Metallic Nanoparticles: Differential impact on Fungal vs Bacterial Soil Communities

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Summary

This study investigated the impact of metallic nanoparticles (NPs) containing copper, silver, copper oxide, and zinc oxide, recognized as potential pollutants, on the structural and compositional aspects of soil microbial communities in comparison to their bulk counterparts. The influence of these nanoparticles was examined at two distinct accumulation levels within the soil ecosystem. High-throughput sequencing of PCR-amplified 16S rRNA and ITS2 marker genes was employed to analyze the impact of NPs and counterparts on bacterial and fungal rhizospheric communities using two dosage levels. Bioinformatic analysis of the obtained sequencing results revealed a distinct metal-dependent differentiation in bacterial and fungal soil community structures. Silver-containing treatments exhibited an enhanced ability to induce changes in both bacterial and fungal communities compared to other metals. Furthermore, treatment dose had a profound differentiation effect on the two microbial communities. The low dose notably influenced bacterial communities to a greater extent compared to the high dose, whereas fungal communities exhibited significant alterations under high-dose conditions rather than under low-dose condition

Methodology

The potential effects on soil microbial communities of metallic nanoparticles in comparison to their bulk counterparts were evaluated in a pot experiment under controlled environmental conditions. The influence of these nanoparticles was examined at two distinct accumulation levels within the soil ecosystem. Bacterial and archaeal 16S rRNA genes were amplified with the primer set 515f - 806r which targets the V4 region of the 16S SSU rRNA. Amplification of ITS was performed with the primers ITS7f - ITS4r. The 16S rRNA gene and the ITS region amplicons were sequenced via HiSeq Illumina Rapid Mode generating 2 × 250 bp paired-end read.

The alpha – diversity indices for community richness (observed ASVs or OTUs), diversity (Shannon) and phylogenetic diversity (Faith's PD), were determined with the phyloseq package (McMurdie and Holmes, 2013) on rarefied ASV tables. The Kruskal-Wallis test ($p < 0.05$) was used to investigate the effects of applied treatments on alpha – diversity indices followed by Dunn's test of multiple comparisons.

Differences of the beta–diversity of prokaryotes and fungi according to the applied treatments were visualized with PCoA based on the weighted UniFrac for prokaryotic community and Bray-Curtis dissimilarity for the fungal community. Permutational multivariate analysis of variance (PERMANOVA; adonis function, in the vegan package with 999 permutations, Wagner, 2018) was applied to assess the effect of applied treatments on the beta–diversity.

Conclusions

For both doses of nanoparticles prokaryotic communities were affected by the metal ions regardless the formulation. Conversely, fungal communities are affected differently depending on whether the applied ions are in the form of nano or not.

Figure 1

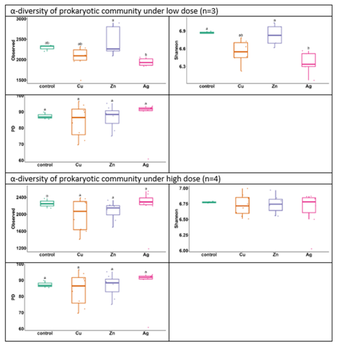


Figure 2

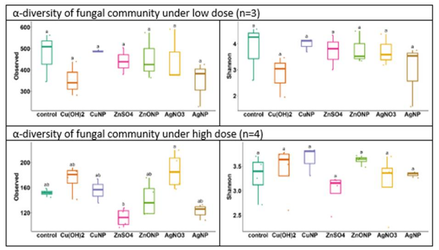


Figure 3

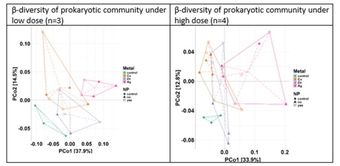


Figure 4

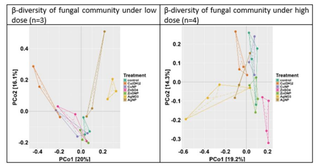
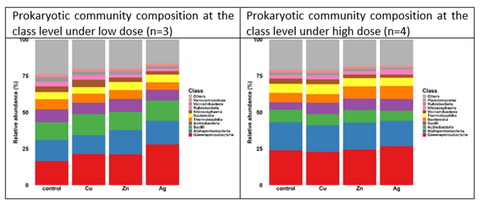


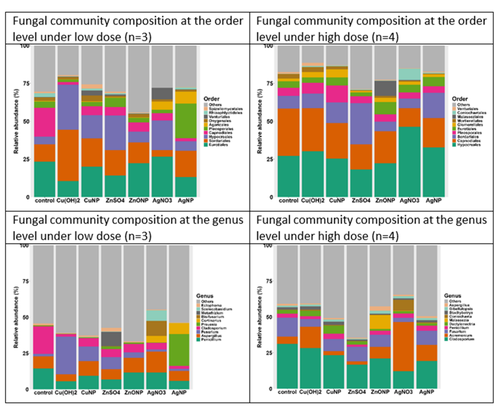
Table 1: Explained variation of the microbial community dissimilarities based on PERMANOVA analysis. For prokaryotic community dissimilarities calculated with weighted UniFrac metric, while for fungal community dissimilarities calculated with Bray-Curtis metric

	Low dose		High dose	
	Prokaryotic community	Fungal community	Prokaryotic community	Fungal community
Metal identity	36%***	26%***	30%***	26%***
Formulation	6.4%ns	6.4%*	3.9%ns	5.7%**
Interaction	9.4%ns	12.3%*	10%ns	14%***

α-diversity

The application of Ag, regardless formulation, nano material or not, decreased the α-diversity of the prokaryotic community compared to control soil, for observed number of species and Shannon index under low dose only (Fig. 1). For fungal community, at high dose only, ZnSO4 application decreased the observed number of species compare to control while AgNO3 increased the observed number of species compare to control (Fig. 2).





β-diversity

PCoA visualization and PERMANOVA analysis attributed the differentiations on β-diversity patterns of the prokaryotic community, based on the phylogenetic metric weighted UniFrac, on metal identity (Cu, Zn or Ag) and formulation was not significant in explaining any dissimilarity in the communities for both doses (Fig. 3, Table 1). Variations on β-diversity patterns, based on the Bray–Curtis dissimilarity metric, of the fungal community were quite similar for both application doses. In both doses nano formulated agrochemicals resulted in more similar to their control, untreated rhizosphere, (highly overlapping polygons) with that to be more profound in high dose. On the other hand, salt and hydroxide forms of the agrochemicals, had a stronger effect on fungal community that led to a clear separation of samples from these treatments for both application doses (Fig. 4). PERMANOVA showed that metal identity (Cu, Zn or Ag) had the highest effect among the main factors on the beta-diversity of the fungal community for both doses, followed by formulation, nano or not (Table 1)

Community composition

Prokaryotic community

For both doses prokaryotic community at the class level consists of γ- and α- *proteobacteria*, *Bacilli, Actinobacteria, Thermoleophilia*, *Bacteroidia* and the ammonia oxidizing archaea *Nitrososphaeria*. At low application dose, γ- proteobacteria increased in all treatments, while in high dose, *Bacilli* increased in all treatments. *Nitrososphaeria* increased at Cu treatment and decreased in Zn and Ag treatments for both doses. Cu is an essential cofactor for ammonia monooxygenase and is also essential for several electron carriers in ammonia-oxidizing archaea.

Fungal community

At the low dose, control soil was dominated at the order level by *Eurotiales, Sordariales, Hypocroales, Capnodiales and Pleosporales* and at the genus level by Penicillium, Aspergillus, Fusarium and Cladosporium. At the genus level, all treatments decreased Penicillium and Cladosporium. Fusarium was increased in Cu and Zn applications, regardless formulation. At the high dose, control soil was dominated at the order level by *Hypocroales, Capnodiales, Sordariales, Pleosporales* and *Eurotiales* and at the genus level by *Cladosporium, Acremonium, Fusarium, Penicillium*, and *Dactylonectria*. At the genus level, all treatments decreased *Cladosporium* and *Fusarium*.

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