STM report of Dr. Gennaro Roberto Abbamondi

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Host Institution: Hasselt University - Centre of Environmental Sciences (CMK)

Program title:

Maize endophytic microbial community changes under nanosilver exposure

This Program continued on a joint Research Project covering the effects of silver nanoparticles on maize and its microbiome, which started in 2015 (Sillen et al. 2015). Previously gathered rhizosphere microbiome data have been supplemented with data covering the endophytic bacterial and fungal community, searching for further correlations between microbiome shifts and host biomass production under nanosilver exposure.

Introduction

Silver nanoparticles are effective anti-microbial compounds used in a myriad of applications (Marin et al. 2015). They end up on agricultural fields intentionally (agrochemical application) and unintentionally (contaminated biosolids, wastewater run-off). Thus, nanosilver's antimicrobial properties affect microorganisms in the plant-associated microbiome. Previous results in this joint Research Project concerned the rhizosphere microbiome, showing nanosilver's direct and indirect impact on plant-associated bacteria and fungi. An even more intimate relation between plants and

microorganisms exists in the plant's inner tissues, which are colonized by microbial endophytes (Hardoim et al. 2015). These endophytes are also affected by nanosilver, as the substance is taken up by the plant. This project examined those changes taking place in endophytic microbiome. Together with previously gathered data on plant parameters and the rhizosphere microbiome, a holistic image of nanosilver's effect in maize agriculture will be created.

Methods

The endophytic bacterial and fungal communities of maize plants exposed to silver nanoparticles were compared to the endophytic communities of control plants by means of ARISA technique (Amplified Ribosomal Intergenic Spacer Analysis).

Four treatments were considered for the analysis:

- CNS = plants grown in control soil and not sprayed with silver nanoparticles
- CS = plants grown in control soil and sprayed with silver nanoparticles
- NPNS = plants grown in silver nanoparticle-containing soil and not sprayed with silver nanoparticles
- NPS = plants grown in silver nanoparticle-containing soil and also sprayed with silver nanoparticles

Total DNA were extracted from harvested roots, stem and leaves. Bacterial 16Se23S ITS DNA were amplified using the primer pair S-D-Bact-1522-b-S-20 (eubacterial rRNA small subunit, 50-TGCGGCTGGATCCCCTCCTT-30) and L-D-bact-132-a-A-18 (eubacterial rRNA large subunit, 50-CCGGGTTTCCCCATTCGG-30) (Normand et al., 1996). Fungal ITS1- 5.8S-ITS2 DNA were amplified with the primer pair 2234C (at 30 end of 18S gene, 50-GTTTCCGTAGGTGAACCTGC-30) and 3126T (at 50 end of 28S gene, 50-ATATGCTTAAGTTCAGCGGGT-30) (Sequerra et al., 1997). Amplified reaction mixtures were loaded onto DNA-1000-chips, allowing the product fragments to be resolved with an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). The 2100 Expert Software was used to digitalize the ARISA fingerprints, resulting in electrophorograms in ASCII formats which were processed using the StatFingerprints package (Michelland RJ et al. 2009), in the 2.13.0 version of the R project (The R Foundation for Statistical Computing, Vienna, Austria).

Results

1. Bacterial communities - Maize leaves

Fig. 1 shows a non-metric Multidimensional Scaling (NMDS) plot constructed from the bacterial ARISA community fingerprints of maize plant leaves exposed or not exposed to nanosilver particles (Ag-NPs).

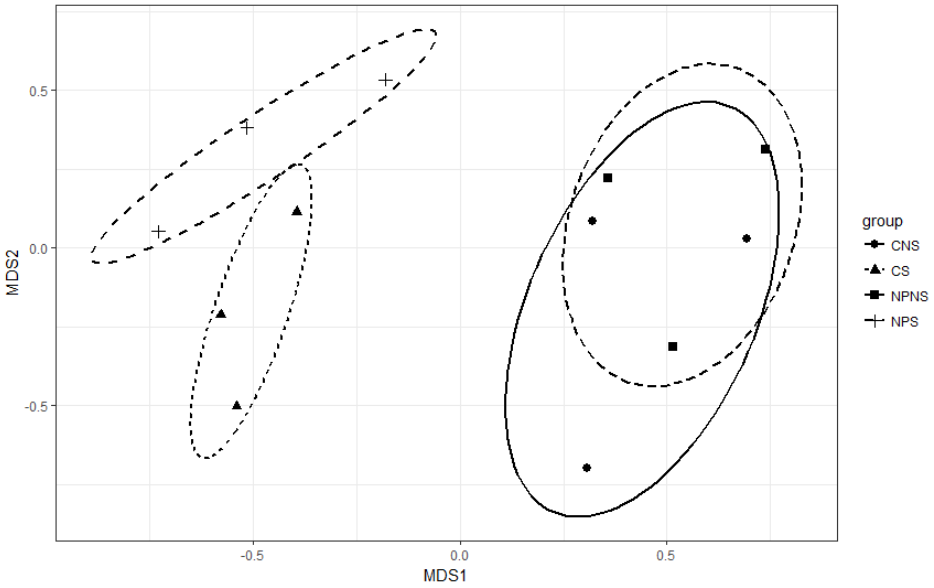

Fig. 1 Bacterial communities of leaves - NMDS plot

CNS = plants grown in control soil and not sprayed with silver nanoparticles; CS = plants grown in control soil and sprayed with silver nanoparticles; NPNS = plants grown in silver nanoparticle-containing soil and not sprayed with silver nanoparticles; NPS = plants grown in silver nanoparticle-containing soil and also sprayed with silver nanoparticles. Circles represent the 68% confidence interval per condition

The more separate the confidence intervals (circles) are for each treatment, the more different the microbial communities are in the specific sample type.

The analysis showed a clear distinction between the bacterial communities of the control (CNS) and those of the plants sprayed with Ag-NPs, in the two cases of soil treated (NPS) or not treated (CS) with the metal nanoparticles (NPs). No significant changes were detected between the bacterial communities of the control (CNS) and those of the plants grown in Ag-NP-containing soil and not sprayed with Ag-NPs (NPNS). On the basis of the obtained result, it seems that the addition of Ag-NPs into the soil did not have a significant impact on the bacterial diversity of maize leaves,

while the treatment with NPs by spraying resulted in significant changes. It was also possible to notice a slight separation between the two groups of plants treated by spray Ag-NPs (CS and NPS), which could be due to the synergistic effect produced by the double treatment with the metal NPs in soil and by spraying.

2. Bacterial communities - Maize roots

Fig. 2 shows an NMDS plot constructed from the bacterial ARISA community fingerprints of maize plant roots exposed or not exposed to Ag-NPs.

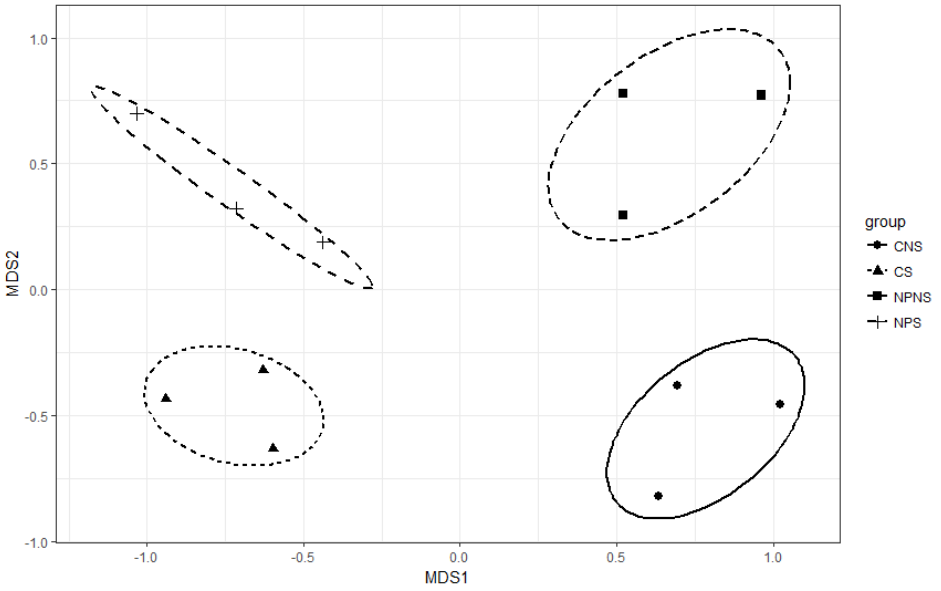

Fig. 2 Bacterial communities of roots - NMDS plot

CNS= plants grown in control soil and not sprayed with silver nanoparticles; CS = plants grown in control soil and sprayed with silver nanoparticles; NPNS = plants grown in silver nanoparticle-containing soil and not sprayed with silver nanoparticles; NPS = plants grown in silver nanoparticle-containing soil and also sprayed with silver nanoparticles. Circles represent the 68% confidence interval per condition

The NMDS plot showed a remarkable separation between the bacterial communities of the roots of maize plants treated with Ag-NPs and those of the control (CNS). It is clearly evident that both the addition of NP in the soil and/or by spraying (CS, NPNS and NPS sample types) influenced the composition of the bacterial communities in the roots. A noticeable separation was also observed between the sample types NPNS and CS, therefore the two treatments with Ag-NPs (added into the soil or sprayed) produced different effects on the bacterial diversity of maize plant roots. Moreover, the NPS treatment is the sample type which deviated more from the control, probably due to the synergistic effect of the treatment with NP in the soil and by spraying.

3. Bacterial communities - Maize stem

Fig. 3 shows an NMDS plot constructed from the bacterial ARISA community fingerprints of maize plant stems exposed or not exposed to Ag-NPs.

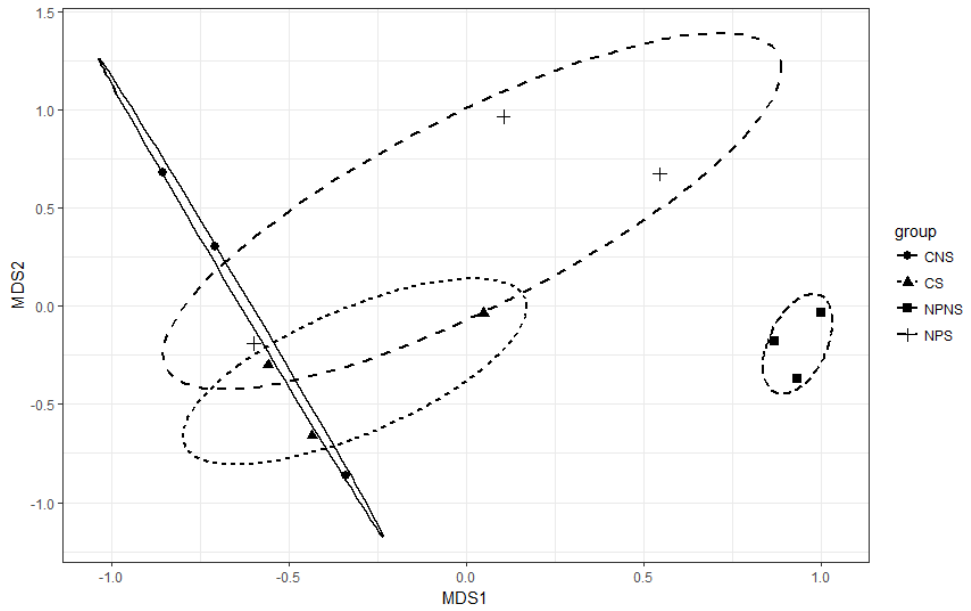

Fig. 3 Bacterial communities of stems - NMDS plot

CNS= plants grown in control soil and not sprayed with silver nanoparticles; CS = plants grown in control soil and sprayed with silver nanoparticles; NPNS = plants grown in silver nanoparticle-containing soil and not sprayed with silver nanoparticles; NPS = plants grown in silver nanoparticle-containing soil and also sprayed with silver nanoparticles. Circles represent the 68% confidence interval per condition

The best separation compared to the control (CNS) occurred with the plants grown in Ag-NP - containing soil and not sprayed with NP (NPNS). A difference was also observed between the control (CNS) and the double Ag-NP treatment (NPS). A slight separation could be also distinguished between the control (CNS) and the plants which were treated with NP by spraying only.

4. Fungal communities - Maize leaves

Fig. 4 shows an NMDS plot constructed from the fungal ARISA community fingerprints of maize plant leaves exposed or not exposed to Ag-NPs.

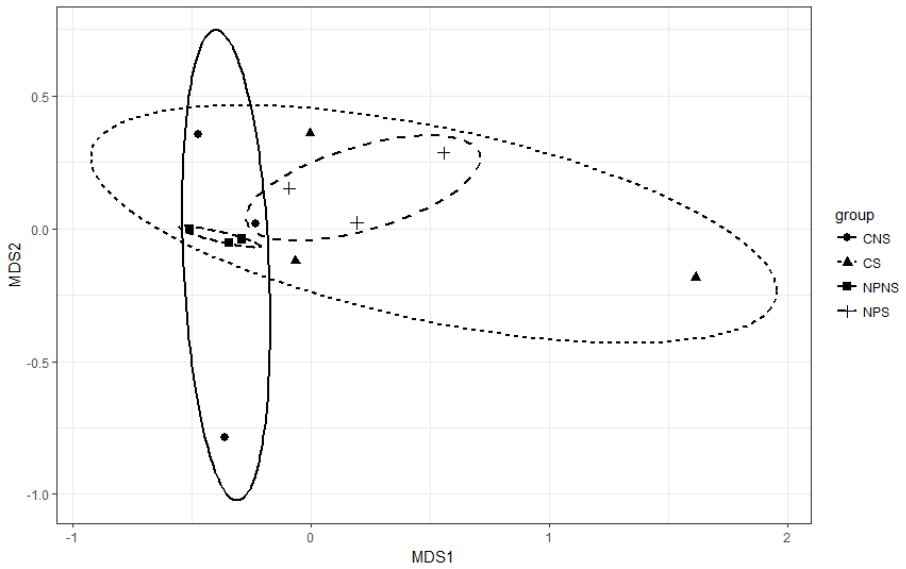

Fig. 4 Fungal communities of leaves - NMDS plot

CNS= plants grown in control soil and not sprayed with silver nanoparticles; CS = plants grown in control soil and sprayed with silver nanoparticles; NPNS = plants grown in silver nanoparticle-containing soil and not sprayed with silver nanoparticles; NPS = plants grown in silver nanoparticle-containing soil and also sprayed with silver nanoparticles. Circles represent the 68% confidence interval per condition

As was the case for bacteria, a difference was noticeable between the control (CNS) and the two treatments with spray NPs (CS and NPS). The separation between the control (CNS) and the plants grown in Ag-NP -containing soil and not sprayed with NP (NPNS) was less evident. As for the bacterial communities, also for the fungal communities of the leaves the treatment with spray NPs produced the strongest effects.

5. Fungal communities - Maize roots

Fig. 5 shows an NMDS plot constructed from the fungal ARISA community fingerprints of maize plant roots exposed or not exposed to Ag-NPs.

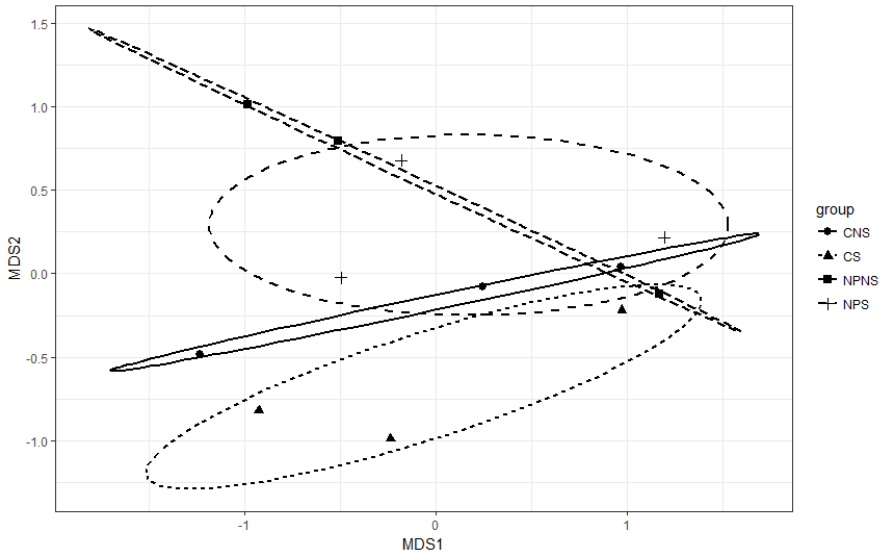

Fig. 5 Fungal communities of roots - NMDS plot

CNS= plants grown in control soil and not sprayed with silver nanoparticles; CS = plants grown in control soil and sprayed with silver nanoparticles; NPNS = plants grown in silver nanoparticle-containing soil and not sprayed with silver nanoparticles; NPS = plants grown in silver nanoparticle-containing soil and also sprayed with silver nanoparticles. Circles represent the 68% confidence interval per condition

As for bacteria, also the fungal communities were influenced by both Ag-NP treatments (NPs added into the soil and sprayed). However, the differences were less evident than the bacteria. Moreover, it was possible to notice a difference between the two treatments with Ag-NPs mixed in the soil (NPNS and NPS) and the one treated by only spray NPs (CS).

6. Fungal communities - Maize stem

Fig. 6 shows an NMDS plot constructed from the fungal ARISA community fingerprints of maize plant stems exposed or not exposed to Ag-NPs.

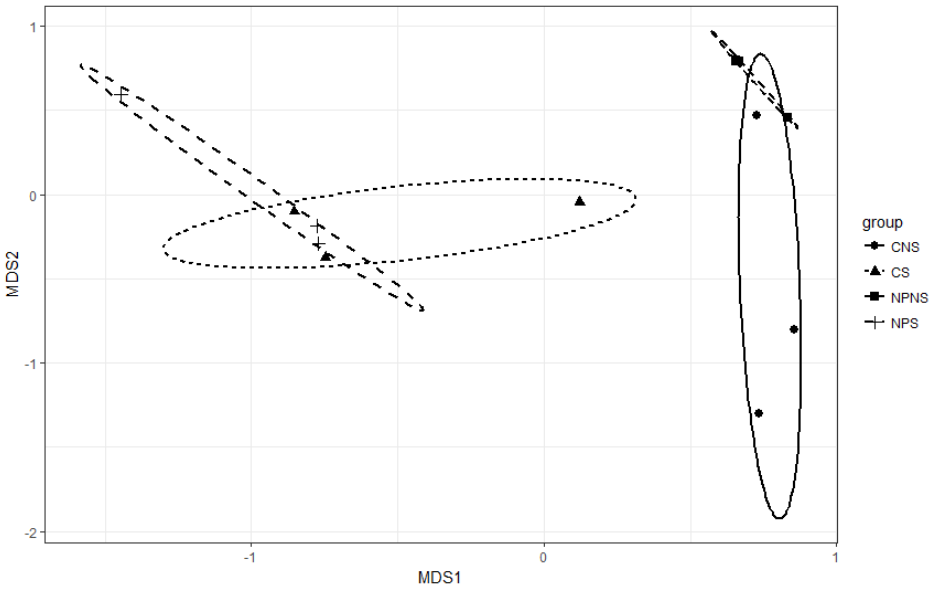

Fig. 6 Fungal communities of stems - NMDS plot

CNS= plants grown in control soil and not sprayed with silver nanoparticles; CS = plants grown in control soil and sprayed with silver nanoparticles; NPNS = plants grown in silver nanoparticle-containing soil and not sprayed with silver nanoparticles; NPS = plants grown in silver nanoparticle-containing soil and also sprayed with silver nanoparticles. Circles represent the 68% confidence interval per condition

In this case, the clearest differences compared to the control (CNS) were recorded for the fungal communities of the plants treated with Ag-NPs applied by spraying (CS and NPS). The largest separation was registered for the samples treated with both NP treatments (NPS).

Conclusions

Based on the obtained results and the consequent analysis with NMDS plots, it was possible to assess that the application of Ag-NPs affected the maize bacterial and fungal diversity in leaves, roots and stems. In particular, it is possible to generalize that spraying mainly affected the communities in stem and leaves, while the addition of Ag-NPs into the soil mainly affected the root communities.

Moreover, a different response to the Ag-NP treatments was observed for bacteria and fungi; bacteria generally were more significantly affected, while fungi were more resilient.

References

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