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Soil Fungal Community Responses to the Silver Nanoparticles Contamination as Assessed by Illumina Next Generation Sequencing (NGS)

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Abstract

The increasing use of silver nanoparticles (AgNPs) due to its excellent antimicrobial activity in commercial products prompting concerns about their fate in the environment. The toxicity of AgNPs is mainly the result of Ag^+ ions. In this study, soil was experimentally contaminated with 100 mg kg^{-1} of $AgNO_3$ to investigate its effect on fungal soil community. Deoxyribonucleic acid (DNA) from the soil was extracted at the 6th, 12th, and 24th month of observation and assessed by Illumina Next Generation Sequencing (NGS). The results show that, the pollutant change fungal community in soil. After 12 months incubated the number of fungal species in the soil reduced significantly and 40% of the community was dominated by one species.

Keywords: AgNPs, $AgNO_3$, fungal community, soil contamination.

1. Introduction

Nowadays, nanoparticles are subject to intense scientific research, due to a wide variety of potential applications in many fields, from textile to electronics industries. Different kinds of nanomaterials are employed to fulfil the purposes of each industry. For example, silver nanoparticles (AgNPs) are used due to its known anti-microbial properties. AgNPs are applied in many everyday products, such as sunscreen, laundry detergent, kitchen utensils and children's toys. Furthermore, researchers suggested the use of AgNPs to control plant pathogenic fungus (Kim *et al*., 2012). The increased manufacture, marketing and use of AgNPs containing household and personal care products is prompting concerns about their fate in the environment.

The properties of the AgNPs can be modified when they interact with the soil environment. As a result, the interaction may change AgNPs stability, availability, and toxicity to organisms (Stone *et al*., 2010; Cornelis *et al*., 2012; Coutris *et al*., 2012; Levard *et al*., 2012; Tourinho *et al*., 2012; Benoit *et al*., 2013). AgNPs have been shown to disrupt the denitrification process by *Bradyrhizobium canariense* (Kumar *et al*., 2014). A similar observation was made by Calder *et al*. (2012) during their research on antimicrobial effect of AgNPs towards beneficial soil bacterium, *Pseudomonas chlororaphis*. Microbes in the soil play important role in soil that any reduction in soil microbial composition results in low soil quality and plant productivity.

There are few publications on the effect of AgNPs on soil fungi and the work carried out has been of short duration (Kim *et al*., 2012; Gavanji *et al*., 2012; Sillen *et al*., 2015; Sweet and Singleton, 2015). Accordingly, the present experiment was carried out for longer duration exposure time (24 months). According to Beer *et al*. (2011), the toxicity of AgNPs is mainly the result of Ag^+ ions. Therefore, another type of silver, silver nitrate ($AgNO_3$), was used in the experiment to examine the soil fungal community at the 6th, 12th, and 24th month of observation and assessed by Illumina Next Generation Sequencing (NGS).

2. Methodology

2.1. Materials

Soil samples (at a depth of 0-14 cm) were obtained from the east headland of Cockle Park Farm, Northumberland, UK. $AgNO_3$ in powder form with 99% purity obtained from Sigma-Aldrich Co. LLC. Fungal DNA from soil were extracted using PowerSoil® DNA isolation kit (MO BIO laboratories, USA). Illumina Next Generation Sequencing (NGS) and processed using UPARSE pipeline to assess the soil fungal community.

2.2. Experimental setup

Soil samples were air dried for 48 h prior to being sieved to 2 mm. Five gram of the soil was mixed thoroughly with 100 mg kg^{-1} of $AgNO_3$. The concentration used in this study represented concentrations suggested for application to control pathogens in agriculture. Control soils were treated identically but without Ag addition. All experiments were carried out in triplicate. All soils were kept in 15 ml conical centrifuge tubes with filter paper as the lids (the paper was pierced using needle to allow airflow) and weighted before being incubated at 25 ^oC. The tubes weight was kept the same over the duration of the experiment by adding water to maintained water capacity in the soil. DNA from the soil was extracted at 6^{th}, 12^{th}, and 24^{th} month using the PowerSoil® DNA isolation kit with slight modifications and sent for sequencing. The DNA extracts were assessed by Illumina Next Generation Sequencing (NGS).

2.3. Soil fungal community assesment

To study fungal communities in soil the data from Illumina Miseq sequencer were processed using single end forward reads following the UPARSE pipeline using USEARCH version 8.1.1756 sequence analysis tool (Edgar, 2013). The sequence quality of each sample was checked using FastQC version 0.11.4 and filtered using fastq_filter command as suggested by Edgar and Flyvbjerg (2015). The sequences number were counted using *grep -c ">" seqs.fa* command line to find the lowest number of sequences to use as sample size. All samples were analysed at the same sequence depth (647 sequences per sample) as this number was the lowest number of the sequences found in samples that didn't exclude a significant number of samples (in 12^{th} month sequences reading). At the end of the pipeline over 300 sequences of forward reads were successfully clustered into operational taxonomic unit (OTU) and assigned for taxonomy.

The taxonomy annotations were added to OTU sequence labels by using the utax command and Unite ITS1 Ver. 7 as the database. In the case the taxonomy assignment did not give satisfy identification, e.g. Fungi sp., the sequence (the output of OTU clustering file) was matched against NCBI GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.).

2.4. Analysis

All data presented are the mean value of three replicates. Values are expressed as means of three replicates ± standard error (S.E) in each group. All statistical analyses were performed using One-way Analysis of Variance (ANOVA) statistical models on Microsoft Excel 2016. Variance analysis was performed on all experimental data and significant differences ($P < 0.05$) between individual means (three replicates) was analysed using a post hoc Least Significant Difference test.

3. Results and Discussion

The changes in soil fungal communities can be assessed by looking at the fungal species diversity in the soil. Species diversity consists of two components; species richness and species evenness. Species richness and species evenness can be identified manually from the OTU table produced (not presented). Species richness is a simple count of species, whereas species evenness quantifies how equal the abundances of each species is.

3.1. Fungal Species Richness

Figure 1 shows the number of fungal species in control soil and in $AgNO_3$ contaminated soil at 100 mg kg^{-1}. At the first six months of incubation time the fungal species number in both control and contaminated soil is insignificant. Fungal species richness in control soil reach a peak after 12 months incubated and decrease insignificantly one year later. Meanwhile, the fungal species number in $AgNO_3$ contaminated soil reduced substantially after being incubated for 12 months and remained steady on the last sampling time.

The reduction in the number of fungal species with the addition of $AgNO_3$ in the soil indicates that silver nanoparticles is toxic to soil fungal species. Batista *et al*. (2017) reported that silver nanoparticles inhibited fungal reproduction and diversity. The species that presence in contaminated soil is consider as tolerant. There are several factors that influence the ability of fungi to survive in the presence of potentially toxic metals. For example, researchers revealed that the survival of fungal species at high metal concentration involved several mechanisms such as extracellular protection, by preventing metal entry into the cell and intercellular, by reducing the heavy metal burden (Anahid *et al*., 2011).

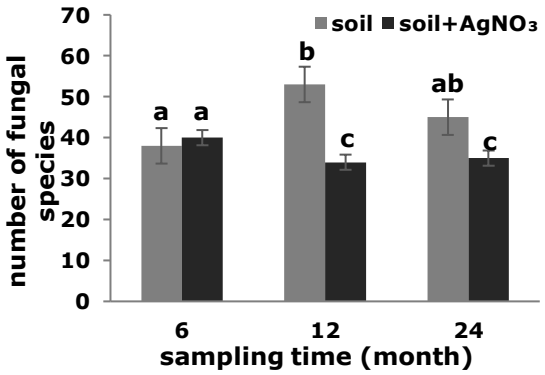

Figure 1. The number of fungal species in control soil and with the application of $AgNO_3$ at 100 mg kg^{-1}. The soils were incubated at 25 °C and sampled at 6, 12, and 24 months after being contaminated. Data represent means of three replicates with standard error. Different letters above the data points indicate significant difference between treatments in one sampling time at the level of $P < 0.05$

3.2. Fungal Species Evenness

Figure 2 indicates the percentage soil fungal relative abundance in control soil. The DNA from the soil were sampled at 6th, 12th, and 24th months after incubated at 25°C.

At the 6th month observation fungal species abundance in control soil is more than 35% of the community is dominated by one species (OTU 3, *Cryotococcus terreus*). The fungal species evenness is altered in month 12th as the percentage of relative abundance among the species found was more even as the curve is sloping. Furthermore, at the 24th month of observation control soil showed the 'best' species evenness curve as there was less domination by one species in the community.

On the other hand, the percentage of relative abundance of each species in $AgNO_3$ contaminated soil at the month 6th is relatively evenly distributed (Figure 3). However, longer term of $AgNO_3$ exposure decreased fungal species evenness in soil. After 12 months incubation 40% of the community was dominated by one species (OTU 1, *Hypocreaceae* sp.). The same species (indicated by same OTU identification) still dominated the community at the 24th month sample time.

From the study it can be seen that species evenness in the soil control is improving with the time while the converse happened in the $AgNO_3$ contaminated soil.

The results of this study indicated that the application of $AgNO_3$ change the fungal community structure in soil. A similar conclusion has been reached that metal nanoparticles indeed change the composition of soil microbial communities (Hänsch and Emmerling, 2010; He *et al*., 2011; Ge *et al*., 2012; Nogueira *et al*., 2012; Shah *et al*., 2014). Others have reported that soil contamination with AgNPs reduces ectomycorrhizal diversity found in bishop pine root (Sweet and Singleton, 2015). Fungal communities in soil are also affected by other types of heavy metal pollutant. For example, zinc and cadmium were strongly correlated with alteration of the fungal community composition (Beeck *et al*., 2015).

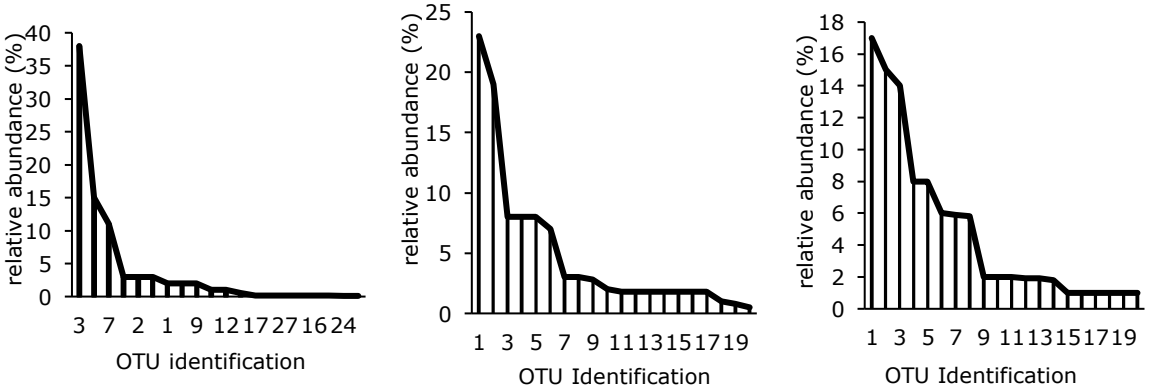

Figure 2. Relative abundance of soil fungi community in soil control sampled at the 6th, 12th, and 24th month, respectively

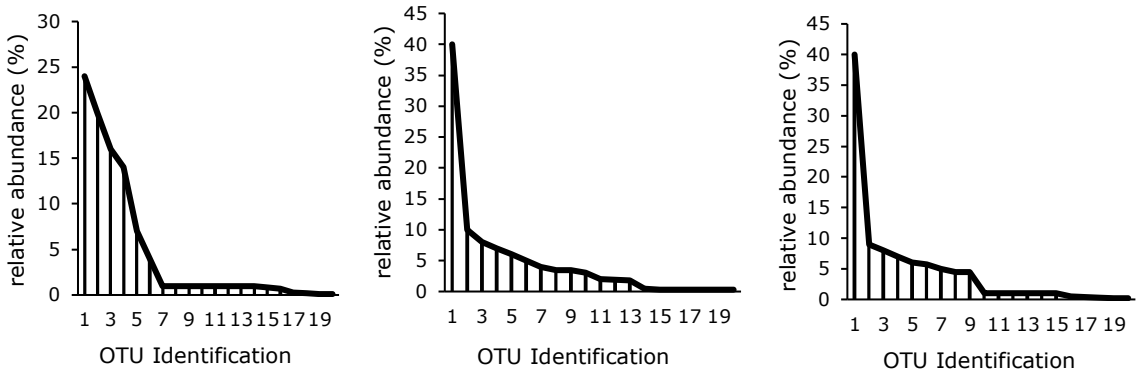

Figure 3. Relative abundance of soil fungi community in $AgNO_3$ contaminated soil sampled at the 6th, 12th, and 24th month, respectively

The study also revealed that sensitivity to Ag is different among fungal genera. Some are more sensitive to silver pollution than others. It has been reported that certain fungi such as *Hypocreales* fungi are abundant in soil treated with the high Ag concentration (Kumar *et al*. 2014). Bacterial communities are more affected by AgNPs as their composition is significantly modified by nanosilver exposure (Sillen *et al*., 2015; Carbone *et al*., 2014). A study confirmed that plant-associating bacteria, *Bradyrhizobium canariense*, appeared to have a marked sensitivity to AgNPs (Kumar *et al*, 2014), showing that the variation in sensitivity to metal pollution shown by fungi is consistent with that seen in other kingdoms.

4. Conclusion

The addition of silver in form of $AgNO_3$ in soil changes the fungi soil community that showed by the reduction in fungal species richness and evenness. Any change in the community has the potential to affect soil decomposition processes, nutrient cycling and finally soil quality and future studies should examine these soil functions. Intentional silver application, for example to control plant pathogenic fungi in agriculture, should be re-considered as it will boost the level of metal pollutant in soil and lead to potentially deleterious effects. As for the future works, the tolerant species can be considered as mycoremediation to remove Ag pollutant in soil.

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