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ORIGINAL ARTICLE

Inhibitory effects of silver silicate ($AgSiO_2$) nanoparticles on uncultivable bacterial phyla present in soil

K. Malabika Singha[1] · L. Paikhomba Singha[2] · Debashish Acharya[3] · Piyush Pandey[1]

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Abstract

The transport of silver nanoparticles (AgNPs) into the soil occurs through various means by wastewater and other anthropogenic sources. The impact of silver silicate nanoparticle ($AgSiO_2$) application on the microbial community structure of rice field soil was evaluated. The results indicate that the introduction of $AgSiO_2$ alters the total bacterial community structure of the soil. Fascinatingly, the phylum WPS2 and *Firmicutes* showed lower abundance in nanoparticle-treated samples, which signifies their susceptibility to $AgSiO_2$ nanoparticles. The relative abundance of uncultivable phyla WPS2 was 8% in untreated soil, which was reduced to 0.09% by treatment of nanoparticles. However, there was no significant impact on the relative abundance of phyla Proteobacteria and Acidobacteria. Members of WPS2 have not been cultivated yet; therefore, information on their physiological and functional roles is not yet available. Functional analysis of the treated sample where WPS2 was abundant, had a higher representation of function related to bacterial motility proteins, nitrogen metabolism, carbon fixation, bacterial chemotaxis, oxidative phosphorylation, and photosynthesis proteins.

Keywords Silver silicate nanoparticle ($AgSiO_2$) · Microbial community structure · WPS2 · Firmicutes · Proteobacteria · Acidobacteria

Introduction

The widespread application of silver in medical devices, along with its increasing use in consumer products like cosmetics, textiles, food packaging, and refrigerators, has led to an estimated annual global production of 500 tons of silver nanoparticles (AgNPs) that subsequently, results in the release of AgNPs into the environment (Ihtisham et al. 2021). In addition, Yu (2013) highlighted that during the production, transport, erosion, washing, or disposal of products containing AgNPs, nanoparticles are released into the environment, including soils. The environmental risks associated with AgNPs are challenging due to their nanoscale size, high reactivity, and poorly defined dissolution properties (Benoit et al. 2013). Moreover, various human activities, intentional releases via remediation technologies, potential agricultural uses, and involuntary releases of sewage sludge introduce AgNPs into the soil (Blaser et al. 2008; Ihtisham 2021). This process may contribute to elevated concentrations of nanoparticles in soils beyond current projections. In the soil, the transformation of AgNPs undergoes via encompassing dissolution, oxidative stress, aggregation, destabilization, and sulfidation reactions (Yin et al. 2015a). The physical and chemical properties of AgNPs may change as they interact with the soil. Furthermore, studies demonstrate that plants grown in aqueous solutions containing AgNPs exhibit accelerated absorption, translocation, and accumulation of these particles within rice tissues (Mirzajani et al., 2013). Soil conditions, such as soil pH, moisture levels, inorganic/organic content, and diversity of microbes, have a significant impact on AgNPs surface properties and life cycle (Yang et al. 2019). As an illustration, in the case of sulfur-enriched soil mixed with AgNPs, a sulfidation reaction can occur, resulting in the formation of Ag°/Ag2S core-shell nanoparticles with lower biological toxicity than AgNPs (Devi et al. 2015). Furthermore, the presence of organic matter (OM) is known to prevent

✉ Piyush Pandey
piyushddn@gmail.com; ppmicroaus@gmail.com

[1] Department of Microbiology, Assam University, Silchar 788011, India

[2] Department of Microbiology, Central University of Rajasthan, Ajmer, India

[3] Department of Physics, Assam University, Silchar 788011, India

AgNPs dissolution by coating the AgNPs particles. However, when soil becomes contaminated, silver is more commonly released on the soil surface in the form of nanoparticles (Eivazi et al. 2018), oxides (Nriagu et al., 1988), and sulfide nanoparticles (Kim et al., 2010). Silver (Ag) content in uncontaminated soil ranges from 0.01 to 1 mg/kg (Eivazi et al. 2018), and 0.07 to 0.1 mg/kg (Bowen 1979). The Ag content in contaminated soils ranges from 8 mg/kg, 9 mg/kg, 19.5 mg/kg (Asylbaev 2013), 23 mg/kg (Puzanov 2015), and 7000 mg/kg in soils associated with ore deposits (Druzhinin 2008; Zhang 2020). Despite the relatively low natural occurrence of silver in soil, its technophilic properties have shown exponential growth over the last half-century, and current projections anticipate further increases in the coming years.

AgNPs have both positive and negative effects since it has been reported to show bactericidal properties even in lower concentrations by inhibiting bacterial respiratory activity, DNA replication, and denitrification activity (Bruna et al. 2021). Also, AgNPs have the property to exhibit anti-phytopathogenic activity, enhance seed germination, and promote plant growth (Park et al. 2009; Mahardika et al. 2021). The action of AgNPs can differ based on particle sizes, nature, and alteration patterns in the environment. Size plays a crucial role, as AgNPs damage depends on its capability to enter cells and undergo oxidation, leading to the generation of reactive oxygen species (ROS) (Pokhrel and Dubey 2013; Singh and Kumar 2015; Ameen et al. 2021). The chemical properties of a particle's coating impact the dissolution and agglomeration of nanoparticles (Furtado et al. 2016). In contrast to other AgNPs, silica coating $AgSiO_2$ has gathered increased attention due to its highly reactive surface-to-volume ratio, antimicrobial properties, and high surface plasmon resonance (SPR) (Acharya et al. 2017). The use of a silica coat is advantageous because, upon absorption into fungal cells, the AgNPs enhance disinfecting activity. Simultaneously, silica triggers a dynamic resistance against diseases, creating a physical barrier against pathogenic fungi, potentially preventing disease recurrence for an extended period after microbial disinfection (Kumari et al. 2024). $AgSiO_2$ are gaining attention for potential usage in agriculture, as they selectively inhibit harmful fungi and bacteria on seeds. They could serve as an alternative fertilizer source, contributing to sustainable agriculture (Parveen and Rao 2014). As most of the silica-coated AgNPs is used in the industries, potential agricultural uses, and involuntary releases of sewage sludge introduce $AgSiO_2$ into the soil. However, the effect of $AgSiO_2$ in rice crop rhizospheric bacterial community has not been studied yet.

The soil microbial community can be affected due to the antibacterial properties of AgNPs, which have been described in previous reports (Lovern and Klaper 2006; Zhang 2020). According to Ihtisham (2021), the impact of AgNPs on soil microbial enzymatic activities can either stimulate or inhibit, depending on factors such as microorganism type, texture of soil, size, shape, and concentration of AgNPs. Because soil microorganisms play an important role in maintaining soil health and ecosystem functionality, the introduction of AgNPs may have negative effects on biogeochemical processes in the soil, including carbon and nitrogen cycling, as well as plant growth-promoting (PGP) attributes (Morones et al. 2005; Diao et al. 2020). In this study, the effect of $AgSiO_2$ was determined on the rhizospheric bacterial community of the rice plant. Rice (*Oryza sativa* L.) is a primary food crop for approximately 65% of the world's population, particularly in Asia, accounting for roughly 20% of total calories consumed (Ijaz, 2019). The toxicity of silica-coated silver nanoparticles remains unexplored, unlike the well-documented effects of silica or silver nanoparticles. Even less is known about the impact of $AgSiO_2$ on rice fields, with no studies conducted on their potential effects. Consequently, the findings of this study will help to determine the fate of AgSiO2 usage in rice fields.

Materials and methods

Synthesis and preparation of $AgSiO_2$

First, AgNPs colloids were made using the citrate reduction procedure. Initially 5 mM $AgNO_3$ was dissolved in 50 ml of deionized water. Then, 25 ml of the produced solution was added while vigorously stirring to 100 ml of water. Upon the onset of boiling with vigorous stirring, 5 ml of a 1% sodium citrate solution was added (prepared as 0.5 g in 50 ml). After a few minutes, the solution's color changed to a faint yellow. Under stirring, 5 ml of the produced AgNPs solution was added to a mixture of 20 ml ethanol and 10 ml H_2O. After 10 mins, 10 l of tetraethyl orthosilicate (TEOS) and 1 ml of dimethylacetamide (DMA) were added drop by drop. The resulting mixture was swirled at room temperature for a full 12 h without being disturbed. Centrifugation was used to separate the products for 30 min at 4000 rpm. The products underwent three more washes with 100% ethanol before being re-dispersed in ethanol, producing a light brown AgSiO2 composite dispersion (Acharya et al. 2017) (Fig. 1).

Soil sample collection and DNA extraction for metagenomic analysis

The soil samples were collected from rice field of Silchar, Assam, India (latitute 24. 833271 and longitude 92.778908) in the month of October, 2022. The samples were collected in a sterilized container, marked sampling site, stored in an ice box, and brought back to the laboratory, kept at 4 °C before nanoparticles were mixed (Dong et al., 2021). $AgSiO_2$ were added to the soil sample to

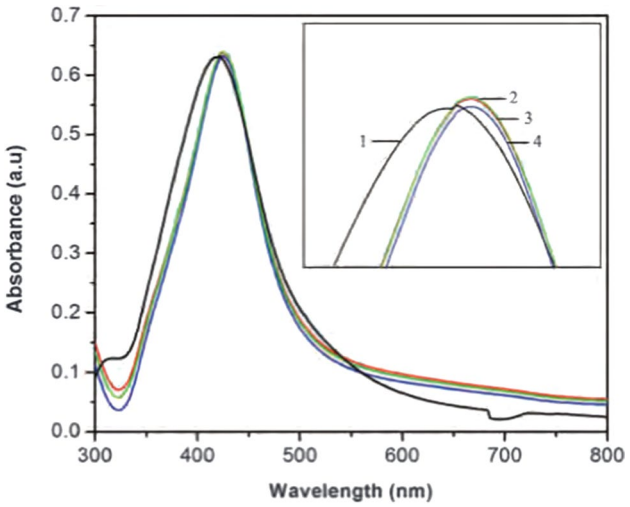

Fig. 1 UV–Vis absorption spectra of AgSiO2 (Acharya et al. 2017)

reach a concentration of 200 mg/kg, followed by intensive mixing with the soil (Yuan 2011). Soil without $AgSiO_2$ was used as the control. The incubation of the microcosm was done at 28 ℃ for 60 days. With the help of a commercially available Kit, metagenomic DNA was extracted from the 250 mg soil samples. 2 µl of the extracted DNA sample was electrophoresed on 0.8% agarose gel at 120 V for about 60 min. Additionally, 0.1 µl of the sample was loaded onto NanoDrop to assess the A260/280 ratio. Sampling was done after 60 days of incubation because most of the soil bacteria showed its effect and a 50% reduction by day 60 of inoculation (Samarajeewa, 2019).

Preparation of 2 × 300 MiSeqlibraries

Preparation of the amplicon library was done by using Nextera XT Index Kit (IIlumina inc.) according to the protocol of 16S metagenomic sequencing Library preparation. Primers for amplifying the 16S rDNA gene specific to bacteria were designed at the Eurofins Genomics Bioinformatics Lab. Amplification of the 16S rDNA gene directing the V3–V4 region specific for bacteria was carried out. An aliquot of 3 µl of the PCR product was assessed on a 1.2% agarose gel, subjected to electrophoresis at 120 V for around 60 min or until three-fourths of the gel length. The amplicons containing Illumina adaptors were amplified using i5 and i7 primers, which incorporated multiplexing index sequences and common adapters essential for cluster generation (P5 and P7), following the standard Illumina protocol. Subsequently, the amplicon libraries were purified using AMpure XP beads and quantified using a Qubit fluorometer.

Quantity and quality check (QC) of the library on Agilent 4200 Tape Station

The Agilent 4200 Tape Station was used to conduct both quantitative and qualitative library assessments. The amplified libraries were examined using the D1000 Screen tape, as per the manufacturer's instructions.

Cluster generation and sequencing

Following the determination of the mean peak size from the Tape Station profile, libraries were loaded onto the MiSeq system at an appropriate concentration (10–20 pM) for both cluster generation and sequencing. Paired-end sequencing was employed, enabling the sequencing of template fragments in both the forward and reverse directions on the MiSeq platform. Kit reagents facilitated the binding of samples to complementary adapter oligos on paired-end flow cells. These adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then utilized to sequence from the opposite end of the fragment. Utilizing information regarding taxonomic assignments, coverage, and GC content, the assembly was further categorized into bacterial taxa.

Results

Succession of taxonomic groups at the phylum levels/ Effect of $AgSio_2$ on specific bacterial populations at the phylum and genus level

The data obtained from Illumina sequencing was utilized to assess how the presence of nanoparticles impacted the diversity and abundance of bacteria. After applying quality filters, a total of 579,858 high-quality reads were attained from two samples. The Illumina data revealed that the untreated sample had 1913 observed operational taxonomic units (OTUs), while the treated sample had 1628 OTUs. Furthermore, numerous diversity indices including Simpson 1-D, Shannon H, Evenness, and Dominance are depicted in Fig. 2 to examine the potential adverse effects of nanoparticles on bacterial richness and diversity. Following a 60 day incubation period, the addition of nanoparticles resulted in a slight but significant reduction in the number of OTUs and the Shannon index when compared to the control treatment. Additionally, the rarefaction curve (Fig. 2C) demonstrated a higher species richness in the untreated sample compared to the treated sample.

To identify specific bacterial populations that may be affected by $AgSiO_2$, the relative abundance and comparison of the phylotypes in treated soil samples were summarized

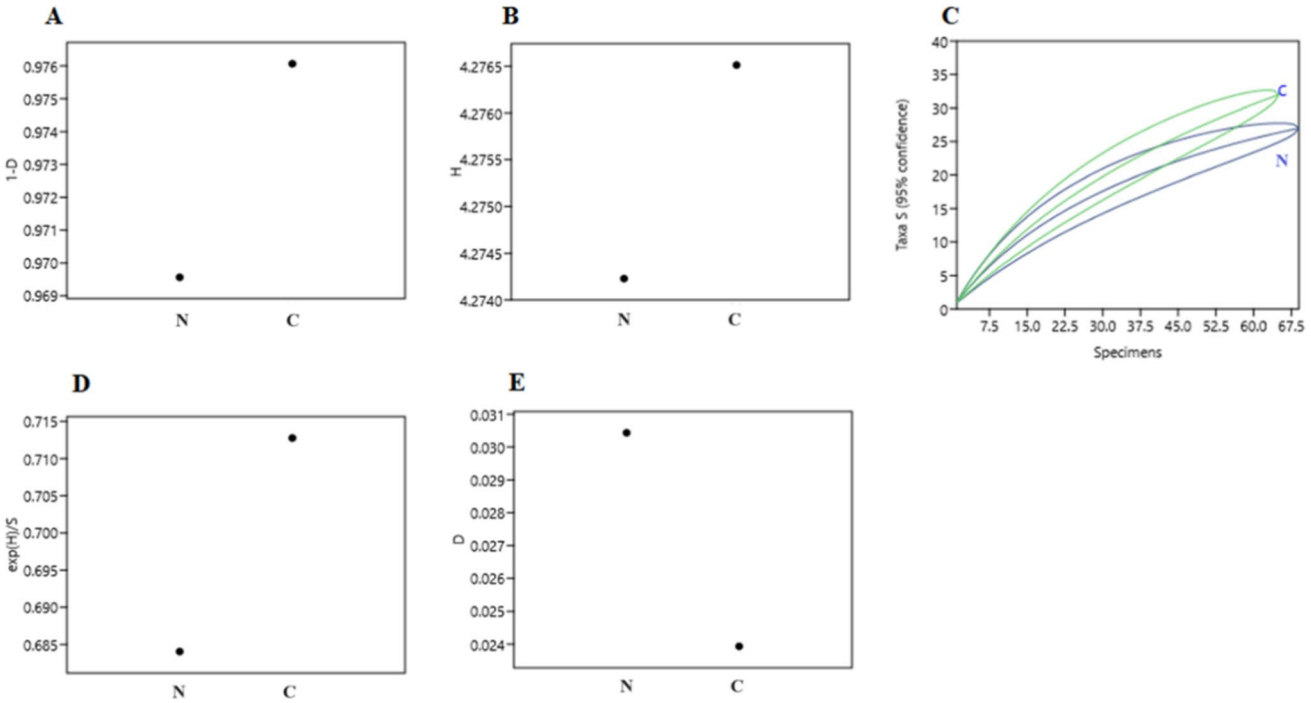

Fig. 2 Diversity indices (**A**. Simpson, **B**. Shannon, **D**. Evenness and **E**. Dominance) and **C**. rarefaction curve among nano treated and untreated sample (C-Control, untreated; N- Nano treated sample

at the phylum, class, and genus levels. The phyla distribution of the 16S rRNA from the treated and untreated samples is shown in Fig. 3. The analysis of the sequences showed the bacterial community was dominated by eight abundant phyla in the control (untreated) sample namely Proteobacteria, Actinobacteria, Firmicutes, Chloroflexi, Acidobacteria, WPS2 (candidate phyla), Planctomycetes and Verrucomicrobia; whereas in treated sample Proteobacteria, Acidobacteria, Actinobacteria and Planctomycetes were abundant. Among the phyla, Proteobacteria was the most abundant, accounting for 30.20% and 28.90% of the total bacterial population in the nano-treated and untreated samples, respectively. Also, the relative abundances of Actinobacteria and Fimicutess were 17.31% and 16.78% respectively, in the untreated sample.

In the case of soil samples treated with $AgSiO_2$ nanoparticles, Proteobacteria, and Acidobacteria were dominant phyla, but their relative abundances changed significantly compared with that of control (untreated). The clones belonging to Proteobacteria and Acidobacteria increased significantly to 30.15% and 20.68% respectively, in the treated sample, whereas in the untreated sample, it was only 29% and 17% respectively. However, the Firmicutes phyla (4.9%) and the Chloroflexi (7.88%) decreased in comparison to control. The other phylum that showed a decrease in abundance were TM7 (0.51%), Euryarchaeota (0.15%), Cyanobacteria (0.07%), Aquificae (0.25%) as revealed by the decrease in relative abundance after the application of $AgSiO_2$ nanoparticles.

Interestingly, the most significant variations in microbial community composition across all treatments occurred in the phyla WPS 2 and TM7, wherein $AgSiO_2$ treated sample the abundance decreased to 0.1% and 4.90% in comparison to the control where the abundance was 7.52% and 16.80% respectively.

Succession of taxonomic groups at the genus level

The soil microbial community at the genus level was analyzed to establish which genera were influenced by $AgSiO_2$ nanoparticles. In the control (untreated) sample, the most abundant genera were unclassified genera from the WPS − 2 (7.52%) phylum and surprisingly the abundance decreased to 0.1% in the treated sample. Moreover, in the $AgSiO_2$-treated sample, the *Candidatus Koribacter* was still dominant. Interestingly, the abundance of the dominant genera is different in two different soil samples.

In the control sample, the genera viz., *Kaistobacter* (6.11%), and *Rhodoplanes* (3.94%) under the phylum Proteobacteria were found to be abundant and relatively diverse. The other abundant genera of Phylum Proteobacteria were *Bradyrhizobium* (2.56%), unclassified *Sinobacteraceae* (2.42%), *Betaproteobacteria* (2.36%), *Rhodospirillaceae* (1.81%), and *Methlosinus* (1.79%). From the Phylum

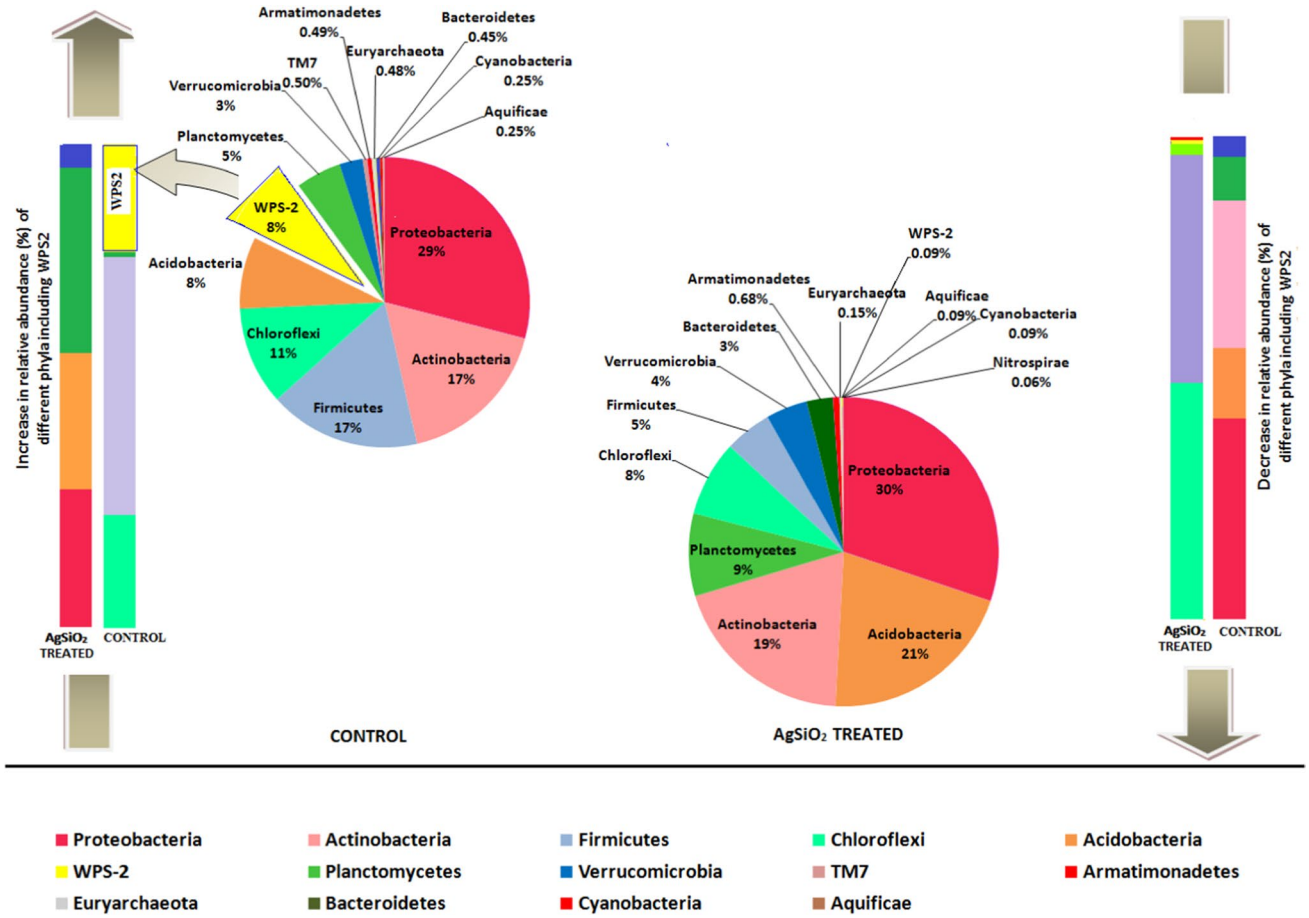

Fig. 3 Relative abundance of bacterial diversity (%) at phylum level in the soil microbiota of the control sample and AgSiO2 treated sample. The control sample indicates the soil without nanoparticles. The pie charts signify the differences in relative abundance (RA) between the control and nano-treated soils for the abundant and rare phylotypes at the phylum level after incubation of 60 days. The bars represent the increase and decrease in relative abundance of the two samples in different phyla. The phylum WPS2 was found to be abundant in the control sample whereas in nano treated sample the abundance was very low

Actinobacteria, mainly unclassified *Streptomycetaceae* (5.67%), and *Streptomyces* (2.37%) were present. Whereas, in phylum Firmicutes, mainly *Clostridium* (2.91%) was found to be abundant.

In contrast, in the $AgSiO_2$ nanoparticle amended sample, members of Acidobacteria namely *Candidatus Koribacter* (11.88%) were highly dominant. The genera of the phyla Actinobacteria and Proteobacteria present in nano treated sample are, *Mycobacterium* (6.64%), *Streptomyces* (3.12%), and unclassified genus of family *Strptomycetaceae* (1.51%) (Fig. 4).

Comparison between control (untreated) and $AgSiO_2$ treated samples at the phylum and genus level

The largest differences in microbial symbiotic community composition among all treatments occurred in the phyla WPS 2. Another unclassified genus from the member of phylum Chloroflexi was completely absent in the $AgSiO_2$ treated sample. Inexplicably, the abundance of some phylotypes including Acidobacteria, Proteobacteria, and Actinobacteria was enriched, after the application of $AgSiO_2$. The phylum Proteobacteria, Actinobacteria, and Firmicutes were located in the top three among all bacterial phyla in the control sample, which made an average of 28.91%, 17.31% and 16.80% respectively, and in $AgSiO_2$ nanoparticle treated sample the abundant phyla were Proteobacteria, Acidobacteria, and Actinobacteria at an average of 30.15%, 20.68% and 19.49% respectively.

A heatmap is produced to envisage the OTU table at the phylum level, with each row representing an OTU and each column representing a sample (Fig. 5). The greater the relative abundance of an OTU in a sample, the more vibrant the color at the corresponding position in the heatmap. In the heatmap red implies a low percentage of OTU contributions

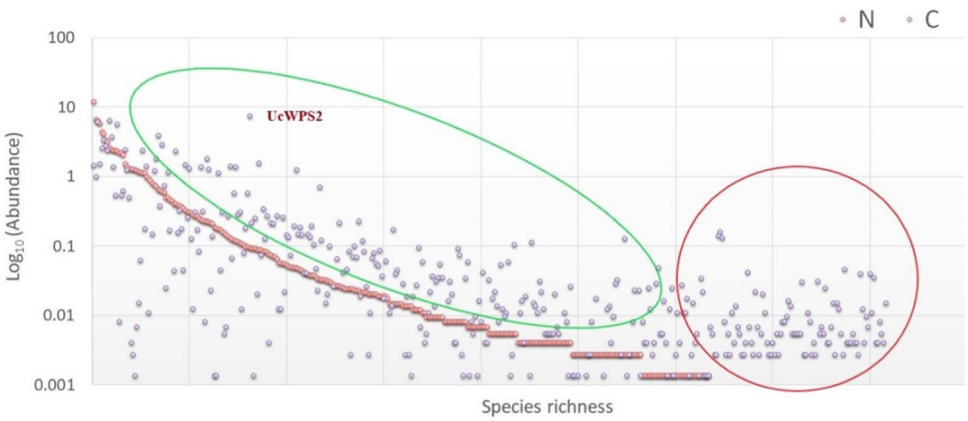

Fig. 4 Relative abundance of bacterial genera in AgSiO2-treated and untreated samples

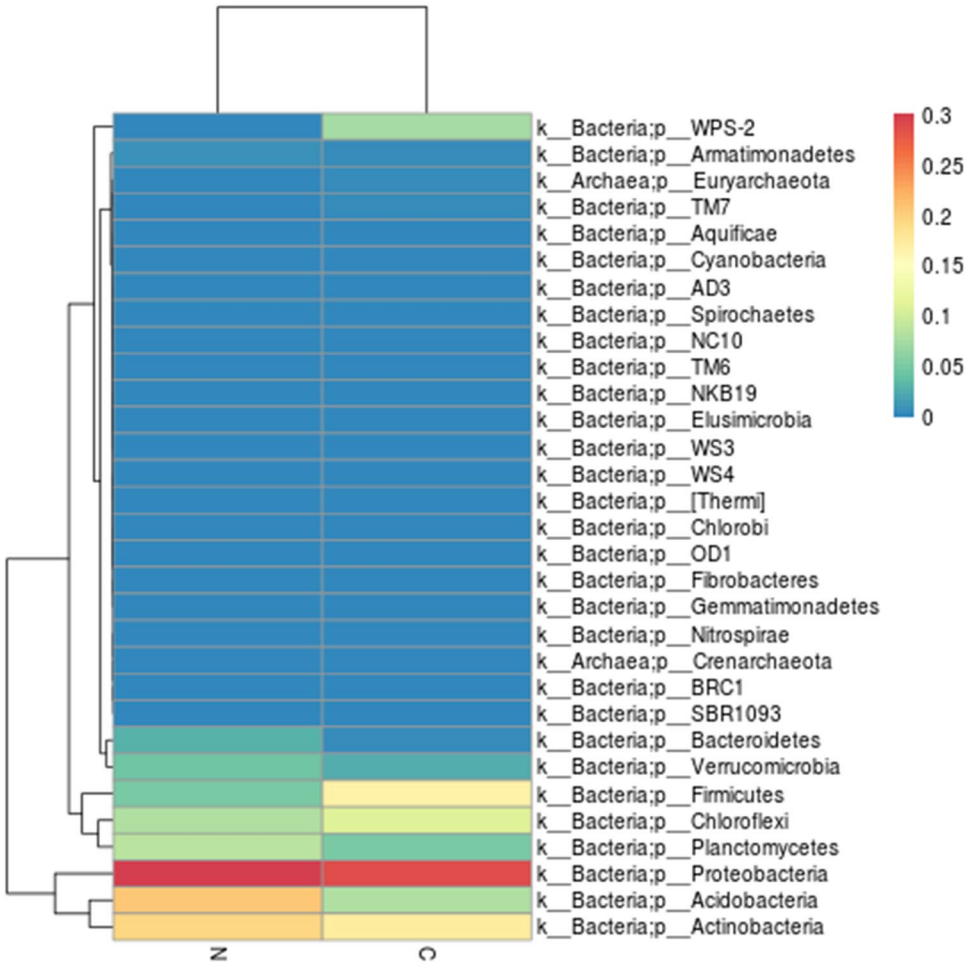

Fig. 5 Heatmap of the metagenomic analysis of the soil samples representing that in the control sample, the abundance of Chloroflexi, Firmicutes, and WPS2 are higher abundance and the phyla Proteobacteria, Actinobacteria, and Acidobacteria were in high abundance in nano treated sample. The remaining bacteria were found to be almost equal in abundance and hence no difference in color was observed

to the sample; while purple implies a high percentage of OTU contributions to the sample.

Correlation analysis between the treated and untreated sample

Correlations among the bacterial genera of the treated and untreated sample were determined based on Spearman's rank correlation (Fig. 6). The phylum WPS2 was positively correlated with the phyla TM7, Nitrospirae, Acquificae, Chloroflexi, Firmicutes ($p < 0.05$). Interestingly, the phylum WPS2 was negatively correlated with the phylum Actinobacteria, Proteobacteria, Acidobacteria Planctomycetes, Bacteroidetes, etc. ($p < 0.05$). In addition, the phylum WPS2 and Firmicutes in the control sample were positively correlated.

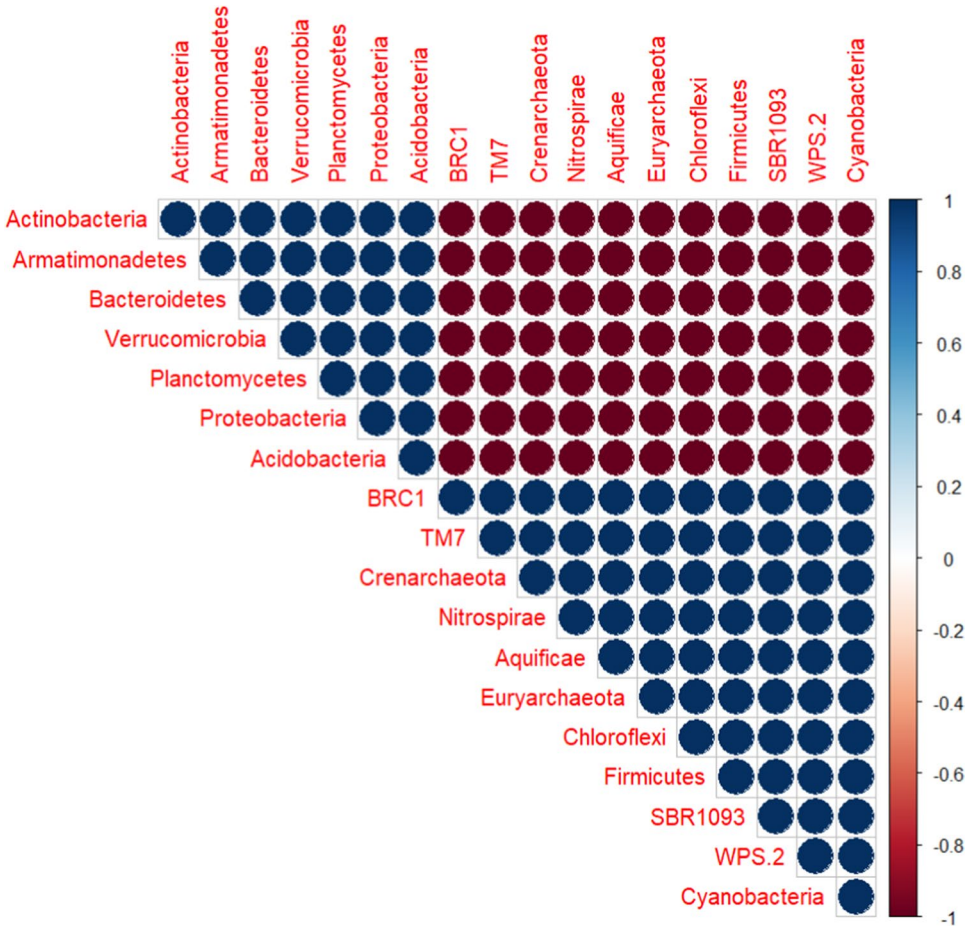

Fig. 6 Correlation matrix depicting relationships among bacterial genera. Spearman's rank correlation coefficients, ranging from 1.0 to − 1.0 (indicating strongly positive to strongly negative correlation), have been organized in hierarchical order and illustrated in the correlation plot

Functional analysis of predicted metabolic pathways of the bacterial community

The metabolic pathways in the bacterial community of both treated and untreated soil samples were predicted using the 16S rRNA gene data through the recently developed software PICRUSt. The predominant metabolic pathways, which account for more than 1% of the predicted genes in the bacterial community, are highlighted in Fig. 7. The carbon fixation, nitrogen metabolism, methane metabolism, oxidative phosphorylation, sulfur metabolism, energy metabolism, peptidases, and specific secretion systems were observed in both the treated and untreated samples. However, the untreated soil sample where WPS2 was abundant, had a higher representation of function related to bacterial motility proteins, nitrogen metabolism, carbon fixation, bacterial chemotaxis, oxidative phosphorylation, and photosynthesis proteins. However, the function of ABC transporter, methane metabolism, porphyrin, and chlorophyll metabolism had a higher representation in nano-treated soil samples.

Discussion

Soil bacterial communities are crucial for delivering numerous ecosystem benefits that directly and indirectly influence the overall functioning of the soil environment. In the present study bacterial communities of soil samples, with and without $AgSiO_2$ amendment had been analyzed. In the present study, a total of 32 phyla including Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes, Chloroflexi, and Planctomycetes were retrieved at varying abundance in both the $AgSiO_2$ nanoparticle treated, and untreated samples. Zhang et al. (2020) also reported similar findings where 11 bacterial phyla were identified from AgNP contaminated soil of China, with remarkable dominance of Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, and Planctomycetes. Moreover, few of the phyla could not be identified pertaining to the limitations of databases used in the analysis.

In the $AgSiO_2$ nanoparticle-treated sample, the most abundant phyla were Proteobacteria (30.15%), whereas in the untreated sample, its abundance was only (28.91%). Also, observation by Mishra et al. (2020) confirmed the

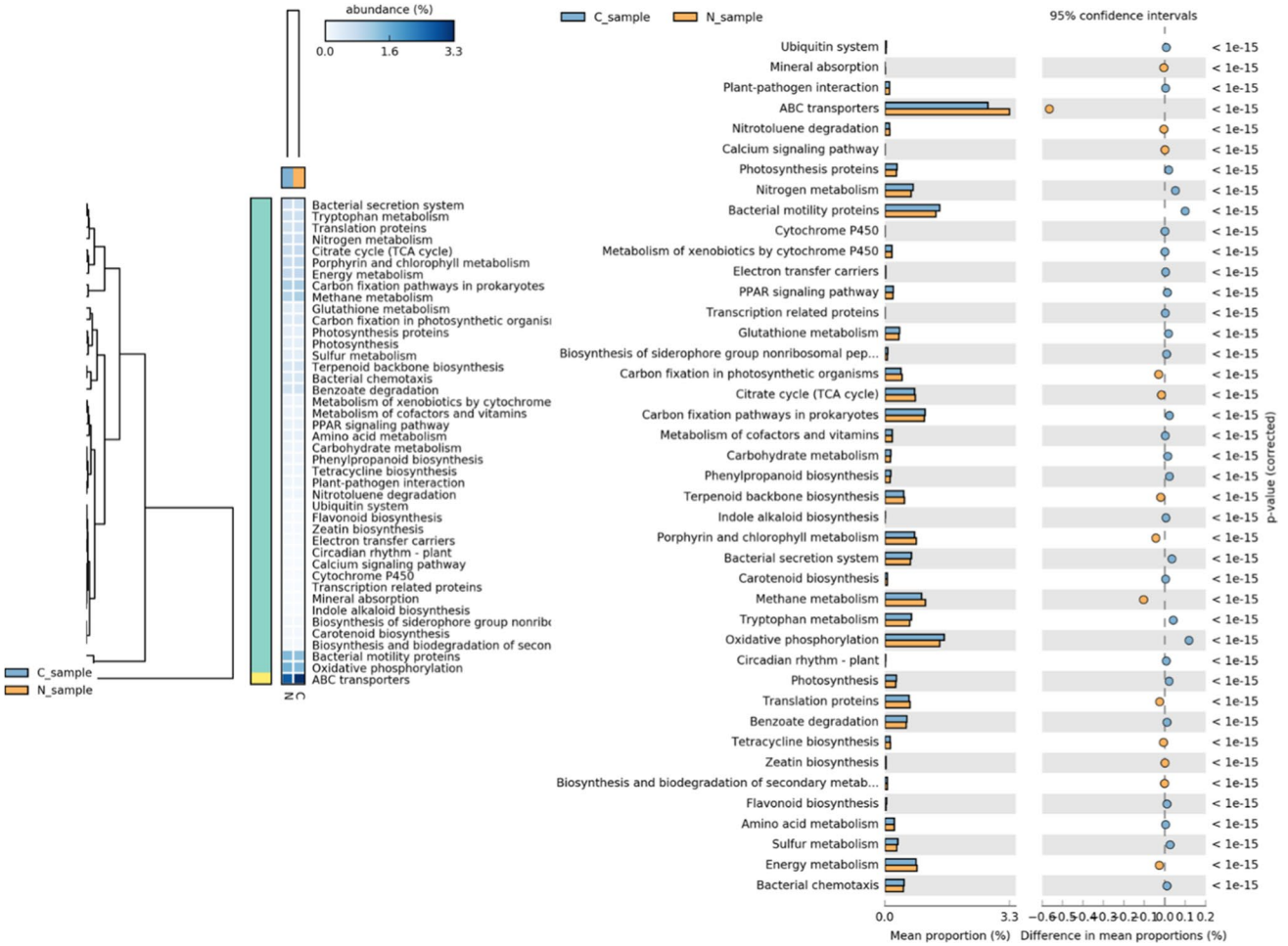

Fig. 7 Relative abundance of genes associated with different metabolic activities in AgSiO2-treated and untreated soil samples

dominance of Proteobacteria in AgNPs contaminated soil samples, establishing the high resistance ability of the bacteria. As Proteobacteria are found in nutrient-rich soils and are important in the nitrogen and sulfur cycle, the resistance of Proteobacteria after the application of nanoparticles might explain the preserving good properties in soil (Begmatov et al., 2021). Other dominant phyla in our dataset were Actinobacteria (17.31%) and Firmicutes (16.78%) in the untreated sample, while in the $AgSiO_2$ nanoparticle-treated sample the abundant phyla are Acidobacteria (20.68%) and Actinobacteria (19.49%), that is similar with the AgNPs contaminated area of China where Acidobacteria and Actinobacteria were among the abundant phyla (Begmatov et al., 2021). The dominance of bacterial genera in AgSiO2-treated samples suggests the resistance of the particular group of bacterial community to $AgSiO_2$ nanoparticles and this is in agreement with Kumar (2011) where the genera under the family Bradyrhizobiaceae were observed to be resistant to AgNPs. This is due to the resistance ability of certain microbes due to genetic mutation or the incompatible concentration of nanoparticles to a few microbial genera that could not interact and show the effects (Bruna et al. 2021). This resistance may facilitate bacterial multiplication in the presence of nanoparticles, coupled with spore formation abilities that contribute to their survival during acute exposures (Qafoku 2010; Yonathan et al. 2022).

Whereas, notably the representatives of Firmicutes (16.78%) and WPS 2 (7.52%) were dominant in the untreated sample, while in the $AgSiO_2$ amended sample, the abundance of the phyla was reduced to (4.9%) and (0.1%) respectively, which might be due to the harmful effects of high concentration of $AgSiO_2$ on the soil microbial community and presence of inorganic ligands and organic chelators matrix as suggested previously (Xiu et al. 2011; Sillen et al. 2020). As WPS 2 helps in the carbon and nitrogen cycle, it could serve as a bioindicator of soil quality (Vandekerckhove et al. 2000; De Mandal et al. 2015). The abundance of certain bacteria in the untreated sample and the decrease in abundance after $AgsiO_2$ exposure suggests that soil microorganisms remained sensitive to minimum levels

of $AgsiO_2$ nanoparticles. According to Ihtisham (2021), the impact of nanoparticles on soil microbial enzymatic activities can either stimulate or inhibit, depending on factors such as microorganism type, soil texture, osmotic potential, size, shape, and AgNPs concentration. Hence, the impact of $AgSiO_2$ nanoparticles on various microbial communities varies.

WPS2 bacteria were first discovered in soil contaminated with polychlorinated biphenyls (PCBs) (Nogales et al. 2001; Shermet et al. 2020), and have subsequently been found in various other soil types (Costello et al. 2009; Grasby et al. 2013). WPS2 is also abundant in several organic-poor soil environments and has a role in autotrophic CO_2 fixation and scavenging of atmospheric H_2 (Ji et al., 2016), therefore it is necessary to study the effect of $AgSiO_2$ on WPS2 phyla. The decrease in abundance of WPS2 after the treatment with $AgSiO_2$ nanoparticles can relate to the idea that the resistance was significantly lower in the $AgSiO_2$ stressed sample. This is mainly because the bacterial group does not exhibit similar reactions to the discussed compounds (Kumar, 2011). In the present study, within the bacterial communities, an uncommonly high predominance of candidate division WPS2 was also observed. Notably, such elevated levels of candidate division WPS2 abundance have not been previously documented whereas in the polychlorinated biphenyl-polluted soil, a low abundance of WPS2, below 2% was observed (Nogales et al. 2001; Grasbyet al. 2013). Therefore, $AgSiO_2$ nanoparticles exert a multifaceted impact on the particular bacterial phylum, they do not exhibit a straightforward toxic effect on the overall soil community. $AgSiO_2$ does influence the bacterial community, emphasizing the need for cautious management and prevention of their environmental disposal (Akafia 2011; Abdulsada et al. 2021).

Besides studying the differences in the composition of microbial communities, we also analyzed the functional genes associated with nitrogen cycling, methane cycling, carbon fixation, bacterial movement in response to $AgSiO_2$ treatment, and the variations in predicted gene counts between two distinct soil treatments. The predicted metagenomes revealed a wide range of functional genes related to nitrogen metabolism, including various nitrogen transformations such as ammonia assimilation, assimilatory nitrate reduction, dissimilatory nitrate reduction, and denitrification (Weigel et al., 2017). A higher number of genes in the function related to bacterial motility proteins, nitrogen metabolism, carbon fixation, bacterial chemotaxis, oxidative phosphorylation, and photosynthesis proteins in untreated control were observed. Following treatment, the functionality of beneficial bacteria like WPS2 might had decreased due to the occurrence of nano silver-induced impairment, which could aid in ROS scavenging cytotoxicity, and consequently, the abundance of beneficial functional genes also decreases (Weigel, 2017). Furthermore, the application of AgNPs might had led to the changes in the carbon and nitrogen cycle-related genes. Therefore, the reduction of beneficial genes in the soil microbial community demonstrates a detrimental effect on the soil environment. Hence, proper assessment and guidelines will be necessary for future nanotechnology research and applications to ensure that $AgSiO_2$ does not harm the ecosystem.

Conclusion

The ecotoxicity of the $AgSiO_2$ nanoparticles on the microbial community was analyzed by a metagenomic approach. Relative to untreated controls and nano-treatment, exposure to $AgSiO_2$ led to more pronounced alterations in community structure, including a significant reduction in species richness and a decrease in the abundance of Firmicutes and WPS 2. Varied susceptibilities to $AgSiO_2$ induced a shift in microbial community structure towards species more tolerant to silver, such as Acidobacteria and Bacteroidetes. The differential impact of $AgSiO_2$ on nitrifying bacteria, such as *Nitrosomonas* and *Nitrosococcus*, and *Chloroflexi* involved in nitrification, highlights a potential threat to nitrogen removal. Additional investigations are warranted to monitor silver fluxes and explore alternative barriers that safeguard against inhibitory effects and potential temporary system failure. In comparison to untreated and nano-treated samples, $AgSiO_2$ exposure resulted in more conspicuous changes in community structure, including a significant reduction in species abundance. Therefore, the mixing of AgNPs with chemicals to act as pesticides should be stopped as it kills beneficial bacteria and the release of AgNPs in the environment should be controlled strictly.

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Author contributions K.M.S. has performed experiments, analyzed data, and written the manuscript. L.P.S. has helped in preparing the manuscript and analyzing the data. D.B. has helped in synthesizing the AgNPs and analyzing the data. P.P. has conceptualized and designed the work, analyzed data; and prepared, reviewed, and finalized the manuscript.

Data availability DNA Sequences are available in NCBI database under the bioproject number (PRJNA411980). The data that support the findings of this study are available on request from the corresponding author.

Declarations

Conflict of interest Authors declare no conflict of interest.

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