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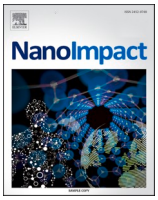

Analytical methods for quantifying PS and PVC Nanoplastic attachment to activated sludge Bacteria and their impact on community structure

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ABSTRACT

Nanoplastics are anticipated to be ubiquitous in various environmental compartments. However, challenges in analytical methods hinder our understanding of risks related to specific nanplastics characteristics such as size and chemical compositions, and interactions between nanoplastics and microorganisms. In this study, we applied fit-for-purpose analytical methods and techniques to understand how nanoplastic chemical composition influences their interaction with bacteria collected from activated sludge. When exposed to polystyrene (PS) and polyvinyl chloride (PVC) nanoplastics for 5 days, the nanoplastics attached to the bacteria. Specifically, on day 1, there was a significant predominance of PS nanoplastics over PVC ones of similar size and shape, possibly due to differences in their chemical composition. After 5 days, there is a substantial decrease in nanoplastics attached to bacteria, suggesting bacterial defence mechanisms may reduce particles attachment over time. The overall bacterial community structure demonstrated a high degree of resilience. This resilience highlights the ability of microbial communities to maintain their structure despite nanoplastic stressors, as evidenced by consistent alpha diversity, PCoA, and PERMANOVA results. Understanding these mechanisms is crucial for assessing nanoplastic fate and thus environmental impacts.

1. Introduction

Numerous studies have highlighted an escalating trend in environmental plastic pollution, with forecasts indicating a potential accumulation of approximately 100–250 million tons by 2025 (Avio et al., 2017). Over time, larger plastics undergo a series of degradation processes in the environment, including photodegradation, biodegradation, hydrolysis, erosion, photooxidation, and mechanical breakdown (Li, 2018). These processes result in the generation of microplastics (MP; 5 mm $>$ size $>1\mu$m) and nanoplastics (size <1 μm) of different sizes, shapes, and chemical compositions, e.g. polystyrene (PS), polyethylene and polyvinyl chloride (PVC) (Gigault et al., 2016). In recent decades, increasing attention has been directed towards the degradation and occurrence of plastic waste in remote environments, particularly in seas (Gigault et al., 2016). This focus has led to reports identifying the presence of microplastics and potentially nanoplastics in various contexts. Documented matrices include sediments (Nuelle et al., 2014), oceanic water (Andrady, 2011) and freshwater (Singh et al., 2021). In urban areas, a primary source of nanoplastics is their direct emission from diverse industrial activities, as well as from degradation and erosion of materials like paints and tires (Liu et al., 2019a; Wright et al., 2020). Only a limited number of studies have investigated the fate and adverse effects of nanoplastic pollution in urban areas. A significant portion of urban nanoplastics ultimately ends up in wastewater treatment plants (WWTPs) (Carr et al., 2016: van Wezel et al., 2016). Although it has been reported that WWTPs effectively remove a high percentage of microplastics from wastewater, predominantly through entrapment within microbial aggregates (Altmann et al., 2023), no data

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has been reported for nanoplastics. It has been estimated, however, that WWTPs could serve as significant sites for the collection and accumulation of nanoplastics (Xu et al., 2023).

The activated sludge (AS) process is a widely used method in WWTPs for treating and purifying wastewater. It is a biological treatment process that relies on the activity of microorganisms to break down organic matter and pollutants from the wastewater (Singh et al., 2022). The microbial communities within the AS system engage in intricate and complex interactions, forming a highly dynamic network comprising a diverse array of microorganisms (Singh et al., 2022). It is within this dynamic network that the AS system gains the capability to carry out a multitude of essential functions, including the removal of oxygen-depleting organic compounds and the processing of nutrients. Thus, bacteria play a pivotal and indispensable role in AS systems (Gu et al., 2008). The presence of nanoplastics in AS has the potential to alter the intricate interaction network among bacterial populations, thereby impacting microbial processes (Liu et al., 2019b). Previous studies have demonstrated that microplastics in AS systems can have a negative effect on the nitrogen transformation process by reducing the diversity of microorganisms (He et al., 2021). The impact of nanoplastics on AS bacteria remains relatively unexplored, despite the expected much higher nanoplastic concentrations compared to microplastics (Xu et al., 2023).

Understanding the interaction between nanoplastics and bacterial composition in AS systems poses a challenge due to the lack of methods and techniques for tracking and quantifying nanoplastics in complex matrices (Abdolahpur Monikh et al., 2021). These methods must navigate the complex matrices of AS, which contain various organic materials that may interfere with nanoplastic detection and characterization (Abdolahpur Monikh et al., 2019b). Since the effects of nanoplastics depend on their type, size, dosage, charge, and exposure time (Abdolahpur Monikh et al., 2022b), the methods must quantify single nanoplastics within or on bacteria and link specific particle characteristics to observed effects. Developing such methods is essential for comprehensively assessing the impacts of nanoplastics on bacterial communities in AS environments.

This study utilizes innovative analytical techniques and establishes nano-specific protocols to examine the effects of two types of nanoplastics, PS and PVC, on four isolated bacteria in AS medium. Additionally, it investigates the attachment of nanoplastics to the bacteria as a function of the chemical composition of the nanoplastics. To address the analytical challenges associated with detecting, tracking, and quantifying nanoplastics in AS, we utilized gadolinium (Gd) entrapped in the polymeric particle structure (Bandekar et al., 2022). As a rare element, Gd serves as a proxy for the particles (Abdolahpur Monikh et al., 2022a). Quantification was achieved using Single Particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS), a powerful method for measuring the number of nanoparticles (Abdolahpur Monikh et al., 2022a). We developed a sample preparation protocol for isolating and characterizing bacteria and their associated nanoplastics. PS and PVC were chosen as model nanoplastics due to their prevalence in the environment.

2. Materials and method

2.1. Chemicals and materials

All chemicals utilized were of analytical reagent grade and obtained from Sigma-Aldrich. The nanoplastics, PS (250 nm, polydispersity Index (PDI): 0.1) and PVC (250 nm, PDI: 0.2), dispersed in Tween 20, were procured from CD-Bioparticles, located in NY 11967. The Gd ions were entrapped within the particles and subsequently, post-preparation, thorough washing was conducted to eliminate any residual free Gd. We requested these specific particles to be synthesized by the company. The amount of Gd in the particles was 9.7% − 11%. We used Gd due to it's rarity prevents natural occurrence within microrganisms' bodies and it's suitability for precise detection and quantification via ICP-MS. Milli-Q water, used throughout the experiments, was provided by a Millipore filtration system, specifically the RiOs Essential 16 Water Purification System. R2A agar (ROTH, Germany) and Tryptone Soya Broth medium (HiMedia, India) were respectively used for isolation of bacteria from AS and maintenance of the isolated strains.

2.2. Particle characterization

Dynamic Light Scattering (DLS) was employed to determine the hydrodynamic diameter and Zeta potential (ζ) of the nanoplastics in Milli-Q water and in the AS water. These measurements were conducted using a Zetasizer Nano-ZS (Malvern Panalytical, Malvern, U.K). For the purpose of imaging using Transmission Electron Microscopy (TEM), sample preparation involved the drop-coating of the nanoplastics suspension following the previous method (Abdolahpur Monikh et al., 2022a), onto carbon-coated copper TEM grids. The coated grids were then scanned using a EOL JEM-2100F (JEOL Corp., Tokyo, Japan) operating at an accelerating voltage of 120 kV. The size of the particles was determined by manual measurement, allowing for the detailed visualization and size characterization of the nanoparticles at the nanoscale level. The stability of nanoplastics against homoaggregation in AS water was assessed by monitoring the number of particles over time using spICP-MS (PerkinElmer NexION 350D) (Supporting information, Section 1). By measuring the concentration of nanoplastics at various time intervals, changes in the number of particles were tracked, providing insights into the degree of homoaggregation occurring within the AS water. The density of the nanoplastics was determined by measuring the contact angle (A KSV Cam 200 contact angle) in Milli-Q water at room temperature.

2.3. Sampling of the activated sludge water

The AS water sample was obtained from the WWTP in Liberec, Czech Republic, situated at the geographical coordinates 50°46′51.9"N 15°01′57.4″E (Supporting information section 2). A volume of 2L of AS water was sterilized via filtration through 0.2 μm pore size filters (Merck Millipore, Germany). This sterilized AS water was subsequently utilized in the experiments as an exposure medium. Non-filtered AS water was used for isolation of single bacterial strains described in section 2.4.

2.4. Isolation of single bacterial strains from AS

Freshly collected AS water samples were subjected to a 10-fold serial dilution and subsequently plated on R2A agar medium (ROTH, Germany). The plates were incubated at 25 °C for 48 h. Based on distinct colony morphologies, 10 single colonies were selected for Sanger sequencing analysis on an ABI3500 system (Thermo Fisher Scientific). Bacterial strains were identified based on a 97% sequence similarity criterion utilizing BLASTn searches in NCBI database. These strains were identified as belonging to the genera *Empedobacter*, *Glutamicibacter*, *Acinetobacter*, and *Rhodococcus*, with respective accession numbers NR_145933.1, NR_025612.1, NR_117628.1, and NR_041775.1. These are common bacteria in AS (Aimale-Troy et al., 2024: Aimale-Troy et al., 2024: Li et al., 2024).

2.5. Bacterial inoculum

Each isolated bacterial strain was cultured in Tryptone Soya Broth medium (HIMEDIA, India) at a constant temperature of 25 °C for a duration of 24 h. Post-incubation, the overnight cultures were subjected to centrifugation, followed by a single washing step. The resulting bacterial pellet was then resuspended in a physiological saline solution (0.89% NaCl). The optical density of the bacterial suspension was adjusted to a value of 1 at 600 nm wavelength (OD600) to standardize cell density. Subsequently, an equal volume of each bacterial strain was

combined and thoroughly mixed in a flask to prepare a homogeneous inoculum containing four strains.

2.6. Exposure of bacteria to nanoplastics in AS

The nanoplastics, i.e. PVC and PS, were separately introduced into two tubes, each containing 15 mL of filter-sterilized AS water, to achieve a final nanoparticle concentration of 50 mg/L ($4{,}7 \times 10^9$ particle /mL for PS and $3{,}7 \times 10^9$ particle /mL for PVC). Subsequently, these tubes were inoculated with the prepared mixed bacterial inoculum, ensuring that the optical density at 600 nm (OD600) was maintained at 0.1. For control purposes, an additional tube containing only filter-sterilized AS water and the mixed bacterial inoculum, without any nanoparticles, was prepared. All experimental samples, including those with PVC and PS nanoparticles and the control without nanoplastics, were set up in quadruplicate. The incubation of these samples was carried out at a temperature of 25 °C over a period of 5 days following a standard method (Kamika and Tekere, 2017). To facilitate 16S rRNA gene sequencing analysis, subsamples were collected at two distinct time points: day 1 and after 5 days.

2.7. 16S rRNA gene sequencing and data analysis

The 16S rRNA sequencing and data analysis were described in detail in previous publications (Nguyen et al., 2021; Nguyen et al., 2023) and Supporting Information section 3. Briefly, DNA extraction from the samples was conducted by using the FastDNA Spin Kit for Soil (MP Biomedicals, CA, USA), adhering strictly to the manufacturer's instructions. After extraction, the DNA concentration was quantified utilizing a Qubit 2.0 fluorometer (Life Technologies, USA). Each sample underwent two consecutive polymerase chain reaction (PCR) processes to amplify the DNA from the V4 region of the 16S rRNA gene. This involved the use of both normal and barcode fusion primers. In silico analysis of these primers was conducted to maximize diversity coverage while maintaining the amplicon size below 400 base pairs. The primer pair used for targeting the V4 region was 515F (5′-TGCCAGCMGCNGCGG-3′) (Dowd et al., 2008), and 802R (5′-TACNVGGGTATCTAATCC-3′) (Claesson et al., 2010). The sequencing of these PCR-amplified DNA fragments (amplicons) was carried out using the Ion Torrent Genexus System (Thermo Fisher Scientific, USA).

2.8. Quantification of nanoplastic attached to bacteria

To quantify the number of particles attached to bacteria, we first separated the unattached nanoplastics from the samples. On day 1 and 5, 1 mL of each treatment was transferred into a 10 mL conical tube containing 0.9 mL of phosphate-buffered saline (PBS). The samples were centrifuged using a Sorvall RC 5B plus centrifuge equipped with a Fiberlixxte F21–8 rotor, at 1500 ×g for 10 min at 4 °C. The supernatants, which were presumed to contain unbound nanoplastics, were then carefully removed. Subsequently, the remaining pellets were diluted with 5 ml of PBS (pH 7.4), re-dispersed by shaking, and then centrifuged again under the same conditions. To ensure the separation of bacteria from particles, we conducted independent experiments and applied a calculation method based on the approach described by (see Supporting Information section 4).

To allow quantification of the particle's number using spICP-MS, we have digested the obtained pellet. Tetramethylammonium hydroxide (TMAH) was utilized for this purpose, following the protocol established in our previous studies (Abdolahpur Monikh et al., 2021). Briefly, the pellets were mixed with 1 mL of 5% TMAH, shaken by hand, and sonicated using a model P30H Elmasonic bath sonicator (Elma Schmidbauer, Singen, Germany) for 10 min to aid in breaking down bacterial cells. The resulting samples kept for 1 h under fume hood. The obtained suspensions were diluted with MQ water and sonicated for 1 min. The dispersions, then were taken immediately after sonication to measure the number of nanoplastics by tracing Gd using spICP-MS. The instrumental setting for spICP-MS is shown in Table S1 (SI).

2.9. Data analysis

The raw sequencing data were analyzed using QIIME 2 version 2021.2 software (Bolyen et al., 2019) (See Supporting Information section 5). Taxonomic classification of Amplicon Sequence Variants (ASVs) was conducted using the q2-feature-classifier, utilizing the classify-sklearn naïve Bayes method against the Silva 138 reference database (Bokulich et al., 2018; Quast et al., 2013). The dissimilarity in bacterial community composition across samples was explored through Principal Coordinates Analysis (PCoA) using the Bray-Curtis distance, which was calculated based on relative abundances derived from non-rarefied data. Statistical comparisons between the samples exposed to nanoparticles and the control samples were carried out using the Permutational Multivariate Analysis of Variance (PERMANOVA) method.

3. Results and discussion

3.1. Nanoplastic characterization

We applied spherical PS and PVC as model of nanoplastics. The SEM images demonstrated that both the PS and PVC exhibit a spherical shape (Fig. 1a). To address the challenges inherent in the characterization and quantification of nanoplastics within cells, we utilized particles embedded with a unique metallic marker. Specifically, these particles were chemically entrapped with a rare element, Gd, as a tracer, following the methodology described in our previous work (Abdolahpur Monikh et al., 2022a; Bandekar et al., 2022). The ζ of the particles was measured, yielding values of -16 ± 2 mV for PS and -18 ± 1 mV for PVC. These values provide insight into the surface charge and stability of colloidal systems (Bandekar et al., 2022).

To assess the potential leaching of Gd from the particles, we conducted a stability test. This involved incubating the nanoplastics in AS water for 72 h at room temperature, followed by analyzing the Gd ions in the dispersion using spICP-MS. The spICP-MS technique is capable of differentiating between particulate and dissolved forms of Gd, thereby providing a clear distinction between Gd ions and Gd entrapped within the particles (Abdolahpur Monikh et al., 2021). Notably, no free Gd ions were detected in the supernatants, indicating the stability of Gd within the particle matrix. No significant changes were measured in the number of the particles in the AS medium on day 1 and 5 (Fig. 1b) indicating the stability of the particles. The hydrodynamic size of these nanoplastics over time was quantitatively assessed using DLS, which suggested the stability of the particles against homoaggregation (Fig. 1b), ensuring the stability of the particles allows interactions with bacteria to occur at the particle level rather than with aggregates. The presence of Gd increase the density of PS nanoplastics from ~1 to 1.3 g/cm^3 and the density of PVC nanoplastics from ~1.38 to 1.7 g/cm^3, respectively as reported by the producer.

3.2. Nanoplastic attachment to bacteria

Our previous study showed that the presence of Tween 20 and Gd at low concentration used is not toxic to bacteria (Bandekar et al., 2022). Fig. 2 shows the number of nanoplastics attached to the bacteria of filtered AS on day 1 and day 5. From the total number of the particles to which the bacteria were exposed, only ~0.01% for PS and ~ 0.007% for PVC were attached to the cells on day 1. Notably, despite having similar size and shape characteristics, the number of PS nanoplastics attached to bacteria is significantly (*t-test* $P < 0.05$) higher than the number of PVC nanoplastics on day 1 of exposure. One plausible explanation could be related to the different in the chemical compositions of the particles. The hydrophobicity and surface properties or affinity of PS nanoparticles

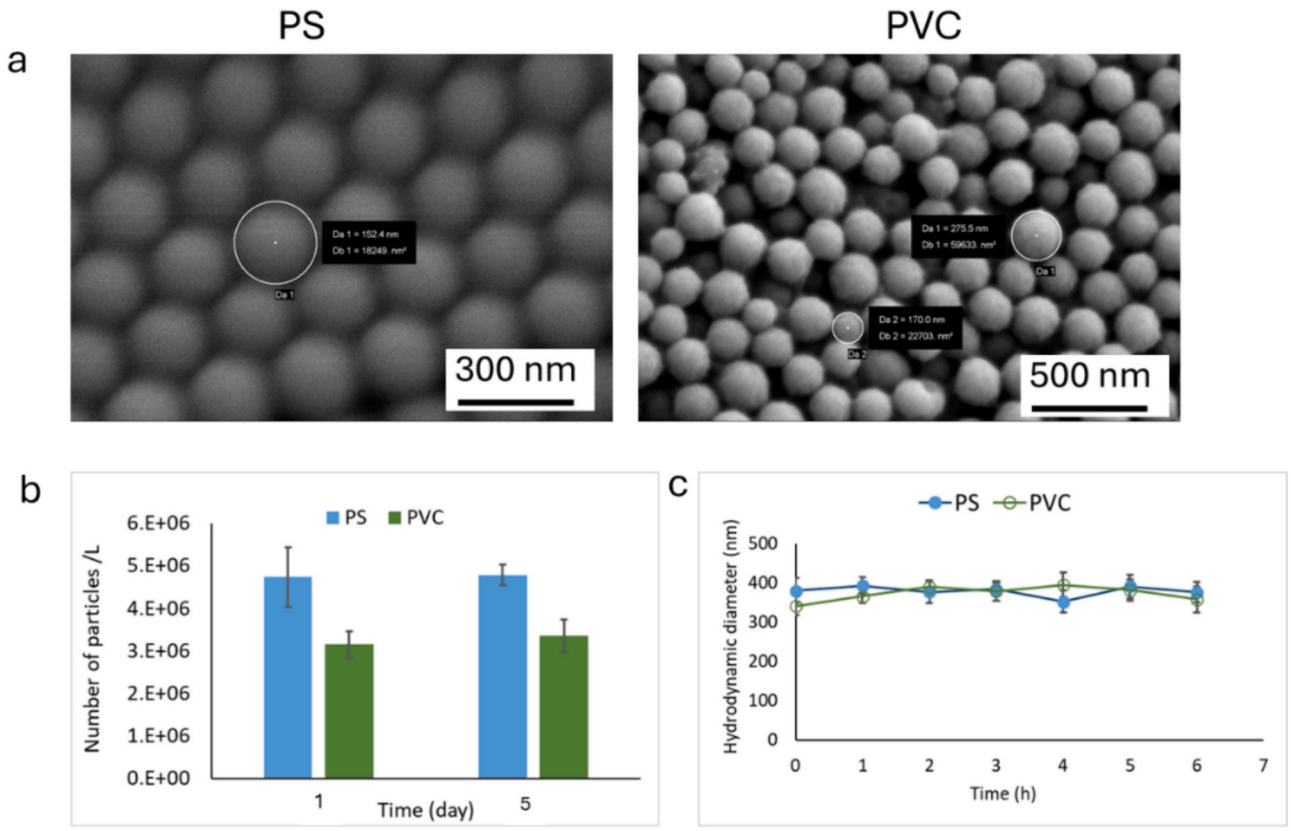

Fig. 1. a) SEM image of PS and PVC nanoplastics. b) The number (mean ± standard deviation) of measured PS and PVC nanoplastics on days 1 and 5 of mixing with AS water using spICP-MS. c) The hydrodynamic size (mean ± standard deviation) of the PS and PVC nanoplastics measured over time using DLS.

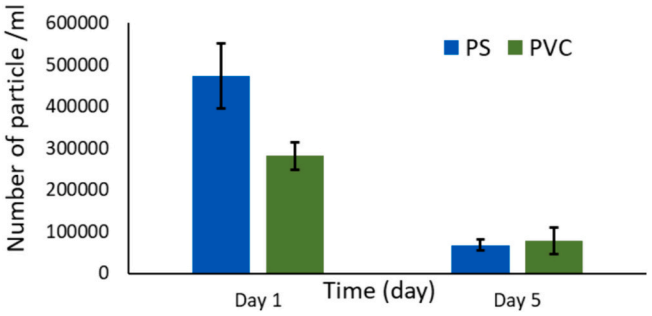

Fig. 2. The number (mean ± standard deviation) of nanoplastics attached to the bacteria on days 1 and 5 of exposure. The number was measured in 1 ml of the bacterial pellet solution after centrifugation.

might make them more prone to attachment or interaction with bacterial extracellular polymeric substances (EPS) than PVC nanoparticles. This emphasizes the importance of considering not only the physical characteristics but also the chemical properties of nanoparticles when studying their interactions with biological entities (Abdolahpur Monikh et al., 2022b).

A significant observation is the substantial decrease in the number of both PS and PVC nanoplastics attached to bacteria after 5 days. This trend suggests that over time, the bacteria may have mechanisms to eliminate or reduce the attachment of these particles as reported for other nanoparticles (Li et al., 2023) and also reported for algae (Abdolahpur Monikh et al., 2019a). The decrease in nanoplastic attachment might be attributed to various bacterial processes, including excretion of EPS, biofilm formation, or other cellular activities that modify the interactions with nanoparticles (Dey et al., 2023). One may hypothesize that the number of bacteria increases over the duration of exposure, potentially leading to a decrease in the number of particles on the cells. This scenario could occur if the initial number of nanoplastics to which cells are exposed is comparable to the number of the bacteria. However, in the context of this study, the number of nanoplastics greatly exceeds that of the cells. Therefore, cell growth would not significantly influence the number of the particles attaching to each cell. Understanding these mechanisms is crucial for evaluating the fate and potential environmental impacts of nanoplastics, especially considering their prevalence in various ecosystems.

3.3. *Influence of nanoplastics on bacteria*

Bacterial inoculum (*Empedobacter, Glutamicibacter, Acinetobacter*, and *Rhodococcus*) was exposed to PS and PVC nanoplastics in filter-sterilized AS. We have anticipated that by filtering the AS water through a 0.2 μm filter, only the bacteria from bacterial inoculum will be detected. However, sequencing analysis revealed a diverse array of bacterial taxa. This discrepancy might be due to ultramicrobacteria which are reportedly smaller than 0.2 μm, and can pass through the filter (0.2 μm) into the filtrate (Hahn, 2004; Maejima et al., 2018; Nakai, 2020; Rösel et al., 2012). This unexpected outcome suggests a complex microbial community in the AS water, highlighting the need for further exploration of overall microbial diversity and interactions in such environments. To better understand the complex microbial community, we analyzed the results of mixed bacterial inoculum taxa separately (in section 3.4) from the whole bacterial community. We only considered ASVs with a mean abundance of at least 0.1% for further analysis.The alpha diversity indices of the whole bacterial community (without bacterial inoculum) show an increase in diversity on day 5 for both control samples (Ctrl) and exposed samples (PS and PVC), as compared to the diversity levels observed on day 1 (Fig. 3a and b). Despite significant changes in bacterial relative abundances, no noticeable

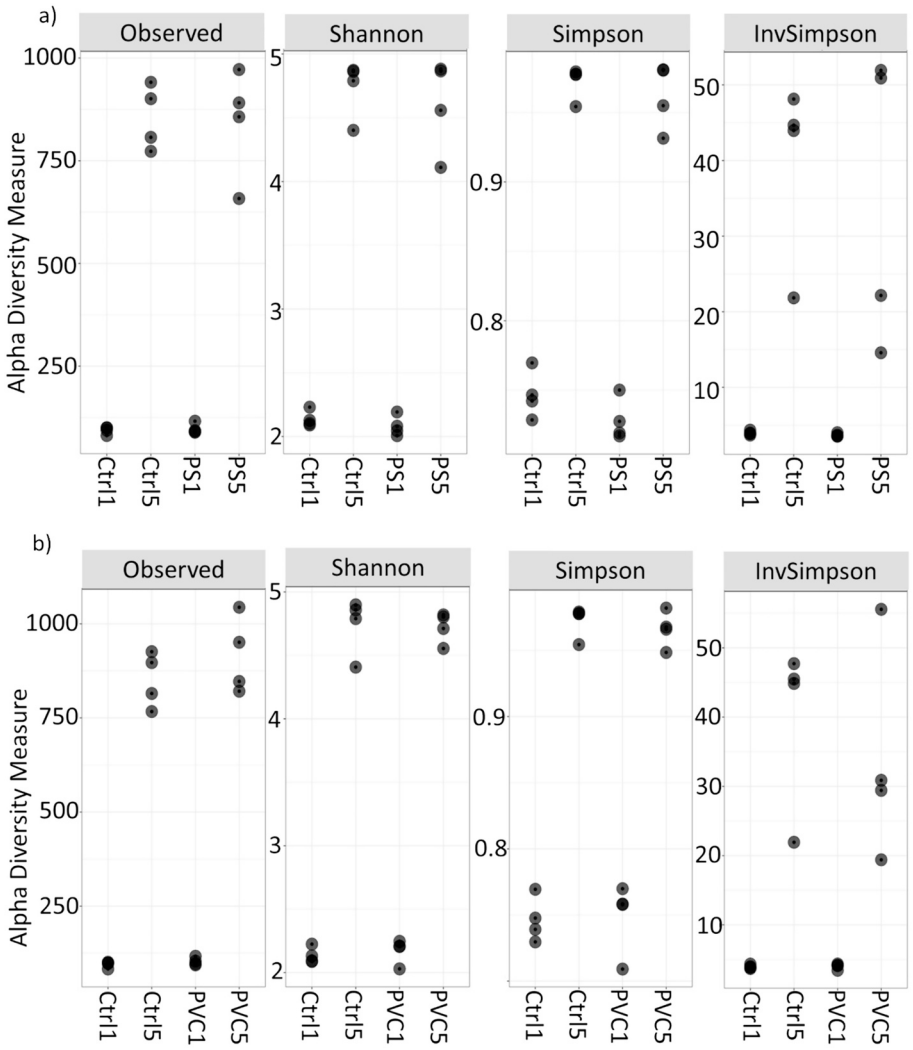

Fig. 3. Alpha diversity indices showing bacterial diversity in the control and in the media contain nanoplastics, i.e. PS (a) and PVC (b) on days 1 and 5. Each point is presented as a replicate in each sample. Each sample was quadruplicate.

distinctions in bacterial diversity were observed between the exposure to PS and PVC nanoplastics (Fig. 3a and b). This implies a degree of resilience in the bacterial community to nanoplastic exposure. The PCoA results further support the notion that the overall structure of the bacterial community remained relatively stable during exposure to PS and PVC compared to the control (Fig. 4). This stability, as reflected in the lack of any significant impact on community structure, suggests that the introduced nanoplastics may not have induced substantial shifts in the composition of the bacterial community. Previous studies showed that the richness (Chao 1) and the diversity (Shannon index) exhibited similar values between the control and PS nanoplastics (Zakaria et al., 2023; Zou et al., 2022). It was reported that, PVC did not significantly affect overall bacterial community diversity and composition in soil over the course of 35 days (Yan et al., 2021). Some specific bacterial phylotypes might be more abundant on PVC plastics (Pinto et al., 2019). The non-significant findings in PERMANOVA analyses (Table 1) reinforce the notion that, at the community level, there were no statistically discernible differences in bacterial composition among the treatment groups.

The absence of significant differences in the relative abundance of bacterial taxa between samples exposed to PVC and PS nanoplastics is an intriguing observation. At the starting time (day 1), the bacterial community (except for bacteria in the inoculum *Empedobacter, Glutamicibacter, Acinetobacter*, and *Rhodococcus*) featured the presence of six taxa, with a predominant affiliation to *Pseudomonas* (60%), followed by *Flavobacterium* (9.9%). Additionally, minor percentages of sequences were associated with bacterial groups *Pseudarcobacter*, *Janthinobacterium*, *Rhodoferax*, and *Aeromonas*. Interestingly, by day 5, all samples (Control, PVC, and PS) demonstrated an increased number of bacterial taxa. The control group primarily featured sequences affiliated with *Prosthecobacter* (14.7%) and *Pseudomonas* (12.6%). In contrast, PVC samples exhibited a higher abundance of *Prosthecobacter* (16%), while PS samples showed a predominant affiliation with *Prosthecobacter* (12.5%) and *Micavibrionales* (10.9%). Notably, the bacterial community displayed similar trends in the presence of both PVC and PS nanoparticles (Fig. 5a and b). This unexpected convergence in bacterial community dynamics suggests a potential common response to nanoplastic exposure, regardless of the specific type of nanoplastic. The shift in bacterial taxa over time indicates a dynamic and adaptive microbial community that could potentially play a role in the degradation or transformation of nanoplastics in the environment. Further investigations into the functional roles of these bacterial taxa and their interactions with nanoplastics are warranted to elucidate the underlying mechanisms driving the observed community dynamics.

3.4. Influence of nanoplastics on bacterial inoculum

Analyzing the relative abundances of the mixed inoculum revealed

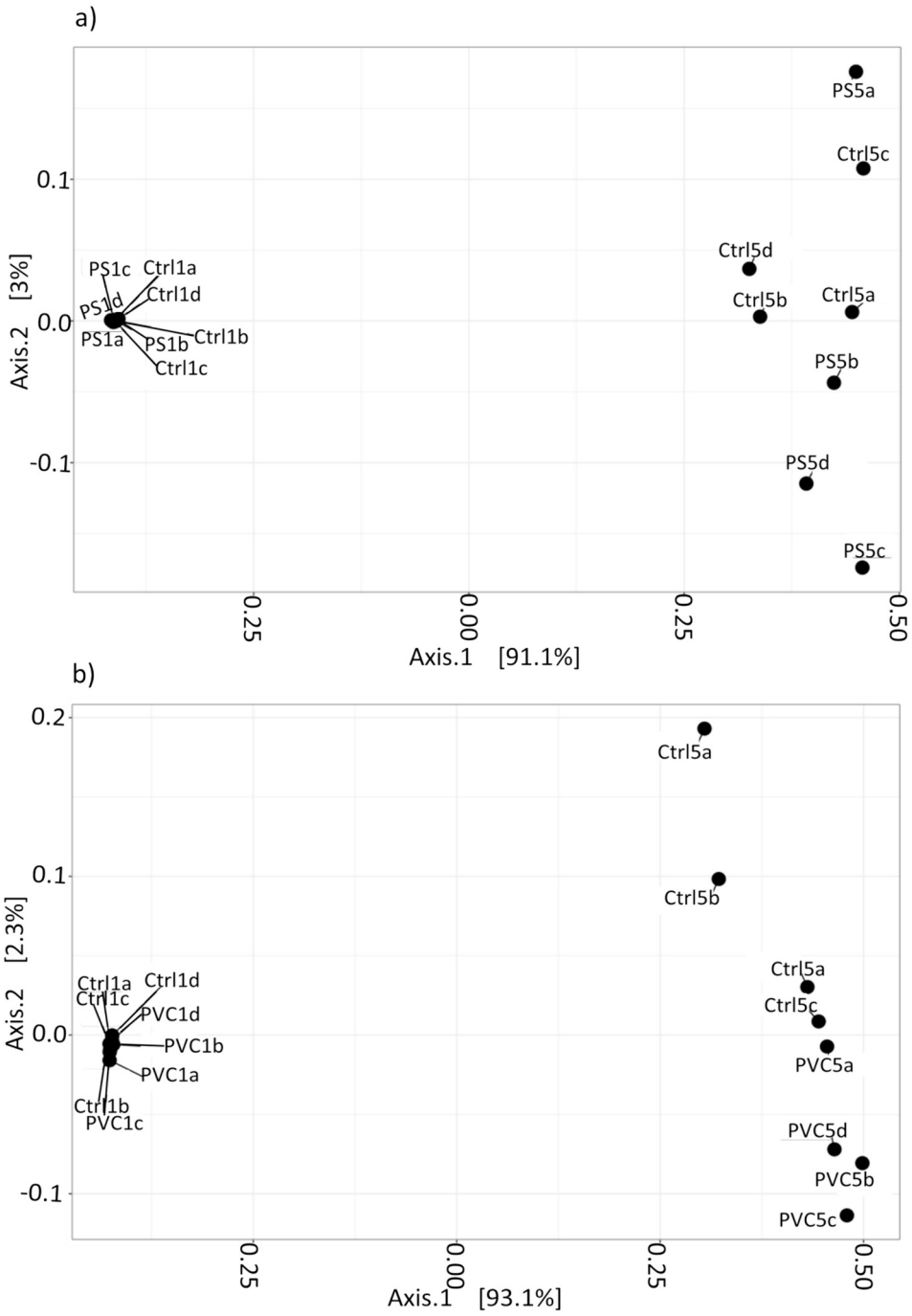

Fig. 4. Principal coordinates analysis (PCoA) of bacterial communities in the control and in the media contain nanoplastics, i.e. PS (a) and PVC (b) on days 1 and 5.

variations across samples. Initially, on day 1, *Empedobacter* dominated with the highest abundance at 62%, trailed by *Acinetobacter* at 36%. However, noteworthy shifts occurred in subsequent days, particularly at day 5, where all samples (Control, PVC, and PS) consistently displayed an increased prevalence of *Acinetobacter*, succeeded by *Rhodococcus*. Notably, this bacterial community trend persisted in the presence of both PVC and PS nanoparticles (Fig. 6a and b). *Rhodococcus* and *Acinetobacter* are commonly reported in AS (Cinà et al., 2019; Carr et al., 2003) and can metabolize various organic compounds in the AS (Zhao et al., 2023) rendering them better adapted to utilize resources available through introduction of nanoplastics, i.e. PVC and PS. Studies show different bacterial species in the mixed inoculum may participate in synergistic relationships and overtime, i.e. when conditions change, species better suited to the new environment (e.g., *Acinetobacter* and *Rhodococcus*) may outcompete others leading to shifts in relative abundances (Islam et al., 2023). The observed fluctuations in bacterial abundance within the mixed inoculum suggest dynamic interactions influenced by temporal factors and the introduction of PVC and PS nanoparticles. The initial prevalence of *Empedobacter* on day 1 may be attributed to specific conditions or substrate availability promoting its growth. However, the subsequent dominance of *Acinetobacter* across all samples on day 5 raises questions about the resilience and adaptability of certain bacterial species under changing environmental conditions.

The consistent prevalence of *Acinetobacter* and the concurrent increase in *Rhodococcus* abundance, despite variations in the samples and the presence of PVC and PS nanoplastics, hint at potential species-specific responses or competitive advantages in the presence of these materials. *Acinetobacter*, known for its versatile metabolic capabilities,

Table 1
The statistics was performed to compare samples exposed to nanoplastics against control samples without any nanoplastics using the PERMANOVA.

Df	SumsOfSqs	F.Model	R^2	p.value	p.adjusted	sig		
Ctrl1	vs	Ctrl5	1	1.887	50.132	0.893	0.031	0.031
pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig	
PVC1	vs	PVC5	1	1.332	8.367	0.582	0.030	0.030
pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig	
PS1	vs	PS5	1	1.502	34.622	0.852	0.034	0.034
pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig	
Ctrl1	vs	PVC5	1	0.157	2.215	0.270	0.053	0.159
Ctrl1	vs	PS5	1	0.091	1.150	0.161	0.356	1.000
PVC1	vs	PS5	1	0.133	1.760	0.227	0.059	0.177

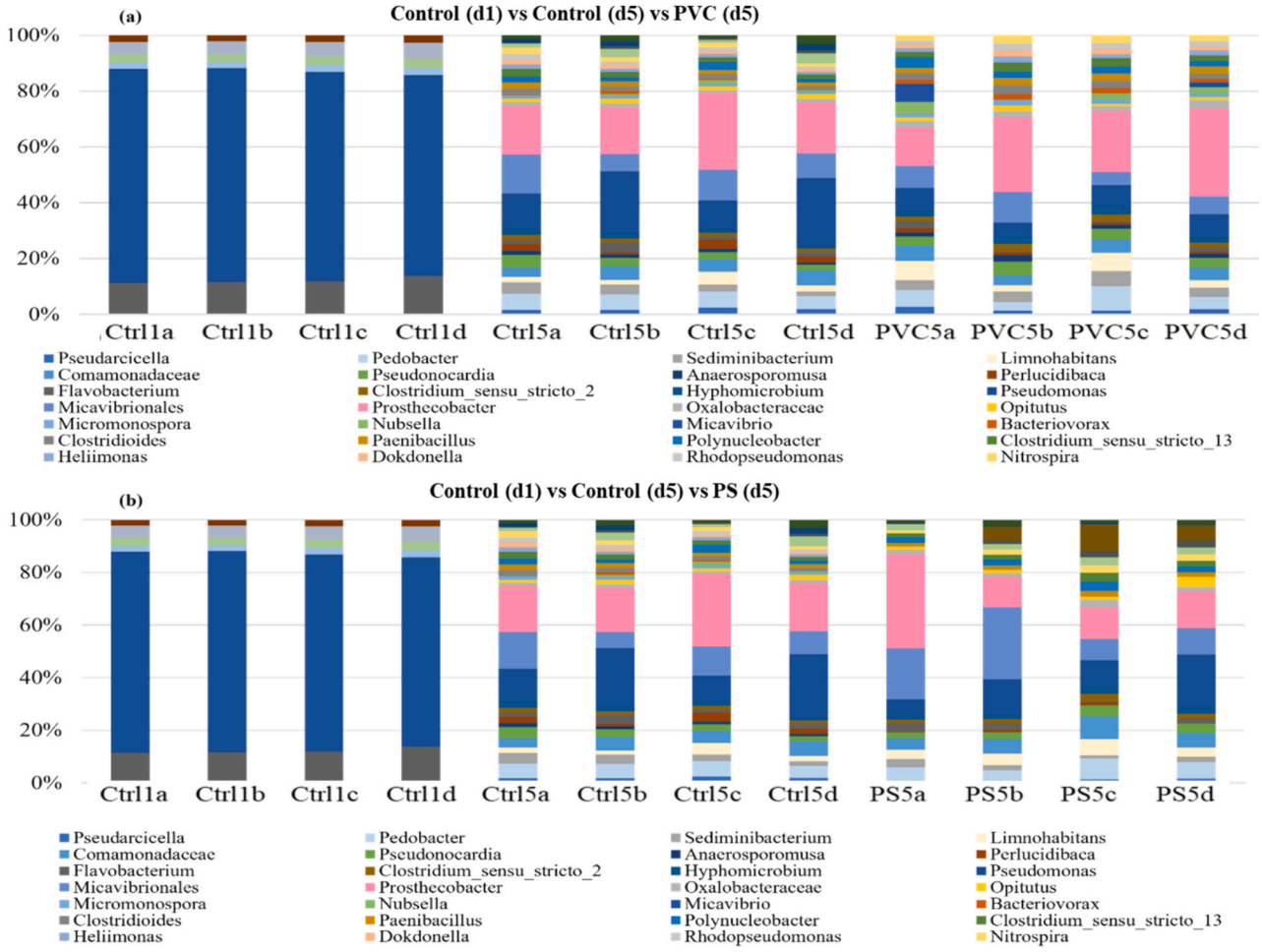

Fig. 5. Relative abundance of bacterial communities in Control (D1, D5), and after exposure to nanoplastics, i.e. (a) PVC (D5) and (b) PS (D5).

may have exhibited an enhanced adaptability or preferential utilization of resources introduced by the nanoparticles.

4. Conclusion

Despite having similar size and shape characteristics, PS nanoplastics were more likely to attach to bacteria than PVC nanoplastics, probably due to more hydrophobic and other surface properties that make them more prone to interact with cells. Another important observation is that there was a substantial decrease in the number of both PS and PVC nanoplastics attached to bacteria over time. This suggests that bacteria may have mechanisms to remove nanoplastics from their surface, or the attachment is not too strong. Alternatively, new bacterial cell generations may not interact with PS and PVC nanoplastics due to the formation of biofilm.

Despite the observed fluctuations in bacterial diversity and relative abundances, the overall bacterial community structure demonstrated resilience to the exposure of PS and PVC nanoplastics, as indicated by increased alpha diversity, PCoA, and PERMANOVA results. This robustness suggests that the microbial community was not affected despite the presence of potential nanoplastic stressors. To unravel the intricacies of this resilience, further investigations into the specific mechanisms governing bacterial responses to nanoplastic exposure are warranted, offering valuable insights into the nuanced interactions

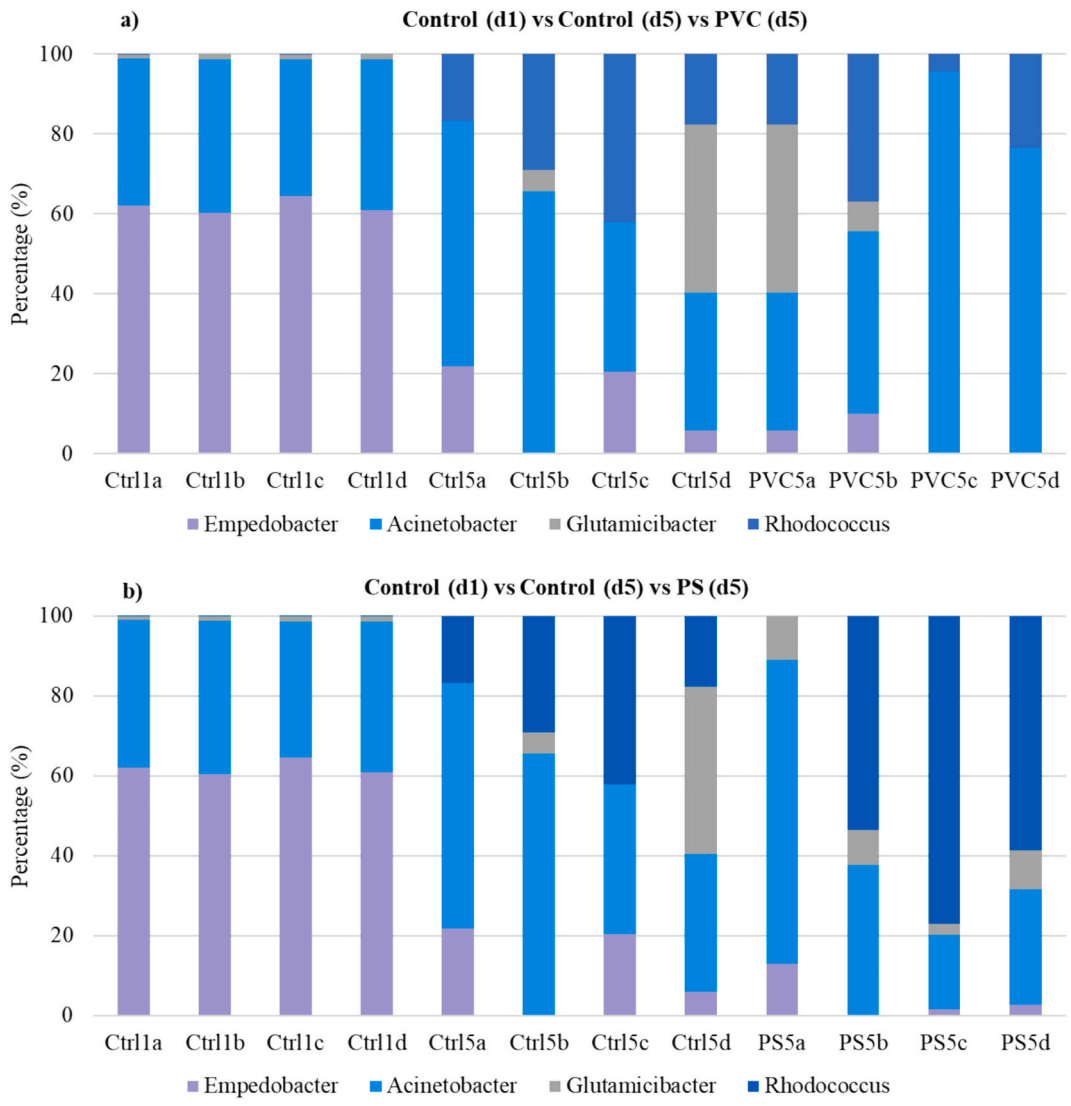

Fig. 6. Abundance of bacteria from a mixed inoculum in Control (D1, D5), and after exposure to nanoplastics, i.e. (a) PVC (D5) and (b) PS (D5).

between microorganisms and nanoplastics within environmental systems. Understanding the broader impacts of nanoparticles on microbial communities is imperative for evaluating potential ecological consequences and guiding responsible nanomaterial design and usage. Consequently, additional research is essential to delve into the molecular mechanisms that underlie the observed trends and to broaden our understanding of the ecological implications arising from nanoparticle-bacterial interactions.

CRediT authorship contribution statement

Fazel Abdolahpur Monikh: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nhung H.A. Nguyen:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Mandar Bandekar:** Writing – review & editing, Methodology. **Jakub Riha:** Software, Formal analysis, Data curation. **Sara Bogialli:** Writing – review & editing, Supervision. **Paolo Pastore:** Writing – review & editing, Supervision. **Hans-Peter Grossart:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Alena Sevcu:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing interest.

Data availability

Data are available in the manuscript and the supporting information.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.impact.2024.100514.

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