Supporting Information for

Silver Nanoparticles Alter Soil Microbial Community Compositions and Metabolite Profiles in Unplanted and Cucumber-planted Soil

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Supporting Information for Method

Soil Chemical Analysis. Soil pH, dissolved organic carbon and extractable macro/micro element content were determined according to the protocol of Houba et al. Briefly, 3 g soil mixed with 30 mL 0.01 M CaCl₂ was shaken for two hours at 200 rpm at 20°C. The pH of the suspension was then measured using a pH meter (Mettler-Toledo, Switzerland). After centrifugation at 10000 rpm for 10 min, the supernatant was collected and filtered through 0.45 μm. The dissolved organic carbon was measured by VarioTOC Analyzer (Elementar, Germany) with Potassium phthalate monobasic (KHP) for calibration. The water extractable element content (K, Ca, Mg, Si, Mn, Al, Cu, Ag, Ti, Cd) was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 8300, Perkin Elmer, USA) and ICP- mass spectrometry (MS) (NexION-300, PerkinElmer, USA). An ICP QC standard solution (NSI Solution Incorporated, USA) were used for calibration and quantitation.

Photosynthetic Pigments Determination. One hundred mg fresh leaves were extracted with 5 mL 80% acetone and ethanol (v:v=1:1) for 12 h. After centrifugation at 4000 rpm for 10 min, the absorbance of chlorophyll a, chlorophyll b and carotenoid at 663 nm, 645 nm and 470 nm, respectively, in the supernatant were measured by microplate reader (Synergy H4 Hybrid Reader, Biotek, America).

Total Phenolic Content. Total phenolic content was determined based on the method of Singleton and Rossi. Briefly, the leaf powder was extracted with 80% acetone and ethanol (v:v=1:1). Then, 50 μL extracts was mixed with 450 μL water, followed by the addition of 250 mL Folin phenol reagent and 1.25 mL 20g/L Na₂CO₃ solution.
Absorbance at 750 nm was measured by microplate reader (Synergy H4 Hybrid Reader, Biotek, America) and the total phenolic content was expressed as mg Gallic acid equivalent g\(^{-1}\) of fresh weight.

**Lipid Peroxidation Assay.** Lipid peroxidation was determined according to the protocol of Jambunathan (2010).\(^3\) Lipid peroxidation was expressed as μmol MDA equivalent g\(^{-1}\) of fresh weight. Briefly, 0.1 g of cucumber leaves was mixed with 4 mL of 0.1% TCA, followed by centrifugation at 10000 rpm for 15 min. One mL of the supernatant was mixed with 2 mL of 20% TCA and 2 mL of 0.5% TBA; the mixture was then heated in a water bath at 95 °C for 30 min. After cooling, absorbance at 532 nm and 600 nm was measured with microplate reader (Synergy H4 Hybrid Reader, Biotek, America).

**GC-MS based Metabolomics.**

**Soil Metabolite Extraction (Sample preparation).** At harvest, a 1000 mg soil sample from each treatment (pot) was sieved to 2 mm and then ground to a fine powder in liquid N\(_2\) and stored at -80 °C for later extraction. One gram of soil was weighed and transferred to a 1.5-mL Eppendorf tube, followed by the addition of 1mL of methanol and water (1: 1 = v: v) and 20 μL of 2-chloro-l-phenylalanine (0.3 mg/mL, dissolved in methanol as internal standard). The mixture was sonicated at 60 HZ for 2 min. The extraction was repeated three times and was centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant was transferred to a 5-ml Eppendorf tube and freeze dried. The dried powder was resuspended in 400uL methanol: water (v: v= 1:1), vortexed for 60s followed by sonication for 30s. The suspensions were centrifuged at 12000 rpm for 10
min at 4 °C.

A QC sample was prepared by mixing aliquots of the all samples to obtain a pooled sample. An aliquot of the 300 μL supernatant was transferred to a glass sampling vial for vacuum-drying at room temperature. Eighty μL of 15 mg/mL methoxylamine hydrochloride in pyridine was subsequently added. The resulting mixture was vortexed vigorously for 2 min and incubated at 37 °C for 90 min. Eighty μL of BSTFA (with 1% TMCS) and 20 μL n-hexane was added to the mixture, which was vortexed vigorously for 2 min and then derivatized at 70 °C for 60 min. The samples were placed at ambient temperature for 30 min before GC-MS analysis.

**GC-MS Analysis.** The derivatized sample extracts were analyzed on an Agilent 7890B gas chromatography system coupled to an Agilent 5977A mass selective detector (single quadrupole) (Agilent Technologies Inc., CA, USA). The column employed was a DB-5MS fused-silica capillary column (30 m × 0.25 mm × 0.25 μm; Agilent J & W Scientific, Folsom, CA, USA Agilent Technologies, Santa Clara, CA). Helium (> 99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL/min through the column. The initial oven temperature was 60 °C, ramped to 125 °C at a rate of 8 °C/min, to 210 °C at a rate of 4 °C/min, to 270 °C at a rate of 5 °C/min, to 305 °C at a rate of 10 °C/min, and finally, held at 305 °C for 3 min. The injection volume was 1 μL with the injector temperature 260 °C in splitless mode. The temperature of MS quadrupole and ion source (electron ionisation) was set to 150 and 230 °C, respectively. The ionisation energy was 70 eV. Mass data was acquired in a full-scan mode (m/z 50-500), and the solvent delay time was set to 5 min. Quality control samples, which were
prepared by applying small aliquots from each sample with L-2-Chlorophenylalanine as internal standard, were injected at regular intervals (every 10 samples) throughout the analytical run.

Reference

Table S1. Soil background elemental analysis (mg/kg dry soil)

<table>
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<tr>
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<th>Al</th>
<th>Fe</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
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<td></td>
<td>21663 ± 1014</td>
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<td>4291 ± 264</td>
<td>3879 ± 164</td>
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<td>276 ± 31</td>
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<tr>
<td>Ti</td>
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<td>31.1 ± 5.0</td>
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<td>16.1 ± 1.5</td>
<td>0.19 ± 0.02</td>
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</table>
Figure S1. SEM image of silver nanoparticles
Figure S2. Soil pH (I) and dissolved organic carbon content (II). A: soil, B: soil+AgNPs, C: soil+plant, D: soil+plant+AgNPs. The data were represented by means±SD by four replicates. * represent the significance is below 0.05, ** represent the significance is below 0.01.
Figure S3. Bacterial community composition with the relative abundance of the bacterial phyla. A: soil, B: soil+AgNPs, C: soil+plant, D: soil+plant+AgNPs.
Figure S4. Bray–Curtis distance of bacterial communities. A: soil, B: soil+AgNPs, C: soil+plant, D: soil+plant+AgNPs.
Figure S5. Partial least-squares discriminant (PLS-DA) scores plot of metabolites in soil. AgNPs at 100 mg/kg were amended in soil in absence or in presence of cucumber plants for 60 days.
Figure S6. Biological pathways of microorganisms in soil altered by AgNPs exposure.
Figure S7. Cucumber fresh biomass (I), photosynthetic pigments (II), MDA content (III) and total phenolics content (IV). C represent “soil+plant”, D represent “soil+plant+AgNPs”. The data were represented by means±SD by four replicates. * represent the significance is below 0.05, ** represent the significance is below 0.01.