Journal of Environmental Management 253 (2020) 109584

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Evidence for positive response of soil bacterial community structure and functions to biosynthesized silver nanoparticles: An approach to conquer nanotoxicity?

Sandhya Mishra[a], Xiaodong Yang[a,**], Harikesh Bahadur Singh[b,*]

[a] CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Yunnan, 666303, China
[b] Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, 221005, India

ARTICLE INFO

Keywords:
Silver nanoparticles
Functional genes
Bacterial community
Soil
Toxicity
Ecosystem functions

ABSTRACT

The environmental impacts of biosynthesized nanoparticles on the soil bacterial community assemblage and functions are not sufficiently understood. Given the broad application of silver nanoparticles (AgNPs), the present study aims to reveal the effects of biosynthesized AgNPs (~12 nm) on the soil bacterial community structure and functions. Specifically, we used a quantitative real-time PCR (qPCR) approach to quantify the relative abundance of bacterial taxon/group and representative functional genes (*AOA, AOB, NirK, NirS, NosZ,* and *PhoD*). Results showed high relative abundance of *Actinobacteria* (1.53×10^7, $p = 0.000$) followed by *Alphaproteobacteria* (1.18×10^6, $p = 0.000$) and *Betaproteobacteria* (2.01×10^6, $p = 0.000$) in the soil exposed to biosynthesized AgNPs (100 mg/kg soil) after 30 days of treatment. *Bacteroidetes* group was observed to be negatively affected by AgNPs treatment. In the case of functional genes abundance, more pronounced impact was observed after 30 days of application. The biosynthesized AgNPs treatment accounted for significant increase in the relative abundance of all targeted functional genes except *NirS*. We conclude that the biosynthesized AgNPs did not cause toxic effects on nitrifiers, denitrifiers and organic phosphorus metabolizing bacterial community. While $AgNO_3$ caused higher toxicity in the soil bacterial community structure and function. Based on our findings, we propose two key research questions for further studies; (i) is there any adaptation strategy or silver resistance embraced by the soil microbial community? and (ii) are biosynthesized nanoparticles environmentally safe and do not pose any risk to the soil microbial community? There is a necessity to address these questions to predict the environmental safety of biosynthesized AgNPs and to apply appropriate soil management policies to avoid nanotoxicity. Since this study provides preliminary evidence for the positive response of the soil bacterial community structure and functions to biosynthesized AgNPs, additional investigations under different soil conditions with varying soil physico-chemical properties are required to authenticate their environmental impact.

1. Introduction

The silver nanoparticles (AgNPs) have become increasingly popular due to their demand in multipurpose sectors such as medicine, electronics, cosmetics, energy, agriculture, etc. The recent scenario demonstrates a great impact of AgNPs on humankind owing to their inflated utilization in almost every field of daily life (Mishra and Singh, 2015a; Grün et al., 2018). Earlier reports suggest that about 320 tons/year of nanosilver are being produced worldwide (Gottschalk et al., 2010; Nowack et al., 2011). Considering the ever-increasing commercial demand of AgNPs, Sun et al. (2014) reported an annual increase of AgNPs concentrations in the soil from an initial value of 1.2 ng–23 ng/kg•year indicating a high rate of increase over time. AgNPs are known for extraordinary antimicrobial properties that add strength to their accelerating applications for beneficial purposes. With a unique combination of physical properties, ultra-small size and high surface to volume ratio, AgNPs embrace a great potential to kill a wide range of pathogenic microbes including fungi, viruses, bacteria, etc. (Shahrokh and Emtiazi, 2009; Reidy et al., 2013; León-Silva et al., 2016; Mishra et al., 2017b). Consequently, AgNPs are widely used in more than 1827 commercial products ranging from medical devices, medicines, cosmetics to home disinfectants (Consumer Products Inventory, 2015).

* Corresponding author.
** Corresponding author.
E-mail addresses: sandhya@xtbg.ac.cn (S. Mishra), yangxd@xtbg.ac.cn (X. Yang), hbs1@rediffmail.com, hbsbhu@bhu.ac.in (H.B. Singh).

https://doi.org/10.1016/j.jenvman.2019.109584
Received 2 June 2019; Received in revised form 9 September 2019; Accepted 15 September 2019
Available online 18 October 2019
0301-4797/

Accordingly, it is estimated that by 2025, the global production of AgNPs would be approximately 800 tons (Pulit-Prociak and Banach, 2016). The rapid upsurge in the synthesis and application of AgNPs has raised environmental safety concerns, the most critical issue of the present time (Ge et al., 2014).

Mounting evidence strongly suggests potential agricultural applications of AgNPs as efficient nanopesticides for controlling plant diseases (Mishra et al., 2014, 2016; 2017a; Singh et al., 2018). Taking into consideration the biocidal effects of AgNPs against a myriad of pathogens, it is inevitable to assume their potential risk to non-target microorganisms as well. Thus, it is very important to know the probable impact of AgNPs on the soil microbial community assemblages and their functional traits important for carrying out several soil ecological processes such as nutrient cycling, decomposition, xenobiotics degradation, etc. (Emmerling et al., 2002; Sillen et al., 2015; Mishra et al., 2019). The amount of work done so far shed light on the possible impact of AgNPs application on certain groups of the bacterial community. For instance, Gajjar et al. (2009) examined the toxic behavior of AgNPs against plant growth promoting *Pseudomonas putida* KT2440. Yang et al. (2013) found more sensitivity of *Nitrosomonas europaea* (nitrifier), *P. stutzeri* (denitrifier) and *Azotobacter vinelandii* (nitrogen fixer) to Ag^+ rather than AgNPs. Interestingly, at a lower concentration of 20–25 μg/L, no toxic effects were exerted by both Ag^+ and AgNPs on nitrogen cycling related gene expression patterns. Likewise, Shahrokh et al. (2014) also observed non-toxic effects of AgNPs on nitrate assimilation process of *Rhizobium* and *Azotobacter* at low concentration of 0.2 ppm as nitrate reductase activity remains unaffected. Interestingly, size dependent toxicity was studied by Choi and Hu (2008) indicating higher toxicity of 5 nm AgNPs on the nitrifying bacteria. Recently, Dhas et al. (2014) detected toxic behavior of AgNPs on selected bacterial strains *viz. Micrococcus, B. barbaricus*, *K. pneumonia, B. subtilis* and *P. aeruginosa*, isolated from sewage and soil, under *in vitro* conditions. In addition to this, they also observed development of AgNPs resistant behavior in the bacterial strains after continuous exposure. Altogether, these findings indicate the underlying toxic behavior of AgNPs and their expected consequences on the soil microbial processes. However, the probable environmental risk posed by AgNPs applications is still not well understood due to limitations in approach and research design using an unrealistic environment. In this context, few studies have examined the interactions between soil physico-chemical properties and AgNPs toxicity. For instance, some studies suggested inhibitory impact on soil microbial biomass (Hänsch and Emmerling, 2010), soil exoenzyme activities (Shin et al., 2012), bacterial community structure and soil respiration (Colman et al., 2013; Chunjaturas et al., 2014). Alternatively, other reports also highlighted the key role of soil properties such as organic matter, pH, cation exchange capacity, etc. in circumventing risk factors of AgNPs (Calder et al., 2012; Schlich and Hund-Rinke, 2015). Most importantly, few interesting studies have identified the potential role of polyvinylpyrrolidone coated AgNPs (PVP-AgNPs) for improving the quality and agricultural utility of sewage sludge *via* co-composting with agricultural wastes (Zhang et al., 2017, 2018). Zhang et al. (2017) demonstrated that the treatment of PVP-AgNPs enhanced the overall fertility of compost by reducing loss of organic matter and total nitrogen during co-composting process. The probable mechanisms for PVP-AgNPs mediated reduction in total nitrogen loss in compost were found to be modulation of nitrifying and denitrifying bacterial genes abundance (Zeng et al., 2018). Recently, the authors provided evidence to show the promising correlation between bacterial genes abundance and physico-chemical properties such as pH, water soluble organic carbon, total carbon & nitrogen and available forms of nitrogen (NH_4^+-N, NO_3^--N) during composting process. This study identified pH, total N and NO_3^--N as key drivers of nitrifying and denitrifying genes abundance (Zhang et al., 2018).

It is important to note that AgNPs obtained from non-biological sources have been extensively studied in the majority of the reports as mentioned above. As a result, our knowledge and understanding of the actual environmental impacts of biosynthesized AgNPs on the soil bacterial community functions are not sufficiently understood. The green synthesis of nanoparticles using plants, microbes and natural biomolecules provides advancement over chemically synthesized nanoparticles by overcoming the toxic effects imposed by toxic chemicals used during the synthesis process (Roy et al., 2013; Kharissova et al., 2013; Das et al., 2017). In view of the ongoing debate on the environmental safety concerns of nanoparticles, the toxicity assessment of biosynthesized nanoparticles is urgently required. Therefore, the present study as an extension of our previous report (Mishra et al., 2017b), aims to reveal the significant impact of biosynthesized AgNPs using *Stenotrophomonas* sp. BHU–S7 (MTCC5978) on the soil bacterial community functions by quantifying important functional genes. Functional genes in the microbial genome are the basic unit linking the microbial community to ecosystem functions *via* mediating synthesis of enzymes catalyzing key steps in biogeochemical pathways (Jackson et al., 2003; Trivedi et al., 2013; Rocca et al., 2015). Hence, soil biogeochemical processes are greatly influenced by the abundance of a particular functional group of microbes within the community and their study can reveal the underlying biological dynamics. Therefore, disentangling the potential impact of biosynthesized AgNPs on the functional role of the soil microbial community is the ecological indicator for determining their environmental toxicity. As a first step towards understanding the environmental compatibility of biosynthesized AgNPs, we think that measuring the relative abundance of functional genes is the best suited approach. Moreover, this preliminary investigation would help us to draw some conclusions regarding the toxicity behavior and biosafety concerns associated with biogenic AgNPs usage and consequences for fundamental ecosystem processes.

2. Materials and methods

2.1. Bacterial strain, identification and culture conditions

Isolation and identification procedure has already been described in our previous study (Mishra et al., 2017b). Briefly, we isolated this bacterial strain from agricultural farm soil and further identified using 16S rDNA sequencing approach. The pure culture of this strain was grown on Nutrient agar culture media. This bacterial culture has been deposited at MTCC (Microbial Type Culture Collection), IMTECH, Chandigarh under the deposition number MTCC 5978.

2.2. AgNPs biosynthesis and characterization

The biosynthesis procedure of AgNPs using *Stenotrophomonas* sp. BHU–S7 culture supernatant has been explained in detail in our previous study (Mishra et al., 2017b). Furthermore, biosynthesized AgNPs were characterized for structural, functional, elemental, optical, etc. properties using different analyzers. The details of analyses have already been documented in our previous article.

2.3. Impact of biosynthesized AgNPs on seed germination

The seed germination assay was performed using okra and wheat as host plants. The seeds were surface sterilized using 0.1% $HgCl_2$ and rinsed ten times with sterile distilled water under aseptic conditions. After this seeds were soaked in diluted AgNPs suspension (1:1 dilution in sterile distilled water) for 1 h. Further, seeds were kept on the Petri dishes containing moist blotting sheet (moist chamber) for germination at room temperature. Seeds soaked in water served as control. Three replicates were maintained for each set.

2.4. Experimental setup

The topsoil (0–20 cm depth) sample was collected from the agricultural field located at Institute of Agricultural Sciences, Banaras

Hindu University campus, cultivated with vegetable crops. The soil texture was characterized as sandy loam with a pH of 7.38, bulk density of 1.37, water holding capacity of 39.29, organic carbon content 3.77 g/kg, available nitrogen 228.37 kg/ha and available phosphorus content of 18.36 kg/ha. After sampling, soil was thoroughly sieved through 2 mm mesh and microcosm setup was prepared by measuring 25 g soil in a sterile Petri dish. In total, 18 microcosms (3 treatments x 3 replicates x 2-time interval) were prepared including 3 independent biological replicates for each treatment. Fresh stock of AgNPs suspension was prepared by dissolving in sterile milli-Q water using an ultrasonic bath for 5 min. Further, soil in the Petri dishes was moistened with water to maintain 15% moisture level and then exposed to one dose of 100 mg/kg of biosynthesized AgNPs. We selected one dose (100 mg/kg) of AgNPs for the soil treatment based on prior studies suggesting this as high concentration (Sillen et al., 2015; Simonin and Richaume, 2015; Wang et al., 2018). The total silver concentration is estimated to be highest in the soil environment as soil is the main sink for environmentally released nanoparticles. Therefore, we used this high dose to get a clue about possible toxicity (if any) of biosynthesized AgNPs. For soil treatment, 1 mL of AgNPs suspension was added homogenously onto the soil surface and mixed slowly for uniform distribution of AgNPs. In the control set, only water was added at the same moisture level while positive control set was maintained by adding same concentration of $AgNO_3$. After this, Petri dishes were incubated at room temperature under dark condition for 30 days. During this time period, moisture content was maintained by adding milli-Q water. Soil sample was collected from each biological replicate of each treatment after 15 and 30 days of incubation. The collected soil samples were immediately processed for soil DNA extraction.

2.5. DNA extraction and quality check

DNA was extracted from fresh soil (0.5 g) using PowerSoil® DNA Isolation Kit (MoBio, USA) following the manufacture's protocol. Soil DNA extraction was done independently for each of the biological replicates from each set of treatments to improve accuracy and to reduce DNA extraction bias (Lanzen et al., 2017). DNA quality was checked using 1.0% gel electrophoresis and quantified using NanoDrop 2000 (Thermo Scientific Nanodrop 2000).

2.6. PCR amplification and cloning

PCR was performed by using taxon-specific and functional gene specific primers listed in Table S1. The 25 μL reaction mixture contained 1.0 μL template DNA (~25 ng), 0.5 μL of each primer (10 μM), 12.0 μL Premix Taq (TaKaRa, Japan) and 11.0 μL ddH_2O. The PCR conditions were as follows: initial denaturation at 95 °C for 15 min (taxon-specific primers) and 5 min (functional gene specific primers) followed by 40 cycles of 95 °C for 1 min, 50–60 °C (specified annealing temperature, Table S1) for 30 s and 72 °C for 1 min; and final extension at 72 °C for 10 min. PCR products were checked on 1% (w/v) agarose gel by gel electrophoresis and further purified by using EAsyPure® PCR purification kit (TransGen Biotech, China). One PCR product (purified) for each primer set was cloned into pEasy-T1 cloning vector using pEasy®-T1 cloning kit (TransGen Biotech, China) and further transformed into Trans 1-T1 Phage Resistant Chemically Competent Cells (TransGen Biotech, China). Positive clones were picked using blue-white selection procedure and plasmid DNA was extracted from positive white clones using EAsyPure® Plasmid MiniPrep kit (TransGen Biotech, China). Plasmid DNA concentration was determined using Qubit® 2.0 (Invitrogen, USA).

2.7. Real-time quantitative PCR assay for estimating gene abundance

Gene abundance was estimated following the protocol as described earlier (Xiao et al., 2017; Yan et al., 2018). Prior to quantitative PCR (qPCR), same volume (25 μL) of DNA extracted from each biological replicate was pooled to give one DNA sample representative for each treatment and further used as template for qPCR. This DNA pooling method has been employed in several studies for qPCR estimation (Lammel et al., 2015; Wafula et al., 2015; Doolette et al., 2016). Firstly ten-fold serial dilutions of plasmid DNA were done to generate an external standard curve. All samples and standard DNA were subjected to qPCR using fluorescent quantitative PCR (model: LightCycler 480II, Roche, Switzerland) in triplicate. The qPCR reaction mixture (20 μL) consisted of 10 μL TransStart Top Green qPCR supermix (TransGen Biotech, China), 0.4 μL of each primer (10 μM), 1.0 μL of template DNA and 8.2 μL of ddH_2O. The qPCR was performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at a given temperature for 30 s and extension at 72 °C for 1 min. Non-template control was also maintained for each primer set. The specificity of qPCR products was examined by melt curve analysis and PCR efficiency ranged from 95.5% to 99.8% with R^2 value > 0.99. The target gene copy number was calculated from the standard curve.

2.8. Statistical analyses

All statistical analyses were conducted using SPSS 16.0 software package. All values represent mean ± standard deviation of three independent replicates. One-way analysis of variance (ANOVA) and posthoc Tukey HSD test were performed to test the significant differences of means between different treatments.

3. Results and discussion

3.1. Biosynthesis and characterization of AgNPs

The biosynthesis procedure of AgNPs using bacterial strain *Stenotrophomonas* sp. is very simple and rapid (Mishra et al., 2017b). Briefly, the visible color change in 1 mM $AgNO_3$ treated culture supernatant indicated positive reaction and synthesis of AgNPs which was further confirmed by UV–vis spectroscopy. The UV–vis spectrum showed surface plasmon resonance (SPR) peak at 440 nm that is specific to AgNPs (Fig. 1A). Previous studies suggest that absorbance range for AgNPs is in the visible range of 400–500 nm due to excitation of SPR (Chung et al., 2016; Zhang et al., 2016). Furthermore, the crystallinity of biosynthesized AgNPs was tested by X-ray diffraction (XRD) that showed face-centered cubic structure due to presence of 4 diffraction peaks at 2Θ angles of 38.39°, 49.25°, 64.10° and 77.87° (Fig. 1B). The average crystallite size, as calculated by Debye-Scherrer equation, was found to be 12.7 nm. Spherical morphology and agglomeration were further confirmed by transmission electron microscopy. The variable size was recorded in the range of 5–30 nm with mean size anticipated to be ~12 nm (Fig. 1C). The purity of biosynthesized AgNPs was estimated by EDAX analysis that showed a strong peak of the silver element at 3 keV which is characteristic of silver nanocrystallites (Scimeca et al., 2018). Notably, EDAX spectrum showed additional peaks representative of carbon and oxygen at 0.2 and 0.5 keV respectively (Fig. 2A). These additional peaks confirmed the possible role of bacterial biomolecules such as enzymes and protein for synthesis and stabilization of AgNPs. In our study, *Stenotrophomonas* sp. BHU–S7 culture supernatant is likely to contain a range of biomolecules that act as reducing and stabilizing agents during AgNPs biosynthesis process. The FTIR spectrum showed broad stretching peaks corresponding to amide, amine and carbonyl functional groups (Fig. 2B). This indicates potential contributions of enzymes and proteins present in *Stenotrophomonas* sp. BHU–S7 culture supernatant for the synthesis of AgNPs and their stability thereafter. Furthermore, TGA confirmed thermal stability of biosynthesized AgNPs as 37.87% weight loss occurred over the temperature range of 0–700 °C leaving behind 62.13% residual weight in two steps. The first step weight loss was recorded to be 9.91% at ~100–200 °C while second step weight loss (27.96%) was achieved at

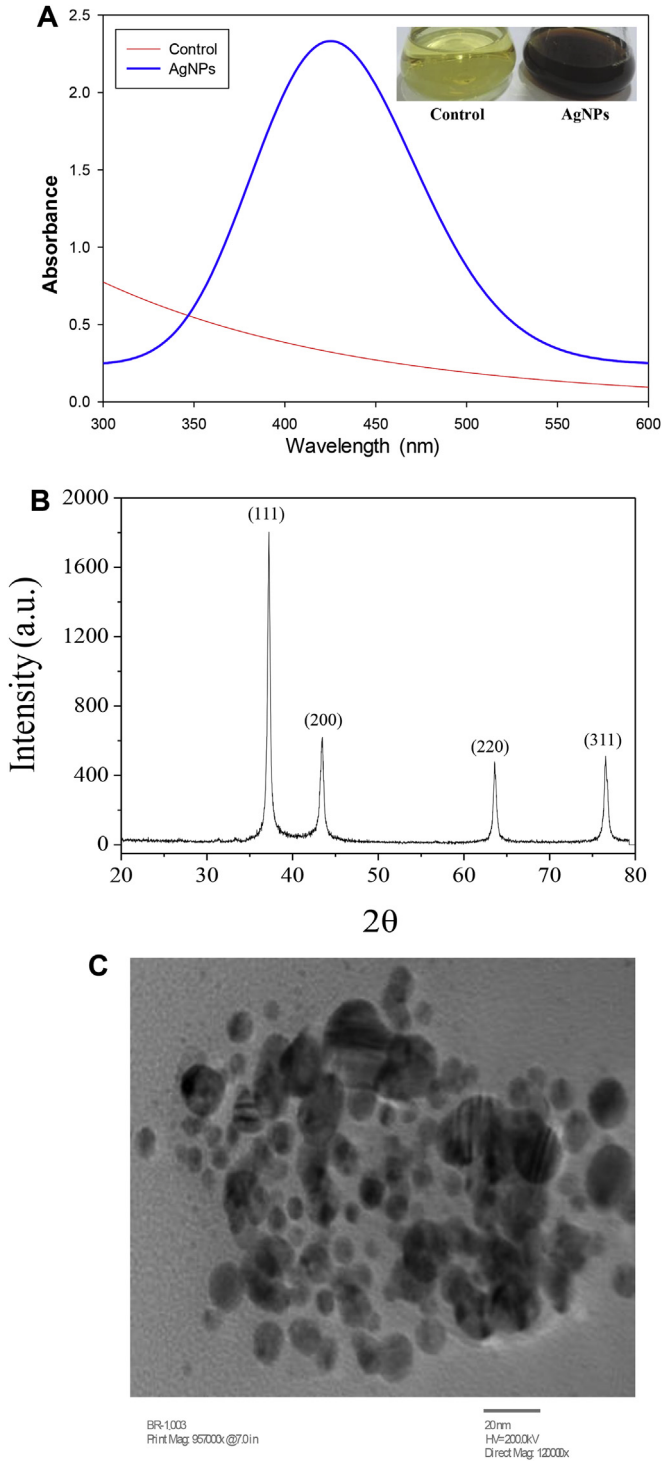

Fig. 1. (A) Biosynthesis of AgNPs (indicated by brown color formation) using *Stenotrophomonas* sp. BHU–S7 (inset picture). UV–vis spectrum of biosynthesized AgNPs showing SPR peak at 440 nm. (B) XRD spectrum and (C) transmission electron microscopy image of biosynthesized AgNPs. (Images reprinted from Mishra et al., 2017b, Sci. Rep. 7, 45154; this manuscript is published under the CC BY 4.0 license link: http://creativecommons.org/licenses/by/4.0/). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

high temperature range of ~300–450 °C (Fig. 2C). Altogether, these data provide enough evidence to show effective synthesis of AgNPs using *Stenotrophomonas* sp. BHU–S7 (MTCC5978).

3.2. Effect of biosynthesized AgNPs on seed germination

As a first step to understanding the environmental impact of biosynthesized AgNPs, seed germination assay was used to evaluate their effects on plant growth. As evident from Fig. 3, the application of biosynthesized AgNPs did not cause negative effects on seed germination. Interestingly, more stimulating effects of biosynthesized AgNPs treatment were observed on the okra plant where we observed enhanced seed germination and radicle length as compared to control. In the case of wheat, seed germination and radicle/plumule length were virtually equal in both control and AgNPs treated set indicating no inhibitory effects. Previously, a few studies have highlighted this beneficial effect of AgNPs synthesized using non-biological sources. For example, Mehta et al. (2016) detected improving effects of AgNPs application on wheat, cowpea and Brassica growth parameters. However, the stimulating effect was observed to be dose specific for different host plants. Likewise, dose-dependent positive effect of AgNPs treatment on *Brassica juncea* was also observed by Sharma et al. (2012). Sillen et al. (2015) also stated growth enhancing effects of AgNPs (100 mg/kg) on maize. In another experiment, Sadak (2019) noticed growth stimulating effects of AgNPs on fenugreek at 20 and 40 mg/L concentration. Based on our previous data and seed germination result in the current study, our findings underline the fact that biosynthesized AgNPs hold promising and robust applications in the agricultural sector, predominantly for plant growth and plant disease management (Mishra and Singh, 2015b; Mishra et al., 2016, 2018). Owing to their antifungal efficacy against foliar & soil-borne phytopathogenic fungi and plant growth stimulating effects, the on-field application is suggested in two ways i.e., soil drenching and plant spraying.

3.3. Impact of biosynthesized AgNPs on the structure of bacterial community in the soil

The high diversity and abundance of the bacterial community in the soil ecosystem can be estimated by the fact that 1 g of soil inhabits ~10^9-10^{10} cells (Fierer et al., 2007; Griffiths et al., 2016; Karimi et al., 2018). The importance of bacterial community for ecosystem multifunctionality has been well documented in several studies (Roger et al., 2016; Delgado-Baquerizo et al., 2016, 2018; Llado et al., 2017). Given the significance of the soil bacterial diversity in governing soil ecological processes, the probable impact of silver nanoparticles on the soil bacterial community needs to be understood. Following the on-field applications, AgNPs enter the soil environment and are likely to affect the residing microbial community. Hence, the main goal of the present study is to explore the potential consequences of biosynthesized AgNPs applications on the soil bacterial community structure by quantifying the relative abundance of important bacterial phyla using a qPCR approach. We used taxon-specific primers to target *Alphaproteobacteria, Betaproteobacteria, Actinobacteria* and *Bacteroidetes* bacterial phyla (Fierer et al., 2005). We selected these phyla due to their ubiquity and abundance in most soil types (Janssen, 2006; Lauber et al., 2009). Moreover, members of these dominant bacterial phyla are known for their significant role in organic matter decomposition, degradation of polysaccharides, carbon & nitrogen cycling, plant growth promotion, plant defense, etc. (Spain et al., 2009; Barka et al., 2016; Wolińska et al., 2017).

The obtained data suggest a noticeable difference in the abundance pattern of *Alphaproteobacteria, Betaproteobacteria, Actinobacteria* and *Bacteroidetes* after 30 days of application. The differential pattern in the gene copy number was observed after 15 days of application, which became more prominent after 30 days of application. *Alphaproteobacteria* group (gene copy number 2.67×10^6) was negatively affected by $AgNO_3$ application as compared to control (gene copy number 3×10^6) and AgNPs (gene copy number 3.09×10^6) treated set respectively, after 15 days of application. The inhibitory impact was found to be significant ($p = 0.019$) whereas a slight increase in the gene

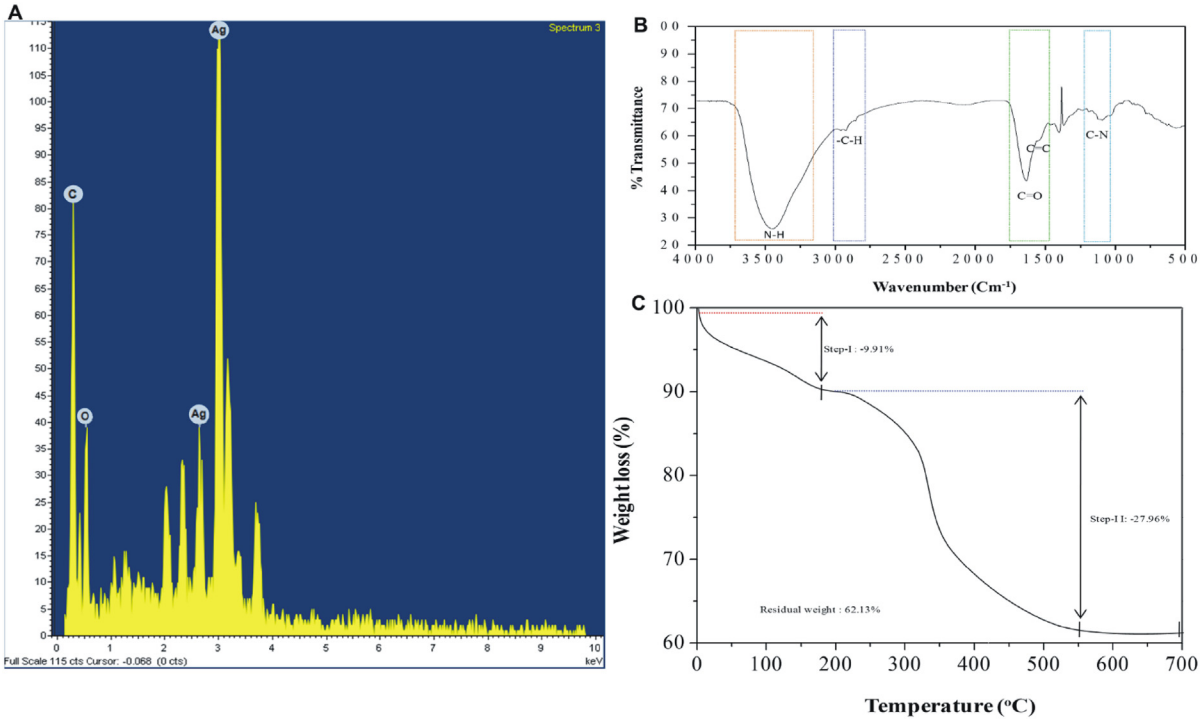

Fig. 2. EDAX (A), FTIR (B) and TGA (C) profiling of biosynthesized AgNPs. (Images reprinted from Mishra et al., 2017b, Sci. Rep. 7, 45154; this manuscript is published under the CC BY 4.0 license link: http://creativecommons.org/licenses/by/4.0/).

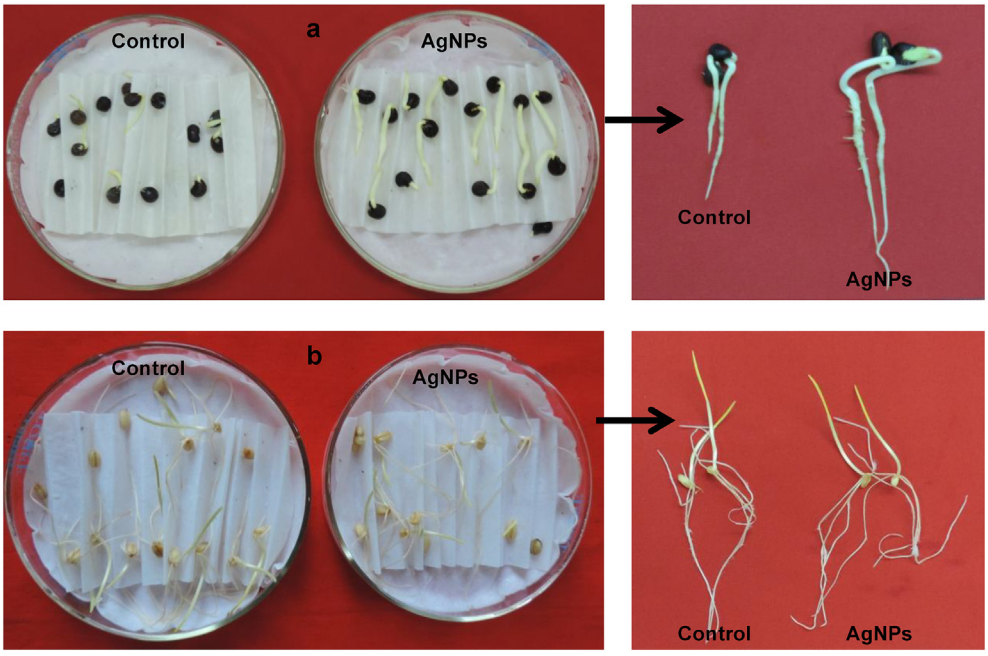

Fig. 3. Effect of biosynthesized AgNPs on the seed germination of okra (a) and wheat (b).

copy number (3.09×10^6) in AgNPs treated set was insignificant ($p = 0.419$). This trend remained same, though it changed vividly after 30 days of application. The $AgNO_3$ application reduced the relative abundance of *Alphaproteobacteria* ($p < 0.008$) whereas AgNPs treatment showed significant increase ($p = 0.000$) compared to control. *Betaproteobacteria* group was found to be positively affected by $AgNO_3$ and AgNPs treatment as significant increase in gene copy number was observed in $AgNO_3$ (8.23×10^6, $p = 0.000$) and AgNPs (7.93×10^6, $p = 0.000$) treatment compared to control (4.23×10^6, $p = 0.010$) after 15 days of application. After 30 days of application, its relative abundance was observed to be same in control (6.74×10^5) and $AgNO_3$ (6.34×10^5) treatments whereas significantly increased in AgNPs (2.01×10^6) treated soil. The relative abundance of *Actinobacteria* was observed to be greater in AgNPs exposed soil than control and $AgNO_3$ treatment. Significant inhibition was noted in $AgNO_3$ treated set after 15 days (5.42×10^6, $p = 0.000$) and 30 days (5.98×10^5, $p = 0.023$) as compared to control where gene copy number was detected to be 1.84×10^7 and 1.54×10^6 after 15 and 30 days, respectively. Furthermore, significant increase ($p = 0.000$) was recorded in AgNPs treated set with gene copy number 1.44×10^7 and 1.53×10^7 after 15

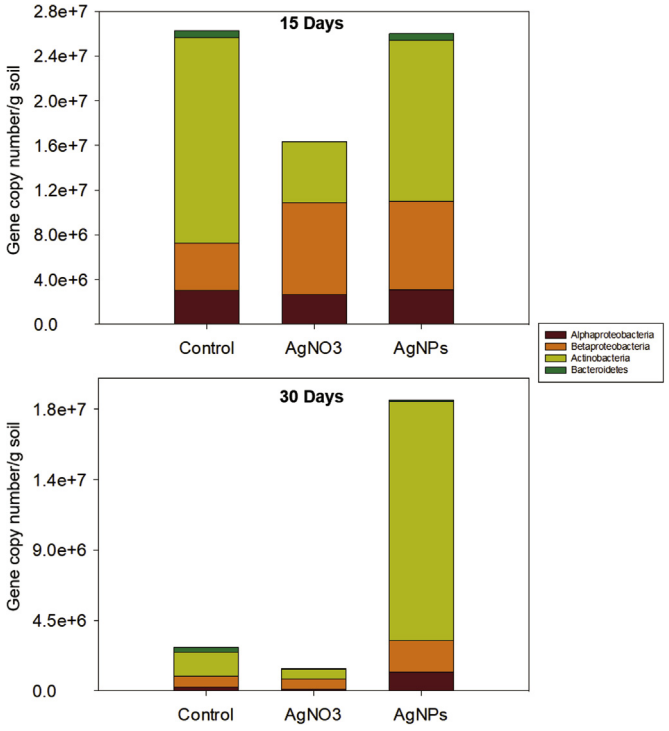

Fig. 4. Relative abundance of the different bacterial groups in the soil exposed to biosynthesized AgNPs and $AgNO_3$ treatments.

and 30 days of application, respectively. In this study, the proportion of relative abundance of *Bacteroidetes* group was found to be lowest compared to other three groups and showed completely different trend. This bacterial group was found to be significantly reduced by both $AgNO_3$ and AgNPs treatment however reduction was more prominent in $AgNO_3$ treated set. After 15 and 30 days, gene copy number was recorded to be 3.48×10^4 ($p = 0.000$) and 5.21×10^4 ($p = 0.000$) respectively in $AgNO_3$ treatment which was much lower than 6.15×10^5 and 3.0×10^5 gene copy number respectively, in control set. The AgNPs treatment negatively affected this bacterial group with gene copy number 5.68×10^5 ($p = 0.0027$) and 7.78×10^4 ($p = 0.000$) as compared to control after 15 and 30 days respectively (Fig. 4). In total, these data suggest that undoubtedly $AgNO_3$ is causing deep negative impact on the relative abundance of the bacterial group. While AgNPs exposure caused varying effects for different bacterial group. For instance, the relative abundance of *Bacteroidetes* group was significantly reduced by AgNPs treatment while other three groups *viz. Alphaproteobacteria*, *Betaproteobacteria* and *Actinobacteria* were observed to be resilient showing significant increase in relative abundance. This result is in contrast with previously published reports which suggest inhibitory impact of AgNPs on the soil microbial community (Grün et al., 2018; Guo et al., 2018). Here, we would like to give emphasis to interesting results obtained by Sillen et al. (2015), as we have used same concentration of AgNPs i.e. 100 mg/kg soil which is recognized as high dose (Simonin and Richaume, 2015). In this study, the authors reported that AgNPs exposure instigated considerable alterations in the bacterial community composition in the maize rhizosphere and also enhanced plant biomass. They concluded that AgNPs mediated alterations in the microbial community benefit plant growth possibly by suppressing the growth of phytopathogenic microbes residing in the rhizosphere. The prevailing discrepancies between different studies can be best understood by considering the fact that there are various factors such as different experimental conditions, dose, exposure time, soil type, AgNPs size, etc., that can influence AgNPs toxicity. Apart from this, another important factor i.e. mode of synthesis of AgNPs (synthesized using biological or non-biological sources) might be playing important role in determining their toxicity behavior. Since studies on chemically synthesized nanoparticles dominate over biosynthesized nanoparticles, we should consider checking the environmental compatibility and toxicity behavior of biosynthesized nanoparticles. Based on previous reports accentuating green synthesis of AgNPs as eco-friendly approach due to their compatibility with the environment (Dhillon et al., 2012; Roy et al., 2013; Kharissova et al., 2013), we propose that biosynthesized AgNPs might pose less or no toxicity to soil microbes. However, this interpretation is based on our preliminary data which requires further validation by elaborated studies in the future. Furthermore, we feel that this study could be useful for designing future experiments with the aim to predict toxicity behavior of biosynthesized silver nanoparticles in the realistic environment.

3.4. Effects of biosynthesized AgNPs on functional genes abundance: an assessment of soil bacterial community functions

Soil microbes are highly diverse and inseparable entities of the soil ecosystem. The dynamic structure and functions of the soil microbial community are critical to maintain soil health and to perform a range of ecosystem functions *viz.* nutrient cycling, decomposition, etc. (Nannipieri et al., 2003; Maron et al., 2018). Most importantly, soil microbes are efficient engineers of biogeochemical cycles and hence support the aboveground plant community. This exchange process between plant and microbes regulates and maintains ecological stability (Fuhrman, 2009; Graham et al., 2016; Liu et al., 2018). Microbial functional genes are the key player for the synthesis of enzymes that catalyze key steps in biogeochemical processes (Trivedi et al., 2013; Rocca et al., 2015). Rapidly growing technological advances allow us to explore the genetic and taxonomic diversity of microbial communities. In addition to this, quantitative real-time PCR is the most suitable and appropriate technique to quantify microbial cells or functional genes. Through quantification of microbial functional genes, we can accurately predict the rate of ecosystem processes (Hallin et al., 2009; Breuillin-Sessoms et al., 2017; Crane et al., 2018). Subsequently, we also made an attempt to quantify certain bacterial functional genes involved in the nitrogen and phosphorus cycling to comprehend the possible impact of biosynthesized AgNPs application on microbial functions (Fig. 5). In this study, we targeted a range of functional genes (*NirK, NirS, NosZ*, *AOA, AOB* and *PhoD*) involved in important soil ecological processes such as denitrification, nitrification and recycling of organic phosphorus (Ragot et al., 2015; Azziz et al., 2017; Trivedi et al., 2019). Our results demonstrated more pronounced impact of AgNPs treatment on the targeted functional genes abundance after 30 days of application. The $AgNO_3$ treatment showed maximum toxic impact on functional genes abundance in comparison to control and AgNPs treatments. Nitrite reductase (NR) enzyme found in denitrifiers, has been categorized into two classes; copper containing NR and cytochrome cd1 containing NR encoded by *NirK* and *NirS* functional genes, respectively (Glockner et al., 1993). The relative abundance of *NirS* gene was found to be 7.39×10^5 ($p = 0.000$) and 1.81×10^5 ($p = 0.000$) in AgNPs treated soil as compared to control exhibiting 2.07×10^6 and 7.12×10^5 after 15 and 30 days, respectively. In contrast, *Nirk* gene abundance was reported to be 1.38×10^6 ($p = 0.408$) and 5.13×10^5 ($p = 0.000$) in the soil exposed to AgNPs in comparison to 1.6×10^6 and 4.53×10^4 gene copy number in control after 15 and 30 days, respectively. It indicates that *NirS* and *NirK* type denitrifier communities responded differently to AgNPs treatment where *NirS* was found to be negatively affected by AgNPs treatment. *NosZ* gene harboring denitrifier community did not show any distinct pattern of alteration in all three treatments after 15 days of application however more prominent impact was observed after 30 days of application. The relative *NosZ* gene abundance was observed to be 5.19×10^6 ($p = 0.000$) and 1.26×10^6 ($p = 0.544$) in AgNPs and $AgNO_3$ treatment respectively, as compared to 1.34×10^6 gene copy number in control after 30 days of application. Taken together, it can be

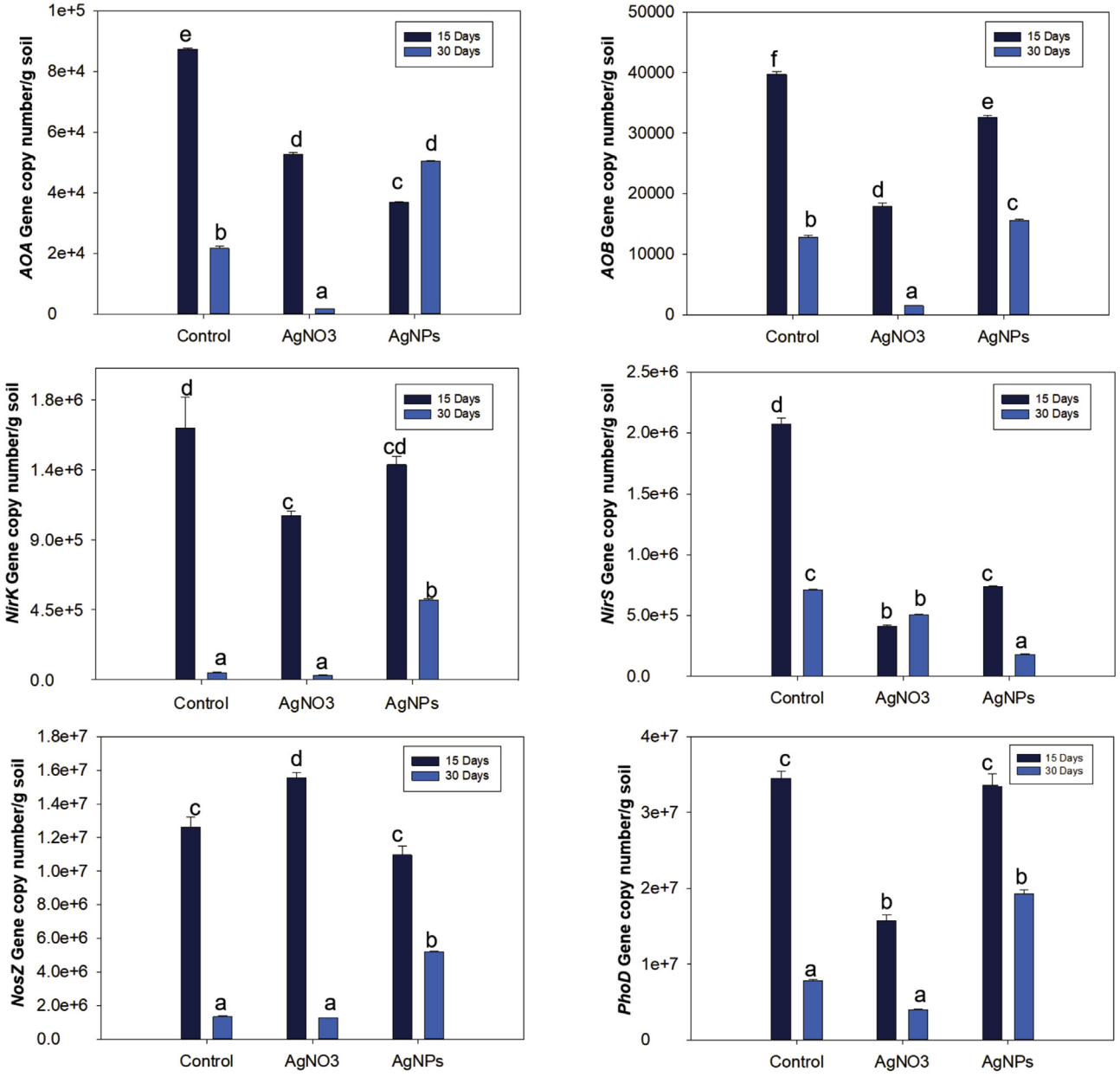

Fig. 5. Effects of biosynthesized AgNPs and $AgNO_3$ on the relative abundance of targeted functional genes involved in the nitrogen and phosphorus cycling in the soil after 15 and 30 days of application. Values represent mean ± standard deviation and different letters indicate significant differences among different treatments as estimated by Posthoc Tukey's test.

postulated that among denitrifiers community, *NirS* type was found to be more sensitive to AgNPs treatment whereas *NirK* and *NosZ* gene harboring community remained unaffected. Previously, Bai et al. (2018) observed the inhibitory impact of AgNPs on denitrification enzyme activity and gene (*NarG* and *NirS*) abundance in the river estuary at dose range of 0–1350 mg/L. Likewise, Zhang et al. (2018) also reported sensitivity of *NirS* gene community to PVP-AgNPs. Interestingly, Throbäck et al. (2007) reported a new possibility of generation of silver resistant novel *NirK* genotypes in the soil in response to 100 mg $AgNO_3$ kg^{-1} soil. They observed high *NirK* diversity but low gene abundance at this high dose of $AgNO_3$ explaining the reason for development of silver resistant population. Furthermore, the impact assessment of AgNPs on the nitrifying bacteria has already been discussed up to some extent. In our study, we also examined the impact of biosynthesized AgNPs on ammonia oxidizing bacteria (AOB) and archaea (AOA) gene abundance as ammonia oxidation is the rate limiting step of the nitrification process. We observed reduced AOA gene abundance after 15 days of application of AgNPs (3.68×10^4, $p = 0.000$) and $AgNO_3$ (5.26×10^4, $p = 0.000$) in comparison to control (8.73×10^4, $p = 0.000$). However, after 30 days of application, we found more prominent toxic impact of $AgNO_3$ (1.70×10^3, $p = 0.000$) and positive impact of AgNPs (5.02×10^4, $p = 0.000$) as compared to control (2.16×10^4). In the case of AOB, the relative abundance was found to be significantly much lower in $AgNO_3$ (1.79×10^4, $p = 0.000$) and slightly lower in AgNPs (3.26×10^4, $p = 0.000$) as compared to control (3.97×10^4) after 15 days of treatment. After 30 days of treatment, more noticeable negative effect was observed in $AgNO_3$ (1.51×10^3, $p = 0.000$) while AgNPs (1.55×10^4, $p = 0.006$) treated set remained same as control (1.28×10^4). Our results are in contrary to previous studies mentioning inhibitory impact of AgNPs on nitrifiers in aquatic environments (Yang et al., 2014; Zheng et al., 2017) and soil (Huang et al., 2018). While our findings are in partial accordance with the study performed by Beddow et al. (2017) where they found AOA gene abundance was not inhibited by nanosilver (50 mg/L) in estuarine sediments. Besides nitrogen

cycling related genes, we also included *PhoD* gene in our study to explore the probable impact of AgNPs on phosphorus cycling. To date, we do not have any direct evidence to show interactions between AgNPs and *PhoD* gene abundance in the soil environment. Our results indicated that in comparison to control (3.45×10^7), relative abundance of *PhoD* gene was not affected by AgNPs (3.35×10^7, $p = 0.582$) treatment after 15 days of application, whereas more pronounced reduction in gene copy number (1.57×10^7, $p = 0.000$) was observed in $AgNO_3$ treatment. More significant and noticeable influence was observed after 30 days of treatment where relative abundance was found to be 4.02×10^6 ($p = 0.000$) and 1.93×10^7 ($p = 0.000$) in $AgNO_3$ and AgNPs treatment respectively, as compared to control showing 7.81×10^6 gene copy number.

4. Conclusions and future perspectives

Our study highlights the beneficial effects of biosynthesized AgNPs on the soil bacterial community structure and functions. We strongly believe that this preliminary study provides a baseline for future research and clearly demonstrates the necessity of environmental risk assessment of biogenic nanoparticles. Moreover, our approach is focused on the functional aspects of the microbial community to know the accurate impact of biosynthesized AgNPs application and to ensure the extent to which their application can affect ecosystem functions. The relative increase or decrease in the abundance of soil microbial functional genes contributing to important ecosystem functions (nutrient cycling in this study) is the ecological indicators for predicting the toxic/non-toxic environment. Our results suggest a differential pattern of biosynthesized AgNPs mediated effects on the relative abundance of bacterial taxon and functional genes after 15 days of application. Interestingly, we found more stimulating effects of biosynthesized AgNPs on the relative abundance of functional genes after 30 days of application indicating the importance of long-term studies to examine the environmental risk posed by nanoparticles. Most importantly, this provides a better clue to further extend our research to know that *is there any adaptation strategy or silver resistance embraced by the soil microbial community?* Further, the second question arises in the view of green synthesis and obtained data in this study, i.e. *are biosynthesized nanoparticles (using plants or microbes) really safe for the environment as they do not pose any risk*? Seemingly, the environmental impact of biosynthesized AgNPs, particularly for functional groups of the soil bacterial community driving important soil ecological processes, is not sufficiently understood. Although, this is the first empirical evidence to show the positive response of the soil bacterial community functions to the application of biosynthesized AgNPs, however, an important limitation is that our interpretation is based on one soil type. Therefore, future studies require elaborated experiments to evaluate nanotoxicity of biosynthesized nanoparticles under different soil conditions with varying properties such as pH, organic matter content, soil texture, water holding capacity, etc. across different spatial scales. Considering that nanoparticles' toxicity is predominantly influenced by various soil factors, this study can provide new perspectives on the environmental safety effects of biosynthesized AgNPs that would be helpful to apply appropriate soil management policies to overcome nanotoxicity.

Author contributions

H.B.S. and S.M. conceived and designed the experiments; S.M. performed the experiments and analyzed data; S.M. wrote the paper; Y.X.D executed the article editing and provided necessary facilities. All of the authors read and commented on the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

Authors gratefully acknowledge the financial support provided by National Natural Science Foundation of China (Grant no. 31700457), China Postdoctoral Science Foundation (Grant No. 2018M631112), and CAS President's International Fellowship Initiative (PIFI) (Grant no. 2019PC0095) awarded to Sandhya Mishra. Xiaodong Yang acknowledges support by CAS 135 program (No. 2017XTBG-T01). We thank Central Laboratory at Xishuangbanna Tropical Botanical Garden for providing necessary facilities and equipment to conduct experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.109584.

References

Azziz, G., Monza, J., Etchebehere, C., Irisarri, P., 2017. nirS-and nirK-type denitrifier communities are differentially affected by soil type, rice cultivar and water management. Eur. J. Soil Biol. 78, 20–28.

Bai, J., Tian, Y.Z., Sun, P.F., Bai, X.Y., Li, K.R., Zhao, Y.G., 2018. Effect of silver nanoparticles on denitrification and functional gene abundances of sediment in Dagu River Estuary and Northwest of Jiaozhou Bay. Huan jing ke xue= Huanjing kexue 39 (11), 4956–4963.

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., Clément, C., Ouhdouch, Y., van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of Actinobacteria. Microbiol. Mol. Biol. Rev. 80 (1), 1–43.

Beddow, J., Stolpe, B., Cole, P.A., Lead, J.R., Sapp, M., Lyons, B.P., Colbeck, I., Whitby, C., 2017. Nanosilver inhibits nitrification and reduces ammonia-oxidising bacterial but not archaeal amoA gene abundance in estuarine sediments. Environ. Microbiol. 19 (2), 500–510.

Breuillin-Sessoms, F., Venterea, R.T., Sadowsky, M.J., Coulter, J.A., Clough, T.J., Wang, P., 2017. Nitrification gene ratio and free ammonia explain nitrite and nitrous oxide production in urea-amended soils. Soil Biol. Biochem. 111, 143–153.

Calder, A.J., Dimkpa, C.O., McLean, J.E., Britt, D.W., Johnsonc, W., Anderson, A.J., 2012. Soil components mitigate the antimicrobial effects of silver nanoparticles towards a beneficial soil bacterium, *Pseudomonas chlororaphis* O6. Sci. Total Environ. 429, 215–222.

Choi, O., Hu, Z., 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ. Sci. Technol. 42, 4583–4588.

Chung, I.-M., Park, I., Seung-Hyun, K., Thiruvengadam, M., Rajakumar, G., 2016. Plant-mediated synthesis of silver nanoparticles: their characteristic properties and therapeutic applications. Nanoscale Res. Lett. 11, 40.

Chunjaturas, W., Ferguson, J.A., Rattanapichai, W., Sadowsky, M.J., Sajjaphan, K., 2014. Shift of bacterial community structure in two Thai soil series affected by silver nanoparticles using ARISA. World J. Microbiol. Biotechnol. 30, 2119–2124.

Colman, B.P., Arnaout, C.L., Anciaux, S., Gunsch, C., Hochella, M.F., Kim, B., Lowry, G.V., McGill, B.M., Reinsch, B.C., Richardson, C.J., Unrine, J.M., Wright, J.P., Yin, L., Bernhardt, E.S., 2013. Low concentrations of silver nanoparticles in biosolids cause adverse ecosystem responses under realistic field scenario. PLoS One 8, e57189.

Consumer Products Inventory, 2015. Resource Document. The project on emergency nanotechnologies. http://www.nanotechproject.org/cpi/products/, Accessed date: 27 October 2015.

Crane, S.L., van Dorst, J., Hose, G.C., King, C.K., Ferrari, B.C., 2018. Microfluidic qPCR enables high throughput quantification of microbial functional genes but requires strict curation of primers. Front. Environ. Sci. 6, 145.

Das, R.K., Pachapur, V.L., Lonappan, L., Naghdi, M., Pulicharla, R., Maiti, S., Cledon, M., Dalila, L.M.A., Sarma, S.J., Brar, S.K., 2017. Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. Nanotechnol. Environ. Eng. 2 (1), 18.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat. Commun. 7, 10541.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D.J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018. A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325.

Dhas, S.P., Shiny, P.J., Khan, S., Mukherjee, A., Chandrasekaran, N., 2014. Toxic behavior of silver and zinc oxide nanoparticles on environmental microorganisms. J. Basic Microbiol. 54, 916–927.

Dhillon, G.S., Brar, S.K., Kaur, S., Verma, M., 2012. Green approach for nanoparticle biosynthesis by fungi: current trends and applications. Crit. Rev. Biotechnol. 32 (1), 49–73.

Doolette, C.L., Gupta, V.V., Lu, Y., Payne, J.L., Batstone, D.J., Kirby, J.K., Navarro, D.A., McLaughlin, M.J., 2016. Quantifying the sensitivity of soil microbial communities to silver sulfide nanoparticles using metagenome sequencing. PLoS One 11 (8), e0161979.

Emmerling, C., Schloter, M., Hartmann, A., Kandeler, E., 2002. Functional diversity of soil organisms—a review of recent research activities in Germany. J. Plant Nutr. Soil Sci. 165, 408.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil

bacteria. Ecology 88 (6), 1354–1364.
Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. The assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71, 4117–4120.
Fuhrman, J.A., 2009. Microbial community structure and its functional implications. Nature 459 (7244), 193.
Gajjar, P., Pettee, B., Britt, D.W., Huang, W., Johnson, W.P., Anderson, A.J., 2009. Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT2440. J. Biol. Eng. 3, 1.
Ge, L., Li, Q., Wang, M., Ouyang, J., Li, X., Xing, M.M., 2014. Nanosilver particles in medical applications: synthesis, performance, and toxicity. Int. J. Nanomed. 9, 2399.
Glockner, A.B., Jüngst, A., Zumft, W.G., 1993. Copper-containing nitrite reductase from Pseudomonas aureofaciens is functional in a mutationally cytochrome cd 1-free background (NirS−) of *Pseudomonas stutzeri*. Arch. Microbiol. 160(1), 18–26.
Gottschalk, F., Scholz, R.W., Nowack, B., 2010. Probabilistic material flow modeling for assessing the environmental exposure to compounds: Methodology and an application to engineered nano-TiO2 particles. Environ. Model. Softw. 25, 320–332.
Graham, E.B., Knelman, J.E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J.M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., 2016. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? Front. Microbiol. 7, 214.
Griffiths, R.I., Thomson, B.C., Plassart, P., Gweon, H.S., Stone, D., Creamer, R.E., Lemanceau, P., Bailey, M.J., 2016. Mapping and validating predictions of soil bacterial biodiversity using European and national scale datasets. Appl. Soil Ecol. 97, 61–68.
Grün, A.L., Straskraba, S., Schulz, S., Schloter, M., Emmerling, C., 2018. Long-term effects of environmentally relevant concentrations of silver nanoparticles on microbial biomass, enzyme activity, and functional genes involved in the nitrogen cycle of loamy soil. J. Environ. Sci. 69, 12–22.
Guo, Y., Cichocki, N., Schattenberg, F., Geffers, R., Harms, H., Müller, S., 2018. AgNPs change microbial community structures of wastewater. Front. Microbiol. 9.
Hallin, S., Jones, C.J., Schloter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J. 3, 597–605.
Hänsch, M., Emmerling, C., 2010. Effects of silver nanoparticles on the microbiota and enzyme activity in soil. J. Plant Nutr. Soil Sci. 173, 554–558.
Huang, J., Chong, C.A.O., Runqing, L.I., Wenzhu, G.U.A.N., 2018. Effects of silver nanoparticles on soil ammonia-oxidizing microorganisms under temperatures of 25 and 5° C. Pedosphere 28 (4), 607–616.
Jackson, L.E., Calderon, F.J., Steenwerth, K.L., Scow, K.M., Rolston, D.E., 2003. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. Geoderma 114, 305–317.
Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl. Environ. Microbiol 72 (3), 1719–1728.
Karimi, B., Terrat, S., Dequiedt, S., Saby, N.P., Horrigue, W., Lelièvre, M., Nowak, V., Jolivet, C., Arrouays, D., Wincker, P., Cruaud, C., 2018. Biogeography of soil bacteria and archaea across France. Sci. Adv. 4 (7), eaat1808.
Kharissova, O.V., Dias, H.R., Kharisov, B.I., Pérez, B.O., Pérez, V.M.J., 2013. The greener synthesis of nanoparticles. Trends Biotechnol. 31 (4), 240–248.
Lammel, D.R., Feigl, B.J., Cerri, C.C., Nüsslein, K., 2015. Specific microbial gene abundances and soil parameters contribute to C, N, and greenhouse gas process rates after land use change in Southern Amazonian Soils. Front. Microbiol. 6, 1057.
Lanzen, A., Lekang, K., Jonassen, I., Thompson, E.M., Troedsson, C., 2017. DNA extraction replicates improve diversity and compositional dissimilarity in metabarcoding of eukaryotes in marine sediments. PLoS One 12 (6), e0179443.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120.
León-Silva, S., Fernández-Luqueño, F., López-Valdez, F., 2016. Silver nanoparticles (AgNP) in the environment: a review of potential risks on human and environmental health. Water Air Soil Pollut. 227 (9), 306.
Liu, Z., Cichocki, N., Bonk, F., Günther, S., Schattenberg, F., Harms, H., Centler, F., Müller, S., 2018. Ecological stability properties of microbial communities assessed by flow cytometry. mSphere 3 (1) e00564-17.
Llado, S., López-Mondéjar, R., Baldrian, P., 2017. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. Microbiol. Mol. Biol. Rev. 81 (2) e00063-16.
Maron, P.A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., Karimi, B., Bernard, L., Dequiedt, S., Terrat, S., Chabbi, A., 2018. High microbial diversity promotes soil ecosystem functioning. Appl. Environ. Microbiol 84 (9) e02738-17.
Mehta, C.M., Srivastava, R., Arora, S., Sharma, A.K., 2016. Impact assessment of silver nanoparticles on plant growth and soil bacterial diversity. 3 Biotech 6 (2), 254.
Mishra, S., Fraceto, L.F., Yang, X., Singh, H.B., 2018. Rewinding the history of agriculture and emergence of nanotechnology in agriculture. In: Singh, H.B., Mishra, S., Fraceto, L.F., de Lima, R. (Eds.), Emerging Trends in Agri-Nanotechnology: Fundamental and Applied Aspects. CABI, UK, pp. 1.
Mishra, S., Keswani, C., Abhilash, P.C., Fraceto, L.F., Singh, H.B., 2017a. Integrated approach of agri-nanotechnology: challenges and future trends. Front. Plant Sci. 8, 471.
Mishra, S., Keswani, C., Singh, A., Singh, B.R., Singh, S.P., Singh, H.B., 2016. Microbial nanoformulation: exploring potential for coherent nano-farming. In: Gupta, V.K., Sharma, G.D., Tuohy, M.G., Gaur, R. (Eds.), The Handbook of Microbial Bioresources. CABI, UK, pp. 107–120.
Mishra, S., Singh, B.R., Naqvi, A.H., Singh, H.B., 2017b. Potential of biosynthesized silver nanoparticles using *Stenotrophomonas* sp. BHU-S7 (MTCC 5978) for management of soil-borne and foliar phytopathogens. Sci. Rep. 7, 45154. this manuscript is published under the CC BY 4.0 license link: http://creativecommons.org/licenses/by/4.0/.
Mishra, S., Singh, B.R., Singh, A., Keswani, C., Naqvi, A.H., Singh, H.B., 2014. Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat. PLoS One 9, e97881.
Mishra, S., Singh, H.B., 2015a. Biosynthesized silver nanoparticles as a nanoweapon against phytopathogens: exploring their scope and potential in agriculture. Appl. Microbiol. Biotechnol. 99 (3), 1097–1107.
Mishra, S., Singh, H.B., 2015b. Silver nanoparticles mediated altered gene expression of melanin biosynthesis genes in *Bipolaris sorokiniana*. Microbiol. Res. 172, 16–18.
Mishra, S., Singh, H.B., Yang, X., 2019. Creating a global database "Nanomaterials in the soil environment": future need for the terrestrial ecosystem. Energy Ecol. Environ. https://doi.org/10.1007/s40974-019-00126-5.
Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54 (4), 655–670.
Nowack, B., Krug, H.F., Height, M., 2011. 120 years of nanosilver history: implications for policy makers. Environ. Sci. Technol. 45, 1177–1183.
Pulit-Prociak, J., Banach, M., 2016. Silver nanoparticles–a material of the future…? Open Chem 14 (1), 76–91.
Ragot, S.A., Kertesz, M.A., Bünemann, E.K., 2015. phoD alkaline phosphatase gene diversity in soil. Appl. Environ. Microbiol. 81 (20), 7281–7289.
Reidy, B., Haase, A., Luch, A., Dawson, K., Lynch, I., 2013. Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials 6, 2295–2350.
Rocca, J.D., Hall, E.K., Lennon, J.T., Evans, S.E., Waldrop, M.P., Cotner, J.B., Nemergut, D.R., Graham, E.B., Wallenstein, M.D., 2015. Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. ISME J. 9 (8), 1693.
Roger, F., Bertilsson, S., Langenheder, S., Osman, O.A., Gamfeldt, L., 2016. Effects of multiple dimensions of bacterial diversity on functioning, stability and multifunctionality. Ecology 97 (10), 2716–2728.
Roy, N., Gaur, A., Jain, A., Bhattacharya, S., Rani, V., 2013. Green synthesis of silver nanoparticles: an approach to overcome toxicity. Environ. Toxicol. Pharmacol. 36 (3), 807–812.
Sadak, M.S., 2019. Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum*). Bull. Natl. Res. Cent. 43 (1), 38.
Schlich, K., Hund-Rinke, K., 2015. Influence of soil properties on the effect of silver nanomaterials on microbial activity in five soils. Environ. Pollut. 196, 321–330.
Scimeca, M., Bischetti, S., Lamsira, H.K., Bonfiglio, R., Bonanno, E., 2018. Energy Dispersive X-ray (EDX) microanalysis: a powerful tool in biomedical research and diagnosis. Eur. J. Histochem.: EJH 62 (1).
Shahrokh, S., Emtiazi, G., 2009. Toxicity and unusual biological behavior of nanosilver on gram positive and negative bacteria assayed by microtiter-plate. Eur. J. Biol. Sci. 1 (3), 28–31.
Shahrokh, S., Hosseinkhani, B., Emtiazi, G., 2014. The impact of nano-silver on bacterial aerobic nitrate reductase. J. Bioprocess. Biotech. 4, 162.
Sharma, P., Bhatt, D., Zaidi, M.G.H., Saradhi, P.P., Khanna, P.K., Arora, S., 2012. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl. Biochem. Biotechnol. 167, 2225–2233.
Shin, Y.-J., Kwak, J.I., An, Y.-J., 2012. Evidence for the inhibitory effects of silver nanoparticles on the activities of soil exoenzymes. Chemosphere 88, 524–529.
Sillen, W.M., Thijs, S., Abbamondi, G.R., Janssen, J., Weyens, N., White, J.C., Vangronsveld, J., 2015. Effects of silver nanoparticles on soil microorganisms and maize biomass are linked in the rhizosphere. Soil Biol. Biochem. 91, 14–22.
Simonin, M., Richaume, A., 2015. Impact of engineered nanoparticles on the activity, abundance, and diversity of soil microbial communities: a review. Environ. Sci. Pollut. Res. 22 (18), 13710–13723.
Singh, H.B., Mishra, S., Fraceto, L.F., De Lima, R., 2018. Emerging Trends in Agri-Nanotechnology: Fundamental and Applied Aspects. CABI, UK.
Spain, A.M., Krumholz, L.R., Elshahed, M.S., 2009. Abundance, composition, diversity and novelty of soil Proteobacteria. ISME J. 3 (8), 992.
Sun, T.Y., Gottschalk, F., Hungerbühler, K., Nowack, B., 2014. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. Environ. Pollut. 185, 69–76.
Throbäck, I.N., Johansson, M., Rosenquist, M., Pell, M., Hansson, M., Hallin, S., 2007. Silver (Ag^+) reduces denitrification and induces enrichment of novel nirK genotypes in soil. FEMS Microbiol. Lett. 270, 189–194.
Trivedi, C., Reich, P.B., Maestre, F.T., Hu, H.W., Singh, B.K., Delgado-Baquerizo, M., 2019. Plant-driven niche differentiation of ammonia-oxidizing bacteria and archaea in global drylands. ISME J. https://doi.org/10.1038/s41396-019-0465-1.
Trivedi, P., Anderson, I.C., Singh, B.K., 2013. Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. Trends Microbiol. 21 (12), 641–651.
Wafula, D., White, J.R., Canion, A., Jagoe, C., Pathak, A., Chauhan, A., 2015. Impacts of long-term irrigation of domestic treated wastewater on soil biogeochemistry and bacterial community structure. Appl. Environ. Microbiol. 81 (20), 7143–7158.
Wang, P., Lombi, E., Menzies, N.W., Zhao, F.J., Kopittke, P.M., 2018. Engineered silver nanoparticles in terrestrial environments: a meta-analysis shows that the overall environmental risk is small. Environ. Sci. Nano 5 (11), 2531–2544.
Wolińska, A., Kuźniar, A., Zielenkiewicz, U., Izak, D., Szafranek-Nakonieczna, A., Banach, A., Błaszczyk, M., 2017. Bacteroidetes as a sensitive biological indicator of agricultural soil usage revealed by a culture-independent approach. Appl. Soil Ecol. 119, 128–137.
Xiao, H., Schaefer, D.A., Yang, X., 2017. pH drives ammonia oxidizing bacteria rather than archaea thereby stimulate nitrification under *Ageratina adenophora* colonization. Soil Biol. Biochem. 114, 12–19.
Yan, L., Wang, G., Ai, S., Huo, Z., Wang, Y., Gu, J.D., Wang, W., 2018. Abundance of

ammonia-oxidizing bacteria and archaea under different ventilation strategies during cattle manure composting. J. Environ. Manag. 212, 375–383.
Yang, Y., Li, M., Michels, C., Moreira-Soares, H., Alvarez, P.J., 2014. Differential sensitivity of nitrifying bacteria to silver nanoparticles in activated sludge. Environ. Toxicol. Chem. 33 (10), 2234–2239.
Yang, Y., Wang, J., Xiu, Z., Alvarez, P.J.J., 2013. Impacts of silver nanoparticles on cellular and transcriptional activity of nitrogen-cycling bacteria. Environ. Toxicol. Chem. 32, 1488–1494.
Zeng, G., Zhang, L., Dong, H., Chen, Y., Zhang, J., Zhu, Y., Yuan, Y., Xie, Y., Fang, W., 2018. Pathway and mechanism of nitrogen transformation during composting: functional enzymes and genes under different concentrations of PVP-AgNPs. Bioresour. Technol. 253, 112–120.
Zhang, L., Zeng, G., Dong, H., Chen, Y., Zhang, J., Yan, M., Zhu, Y., Yuan, Y., Xie, Y., Huang, Z., 2017. The impact of silver nanoparticles on the co-composting of sewage sludge and agricultural waste: evolutions of organic matter and nitrogen. Bioresour. Technol. 230, 132–139.
Zhang, L., Zhang, J., Zeng, G., Dong, H., Chen, Y., Huang, C., Zhu, Y., Xu, R., Cheng, Y., Hou, K., Cao, W., 2018. Multivariate relationships between microbial communities and environmental variables during co-composting of sewage sludge and agricultural waste in the presence of PVP-AgNPs. Bioresour. Technol. 261, 10–18.
Zhang, X.F., Liu, Z.G., Shen, W., Gurunathan, S., 2016. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int. J. Mol. Sci. 17 (9), 1534.
Zheng, Y., Hou, L., Liu, M., Newell, S.E., Yin, G., Yu, C., Zhang, H., Li, X., Gao, D., Gao, J., Wang, R., 2017. Effects of silver nanoparticles on nitrification and associated nitrous oxide production in aquatic environments. Sci. Adv. 3 (8), e1603229.