitory effect on plant growth, cause variations in the bacterial community composition, and reduce the soil enzymatic activity (Dimkpa et al., 2013; Kumar et al., 2011; Sillen et al., 2015). However, most studies evaluating the toxicity of AgNPs have been conducted on plants and microbes separately. Few investigations have simultaneously examined how AgNPs affect both plant growth and the microbial community in rhizospheric soils (Sillen et al., 2015).

The rhizosphere is an extremely active environment that contains exudates from plant roots (Cardon and Whitbeck, 2007). Plant roots and their exudates can alter the rhizospheric microbial community in ways that may change the microenvironment or microbial bioavailability of carbon sources, resulting in a rhizospheric microbial community different from those in bulk soils (Weinert et al., 2011). Rhizospheric microbes can also make contribution to plant growth and health (Kaye et al., 2005). For example, arbuscular mycorrhizal (AM) fungi are ubiquitous soil microorganisms that can form mutualistic associations with an estimated 90% of land plants (Smith and Read, 2008) and are known for their beneficial role in increasing plant nutrient acquisition, notably phosphorus (P), and plant resistance to exotic stresses (Göhre and Paszkowski, 2006; Miransari, 2010; Rillig, 2004). In addition to direct effects on their hosts, AM fungi may

* Corresponding author. Tel.: +86 2586881589; fax: +86 2586881000.
E-mail address: xglin@issas.ac.cn (X. Lin).

http://dx.doi.org/10.1016/j.apsoil.2017.04.011
Received 16 September 2016; Received in revised form 29 March 2017; Accepted 19 April 2017
Available online 19 July 2017

increase soil enzymatic activities because their propagules can synthesize enzymes and their host roots may release enzymes that are important for nutrient cycling in soils (Rillig, 2004; Wang et al., 2006). However, AM fungi themselves are especially sensitive to environmental changes caused by heavy metals and other abiotic stresses (Cui et al., 2016; Hassan et al., 2011; Xiang et al., 2014). Due to the vital role of AM fungi in soil ecosystems, therefore, knowledge regarding the influences of AgNPs on plant–AM fungi interactions is important for evaluating the consequences of introducing AgNPs into soil ecosystem.

In this investigation, the effects of AgNPs on maize plants and AM fungal community composition and diversity in rhizospheric soils, as well as their ecological functions, were studied. Maize was chosen as the experimental plant because of its importance in global agriculture and because it can easily be colonized by AM fungi (Wang et al., 2006). It was hypothesized that (1) maize growth and rhizospheric AM fungal status would differ between control and AgNP-treated soils and (2) the AM fungal community would be shifted by AgNPs through direct toxicity or indirectly by affecting the bioavailable carbon content in rhizospheric soils. Plants and microcosm soils were harvested when the phytotoxicity of AgNPs was manifested, and the AM fungal community composition and diversity were subsequently analyzed by high-throughput sequencing. The results will aid our understanding of plant–AM fungi interactions in the rhizosphere under stress caused by AgNP exposure and the possible consequences to plant–soil systems. The objective of this study was to evaluate the influences of AgNPs on maize growth and the rhizospheric AM fungal community and their ecological functions.

2. Materials and methods

2.1. Experimental design and setup

Surface (0–15 cm) soil was obtained from an agricultural filed after wheat harvest in Fengqiu County (35°00'N, 114°24'E), Henan Province, China. The soil type was aquic inceptisol and had a sandy loam texture. Visible roots, stones and macro-fauna were removed, and the residual soil was mixed and sieved through 2 mm mesh. The following soil properties were then determined: pH (8.58), soil organic matter (OM) (5.83 g kg^{-1}), total nitrogen (N) (0.45 g kg^{-1}), total P (0.55 g kg^{-1}) and total potassium (K) (22.0 g kg^{-1}).

The AgNPs used in this study were synthesized with the chemical reduction method following a previously described procedure (Feng et al., 2013) with a primarily spherical shape; the average size and zeta potential (ζ) were approximately 20.4 ± 3.2 nm and −23.0 ± 1.0 mV, respectively. Bulk Ag powder (purity 99%, AR) with a particle size of approximately 5.0 μm was purchased from Sinopharm Chemical Reagent Co., Ltd, China. Before the amendments, AgNPs or bulk Ag was dispersed and vigorously shaken using a sonicator (600 W, 20 min). Thereafter, the suspensions containing the appropriate amount of AgNPs or bulk Ag were added to 180 g of sieved soil in a 250 mL plastic pot (diameter = 7.2 cm and height = 9.2 cm) to achieve application levels of 0.025 (L), 0.25 (M) and 2.5 (H) mg kg^{-1}, which was commonly used in the studies of AgNPs' effects on ecosystem (Dimkpa et al., 2013; Feng et al., 2013). To make the particles to thoroughly mix into the soils, the suspensions were added drop by drop to the soil surface, and then the pots containing the mixture of soils and suspensions were gently shook up and down five times (He et al., 2011). After mixing, soils were subsampled and stored for evaluating treatments effects on DNA extraction efficiency. Soils without AgNPs or bulk Ag were used as the control. Each treatment had three replicates.

Surface-sterilized maize (*Zea mays* L.) seeds were germinated in filter paper at 28 °C. After 2 days, two healthy seeds were sown into each box at a depth of approximately 0.5 cm. Seedlings were grown in a greenhouse at a 25/18 °C day/night temperature with a 16 h photoperiod and 40–60% relative humidity. Each pot was irrigated with 20 mL of 50% Hoagland's nutrient solution every week. After 20 days of growth, a time at which the effects of AgNPs on plant growth are apparent, all maize plants were harvested. Because the maize roots extend throughout the soil, the entire pot of soil was mixed and collected after the roots were removed. Subsamples were maintained at −40 and 4 °C for molecular studies and chemical determinations, respectively.

2.2. Plant, mycorrhizal colonization, antioxidant enzymes activities, P and Ag concentrations

Shoots and roots of plant were sampled separately, rinsed with tap water and deionized water and subsequently dried at 70 °C for 48 h to obtain the dry plant biomass. Before drying, the weighed subsamples of fresh roots and leaves were used for root mycorrhizal colonization and antioxidant enzyme activity assessment, respectively. The root mycorrhizal colonization was determined using the grid-line intersect technique in terms of the percentage of fresh root length (Giovannetti and Mosse, 1980). To determine the occurrence of oxidative stress in plant leaves caused by AgNPs exposure, the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were determined, which are commonly used in studies of metal-induced oxidative stress (Gratão et al., 2008). SOD activity was assayed by measuring the capacity of the reaction mixture to inhibit the photochemical reduction of nitro blue tetrazolium (Wang et al., 2004). POD activity was measured by following the change in optical density at 470 nm due to guaiacol oxidation (Wang and Yang, 2005). CAT activity was determined by measuring the initial rate of disappearance of H_2O_2 as the decline in optical density at 240 nm (Chaoui et al., 1997). All dried shoots and roots were ground into powder and separated into two parts. Subsamples of shoots and roots were digested by H_2SO_4-H_2O_2 and then diluted with deionized water. The P content was determined using molybdenum blue colorimetry (Thomas et al., 1967). Subsamples of plant shoots and roots were digested with concentrated nitric acid, followed by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific, Waltham, MA, USA) to measure Ag concentrations in plants. Shoot and root P and Ag acquisitions (amounts of P and Ag in a plant shoots and roots) were calculated as shoot and root biomass with P or Ag concentration in the shoots and roots.

2.3. Soil characteristics analysis

Soil pH and electrical conductivity (EC) (with a soil-to-water ratio of 1:5) were determined with a pH meter (Beckman) and EC meter (Orion 160), respectively. Available P and K were measured using the molybdenum blue method and flame photometry (Bray and Kurtz, 1945). The content of OM was measured by Kjeldahl digestion (Nelson and Sommers, 1982), respectively. Soil alkaline phosphatase activities were determined according to the methods of Tabatabai (1982) by incubation at 37 °C with borate buffer (pH 9). Soil DOC was extracted by adding 50 mL of 0.5 M K_2SO_4 to 10 g of soil and determined using a TOC-TN analyzer (Skalar, The Netherlands). The soluble Ag in soils was extracted with 0.005 M diethylene triamine penta-acetic acid and was then analyzed using ICP-MS (Thermo Scientific, Waltham, MA, USA) (Lindsay and Norvell, 1978). All results were expressed based on an oven-dried (105 °C, 24 h) soil weight.

2.4. Soil DNA extraction, polymerase chain reaction (PCR) and Illumina sequencing

For each soil sample, 0.5 g fresh soil was used to extract total DNA with the FastDNA® SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA). DNA concentrations were determined by spectrophotometry (NanoDrop ND-1000, Thermo Scientific, USA).

The extracted DNA (50 ng) was used as template to amplify the 18S rRNA with the primers set AMV4.5NF (5'-AAGCTCGTAGTTGAATTTCG-3')/AMDGR (5'-CCCAACTATCCCTATTAATCAT-3') (Lumini et al., 2010). To distinguish the amplified products, a 5 bp barcode sequence