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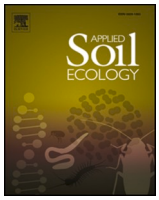

Research paper

Long-term per- and polyfluoroalkyl substances (PFAS) exposure causes selective changes in the rhizosphere bacterial community

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants, yet their impact on soil microbial diversity, function, and plant-microbe interactions remain poorly understood. This study investigates the effects of short-term (3 months, high concentration) and long-term (>30 years, low concentration) PFAS exposure on rhizosphere bacterial communities, incorporating plant interactions and functional gene profiling. Using 16S rRNA amplicon sequencing, selective microbial shifts were observed, where Firmicutes, Bacteroidetes and Gemmatimonadetes were enriched, while Actinobacteria and Acidobacteria declined in PFAS-contaminated soils. LEfSe biomarker analysis identified 33 genera including *Nitrosospira*, *Nakamurella*, *Gemmatimonas*, *Nitrosomonas*, *Nordella* and *Pseudonocardia* present in long-term exposed soils but were absent in short-term exposure, highlighting adaptive microbial responses over time. Functional predictions revealed enrichment of genes associated with xenobiotic degradation, lipid metabolism, and redox processes, inferring possible microbial metabolic adaptations to PFAS. Plant-specific effects further shaped microbial communities, with willow promoting Bacteroidetes and poplar reducing Actinobacteria, emphasizing their potential role in phytoremediation strategies. Overall, this study provides insight into potential microbial biomarkers and functional redundancy associated with PFAS exposure and features the long-term impact of PFAS on rhizoshpere microbial ecosystems, informing strategies for bioremediation and ecosystem recovery.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals that have been widely used in industrial and consumer applications due to their high thermal and chemical stability (Cao et al., 2022). PFAS constitute over 5000 substances that were extensively used since their discovery in the 1930s, primarily in firefighting foams, paints, textiles and coated paper (Herzke et al., 2012; OECD, 2018). Their use has led to a widespread distribution in the terrestrial and aquatic environment through direct sources such as fire training facilities, wastewater treatment plants and landfill facilities, and indirect sources such as atmospheric deposition or runoff (Abou-Khalil et al., 2022; Ahrens et al., 2015; Gobelius et al., 2017; Xu et al., 2022). Despite regulatory restrictions on production and phasing out industrial applications of certain PFAS during 2000s, their presence remains in the environment due to their persistence and historical usage.

PFAS are extremely resistant to degradation due to their strong C—F bonds and have a high bioaccumulation potential, thus pose a threat to terrestrial and aquatic ecosystems (Brusseau et al., 2020; Fenton et al., 2021; Zhang et al., 2022). Their bioaccumulation potential and toxicity depend on chain length and functional groups. Short-chained PFAS tend to leach more readily into the groundwater affecting aquatic ecosystems (Ahrens et al., 2015; Cai et al., 2019). In contrast, long-chain PFAS, such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have higher sorption potential to soil particles, leading to accumulation in the upper soil layers (Xu et al., 2023). The properties of their functional groups, hydrophobicity, and lipophobicity also affects the fate of PFAS in the environment contributing to their persistence and widespread distribution (Ahrens and Bundschuh, 2014; Conder et al., 2008; Wee and Aris, 2023).

Soils are particularly susceptible to PFAS contamination and their influence on soil biota are crucial for assessing their environmental impact. Soil bacteria have a central role in nutrient cycling, organic matter decomposition, and overall ecosystem stability and performance (Jiao et al., 2018). PFAS have shown to impact microbially-driven processes in soil, including soil respiration, litter decomposition, soil

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structure and pH (Cai et al., 2019; Xu et al., 2023). For example, the presence of perfluorobutanesulfonic acid (PFBS) significantly increased litter decomposition and was associated with positive effects on β-glucosidase activities, and these effects increased at higher PFBS concentration (Xu et al., 2023). Such changes affect soil microbial diversity, abundance and metabolism (Bao et al., 2018; Cai et al., 2020; Lai et al., 2018; Li et al., 2017; Xu et al., 2022). Xu and colleagues (Xu et al., 2022) observed changes in soil bacterial abundance among different phyla during PFOS, PFOA and PFBS exposures at environmentally relevant concentrations. Qiao and colleagues (Qiao et al., 2018) also observed an increased abundance of Proteobacteria, Firmicutes, Actinobacteria and Acidobacteria in soil exposed to 1 μg/g dry weight (dw) PFOS for 80 days. Lai et al. (Lai et al., 2018) reported that PFAS exposure led to marked modulations in the abundance of Firmicutes, Bacteroidetes, Proteobacteria and Cyanobacteria, groups involved in metabolism of amino acids and short-chain fatty acids in the gut. Difference in abundance of some bacterial groups is attributed to their adaptation to changes in physiochemical properties of the soil, their ability to cleave key chemical bonds in PFAS and render it harmless or utilize PFAS as an alternative energy source (Chetverikov et al., 2017; Jin et al., 2023). However, the specific mechanisms by which PFAS affect different bacterial communities still remain to be determined (Shahsavari et al., 2021).

Soil microbial communities and their metabolic process have an important function in the rhizosphere and contribute to plant growth, therefore they would be expected to influence plant-microbe-PFAS interactions (Gobelius et al., 2017). Willow (*Salix miyabeana*) and poplar (*Populus trichocarpa*) have been assessed for their suitability in phytoremediation of heavy metals and organic pollutants due to their high biomass production, evapotranspiration, and fast growth rate (Sandil and Gowala, 2022; Tőzsér et al., 2023). Consequently, these plants have also been studied for PFAS uptake efficiency from contaminated soil (Gobelius et al., 2017; Sharma et al., 2020), but the influence of the soil microbial community on this process is not yet understood. While microbial communities in the rhizosphere can affect phytoremediation, the deep roots of poplar and root exudates of willow are shown to enrich the microbiome (Li et al., 2023; Schmidt et al., 2000; Vives-Peris et al., 2020; Wang et al., 2021).

The duration of PFAS exposure also affects soil microbial communities (Bao et al., 2018; Xu et al., 2022). Short-term PFAS exposure has been shown to disrupt microbial metabolic processes, hinder nutrient cycling and reduce soil respiration (Cai et al., 2020; Xu et al., 2022). In contrast, long-term PFAS exposure can result in profound alterations in microbial diversity and functional potential impacting plant-microbe interactions and the overall soil ecosystems. A comprehensive understanding of these temporal effects is crucial for evaluating resilience of microbial communities and the broader ecosystem to PFAS contamination. Moreover, assessing both short- and long-term PFAS exposure allows us to assess how different contamination durations shape microbial dynamics, offering insight for the development of effective remediation strategies, such as phytoremediation. Specifically, microbial taxa that are affected by PFAS may serve as biomarkers of pollution and can guide bioremediation efforts (Xu et al., 2022).

In this study, we examined how exposure duration (short-term, 3–6 months, versus long-term, >30 years) shape soil bacterial communities exposed to PFAS mixtures, while also considering plant effects. Previous studies often tested single PFAS and short-term exposures (Bao et al., 2018; Cai et al., 2020; Xu et al., 2022), whereas PFAS mixtures under chronic field contamination may impose distinct selection on rhizosphere microbiota with consequences for phytoremediation. To address this, we compared greenhouse pot experiments (controlled, short-term exposure) with a historically contaminated field site (long-term exposure) to: i) quantify community shifts; ii) identify differences in community composition across soil types and plant treatments; iii) investigate influence of plants on PFAS affected soil bacterial communities; and iv) identify candidate biomarker taxa enriched under long-term exposure and distinguish predicted functional patterns based on taxonomic inferences.

2. Materials and methods

2.1. Soil samples

Greenhouse and environmental soil samples used in this study are described in Table S1. Environmental soil samples (n = 10) were collected in duplicate 2–10 m apart from a depth of 10–15 cm at a fire training site located 3.5 km southwest of the town of Arboga in central Sweden (59°38′69.04“ N, 15°89’71.08” E) in October 2021 (Fig. S1). The area was used as a firefighting training site where PFAS-containing aqueous fire-fighting foams (AFFFs) were used from the 1950s until the 1990s (Niarchos et al., 2023). The concentration of PFAS in groundwater and surface soil was previously reported (Niarchos et al., 2023). Composite soil was collected from three locations (L1−L3), contaminated with different levels of PFAS and one location outside the contaminated areas to use as a reference soil (L5). The metal and PFAS analysis of the environmental soils is presented Tables S2 and S3.

Commercial greenhouse soil (S-jord garden soil, Hasselfors, Sweden) with and without PFAS spiking and transplanted with willow (*Salix miyabeana*) or poplar (*Populus trichocarpa*) were collected (n = 31) as previously described (Nassazzi et al., 2025). Fifteen PFAS were used for spiking to a final concentration of 250 μg/kg dw soil for willow and 125 μg/kg dw soil for poplar for each individual PFAS (Table S4). To ensure measurable effects within the short-term (3 and 6 months) greenhouse study, while retaining mixture composition, the ∑PFAS concentration was selected from the upper range of reported environmental contamination. The sample groups prepared in triplicate were i) reference pot soil without PFAS and plants (C0 and C3), ii) PFAS spiked soil (Pf0 and Pf3), iii) PFAS spiked soil transplanted with Willow ($W0^+$, $W3^+$, $W6^+$), iv) PFAS spiked soil transplanted with Willow and amended with microbes (W_suppl3^+), and v) PFAS spiked and non-spiked soil transplanted with poplar ($P3^+$ and P3). Commercially available microbial supplements used as soil amendments included a bacterial mixture (Tarantula®, Advanced Nutrients) and an arbuscular mycorrhizal fungus (Mykos®, Xtreme Gardening; *Rhizophagus intraradices*) (listed in Table S5). Reference, PFAS spiked, and willow transplanted soil were sampled at the beginning of the experiment (C0, Pf0, $W0^+$) and after 3-months in greenhouse (C3, Pf3, $W3^+$). Willow transplanted PFAS spiked soil was also sampled after 6-months ($W6^+$). Poplar transplanted soil with ($P3^+$) and without PFAS (P3) were sampled after 3-months. Willow transplanted PFAS spike soil was amended with commercially available microbes (Table S5), following manufacturer's instructions and sampled after 3-months (W_Suppl3^+). For each sample, 1 g of well mixed soil was transferred to 9 mL of DNA/RNA Shield (Zymo Research, USA), initially stored at room temperature for 24 h before being transferred to a −80 °C freezer until DNA extraction.

2.2. PFAS analysis

In the environmental soil samples, 19 PFAS were analyzed including C_3-C_{13} perfluoroalkyl carboxylates (PFCAs), C_4-C_{10} perfluoroalkyl sulfonates (PFSAs) and perfluorooctane sulfoneamide (FOSA). In addition, linear and branched isomers were quantified for PFHxS (L-PFHxS, B-PFHxS), PFOS (L-PFOS, B-PFOS) and FOSA (L-FOSA, B-FOSA) (Table S3). In the greenhouse soil samples, 15 PFAS were spiked and analyzed including C_3-C_{13} PFCAs, C_4, C_6, C_8 perfluoroalkyl sulfonates (PFSAs), and perfluorooctane sulfoneamide (FOSA) (Table S4). After solid-liquid extraction, the instrumental analysis was performed using ultra-high-pressure liquid-chromatography (SCIEX ExionLC AC system) coupled to tandem mass spectrometry (SCIEX Triple Quad™ 3500) (UHPLC-MS/MS) with validated methods as described previously (Nassazzi et al., 2022).

2.3. DNA extraction, library preparation, and sequencing

Soil DNA was extracted with the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, USA) according to the manufacturer's protocol. The concentration, quality (A_{260}/A_{280}), and purity (A_{260}/A_{230}) of the extracted DNA were assessed using a DeNovix spectrophotometer (Thermo Scientific, USA) and agarose gel (2 %) electrophoresis, respectively. To minimize cellular bias, the ZymoBIOMICS Microbial Community Standard was included in the extraction and library preparation. For bacteria, 16S rRNA amplicon-based 300 bp paired-end library was prepared through amplification of the hypervariable region V3-V4 (445 bp) using primers F: 5'-TACGGGAGGCAGCAG-3′-3′ and R: 5′- CCAGGGTATCTAATCC -3′ (Friedle et al., 2021). Amplicons were sequenced using an accredited Illumina MiSeq Personal Sequencer at the European Genome and Diagnostics Center (Eurofins Genomics, Germany).

2.4. Microbiome profiling

Sequenced reads were preprocessed and analyzed using the Microbiome sequence analysis pipeline (Eurofins Genomics, Germany). All reads that passed the standard Illumina chastity filter (PF reads) were demultiplexed according to their index sequences. Primers were clipped from the raw reads and unmatched read pairs were removed retaining only high-quality reads. Overlapping forward and reverse reads were merged and assembled into single full target regions. If this was not possible, the high-quality forward read was retained as a fallback. Merged reads were length filtered according to the expected length and variations of the target region (V3-V4) of 16S rRNA, and those with shorter lengths (<445 bp) were discarded.

Merged and retained reads containing ambiguous bases ("N") were discarded. Chimeric reads were identified and removed based on the de-novo algorithm of UCHIME as implemented in the VSEARCH package. The length filtered, merged and quality clipped forward reads were used for microbiome profiling. High-quality reads were partitioned using minimum entropy decomposition (MED), an entropy-based denoising approach that resolves single-nucleotide variation into homogeneous sequence units (MED nodes), hereafter referred to as Operational Taxonomic Units (OTUs). MED-based denoising, followed by downstream centered log ratio (CLR) normalization (see below) enabled robust comparison of community patterns while minimizing low-abundance noise (≤0.02 %) before downstream statistical analysis. Taxonomic information was assigned to each OTU using DC-MEGABLAST. Cluster representative sequences were aligned to the reference sequences in the database (Reference database: /mnt/nsa3/projects/active/bioit development/ebe transfer/mdxMicrobiomePro_l-ing/ncbi nt/nt 02–03 well classi_ed only/nt._ltered.fa, Release 2020-02-03) and those with 70 % identity and 80 % query coverage were assigned to the OTU as the lowest common taxonomic unit. If the representative sequence of an OTU had no significant database match, no taxonomic unit could be assigned. The total number of reads of these unclassified OTUs is stated as category "Unclassified". Further processing of OTUs and taxonomic assignments was performed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (v1.9.1, http://qiime.org/). Finally, lineage-specific copy number normalization was done for the target marker gene to improve estimates using CopyRighter. The adequacy of the amplicon sequencing dataset and the richness of species in samples was assessed with rarefaction curves (Mao's tau). All analysis shown below were done using these OTU datasets.

2.5. Data analysis

The evolutionary relationship among OTUs was evaluated with Molecular Evolutionary Genetics Analysis version 11 (MEGA11) (Tamura et al., 2021). Sequences were aligned using MUSCLE and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster method. Phylogeny tree is constructed with the Neighbor-Joining method with default settings. The phylogenetic tree, OTUs and taxonomy table results were used for all analysis in MicrobiomeAnalyst 2.0 online tool (https://www.microbiomeanalyst.ca/MicrobiomeAnalyst/) (Chong et al., 2020; Lu et al., 2023). The inferences focus on between-group contrast (PFAS vs reference within each soil type), which are robust to the choice of high-resolution denoising framework, and the key results (beta-diversity, differential abundance) reflect patterns rather than absolute richness counts. Row OTUs count data were normalized with Centered log ratio (CLR) which addresses the issue of compositionality and allows for meaningful interpretation of distances between samples (Gloor and Reid, 2016). Its preferred over rarefaction as subsampling discards a portion of the observed sequences in samples with high species richness (Cameron et al., 2021). To minimize confounding differences inherent in the various soils, all primary comparisons were performed within each soil type (PFAS-exposed vs matched reference within greenhouse or within environmental soil). Alpha and beta diversity were used for comparing the microbiome composition within and between environmental and greenhouse soil samples. 'Rare taxa' were operationally defined for ACE (abundance-based coverage estimator) as OTUs with counts ≤10 in a sample (including singletons/doubletons), consistent with ACE implementation. ACE was used to evaluate richness in the rare fraction alongside Shannon and Simpson indices to evaluate overall diversity and evenness. Weighted and unweighted UniFrac beta diversity distances were calculated and used as input for Principal Coordinates Analysis (PCoA) ordination. The significances on the effect of the PFAS on community composition were measured with Permutational Multivariate Analysis of Variance (PERMANOVA). The multi-testing adjustment was done based on Benjamini-Hochberg procedure (FDR). Non-metric multidimensional scaling (NMDS) was calculated based on the Bray–Curtis distance metrics. Inherent patterns and correlations within the PFAS exposed and reference soil groups were identified with the unsupervised heat tree analysis using the Wilcoxon Rank Sum test and ward clustering. Significant features and potential biomarkers associated with PFAS exposure was identified with supervised Linear Discriminant Analysis (LDA) effect Size (LEfSe) methods in MicrobiomeAnalyst platform. LEfSe employes a Kruskal-Wallis rank sum test to detect taxa with significant differential abundance with PFAS exposure, followed by Linear Discriminant Analysis to evaluate the relevance or effect size of the taxa.

Functional profiles were predicted from 16S rRNA data using Tax4Fun2 (Wemheuer et al., 2020) and interpreted as inferred functional potential (Matchado et al., 2024). Tax4Fun2 (v2020) was used to derive predicted KEGG Ortholog (KO) profiles from 16S rRNA data, where OTU representative sequences were mapped to the nearest 16S reference genomes and the associated KO profiles were aggregated and normalized using Tax4Fun2 defaults. Functional Redundancy Index (FRI) was computed per sample using the calculateFunctionalRedundancy function, yielding a sample × KO matrix of FRI values (predicted/inferred functional potential). For visualization, bias was summarized as the log ratio;

$$LFRI_k = log_{10}\left(\frac{FRI_{k,PFAS}}{FRI_{k,REF}}\right)$$

where $FRI_{\kappa,\ PFAS}$ and $FRI_{\kappa,\ REF}$ are the mean FRI values per-sample for KO in the PFAS-exposed and reference groups, respectively. $LFRI_\kappa > 0$ indicates higher predicted redundancy under PFAS exposure (PFAS-biased), and $LFRI_\kappa < 0$ indicates higher predicted redundancy in reference soils (reference-biased). To show which functions increased most under PFAS exposure, the top 10 PFAS-biased KOs were listed by ranking all KOs in descending order of the log-ratio metric. The FRI was tested for each KO between PFAS and Reference soils using a two-sided Mann–Whitney *U* test and adjusted *p*-values across the listed KOs using the Holm–Šídák method. Enrichment score defined as –log10(adjusted

p-value) where the adjusted $p < 0.05$ was considered significant.

3. Results and discussion

3.1. PFAS levels and composition in environmental and pot soil

In total, 16 out of 19 PFAS were detected in the environmental soil near a firefighting training site at Arboga Airport, while 6:2 FTSA, PFHxDA and PFOcDA were not detected (Table S3). $\sum_{19}$PFAS concentrations in soil ranged from 54 to 187 ng/g dw at contaminated locations, L1-L2 (Fig. S1) and were in the same range reported previously with $\sum_{11}$PFAS levels of 54 ng/g dw at soil depths of 0–0.5 m at the same study site (Niarchos et al., 2023). The concentration of ∑PFAS were comparable to previous ∑PFAS levels reported at firefighting training site at Stockholm Arlanda Airport, Sweden (16–160 ng/g dw) (Gobelius et al., 2017), but lower than previously reported in other AFFF impacted areas (Cao et al., 2022; Gobelius et al., 2017). PFOS (80 % of ∑PFAS) was the dominant PFAS with an average of 37.7 ng/g dw linear-PFOS (66 %) and 8 ng/g dw branched-PFOS (14 %). The uncontaminated reference soil (L5) had a median PFOS level of 0.3 ng/g dw. The distribution of PFAS indicated a contamination with a PFOS-based AFFFs (Ahrens et al., 2015). The ratio PFOS/PFOA$_{soil}$ (83) was higher than the ratio PFOS/PFOA$_{groundwater}$ (~2) (Fig. S1), indicating leaching of the PFOA from soil to groundwater was faster than PFOS, likely due to its higher water solubility (9500 mg/L (PFOA) vs 680 mg/L (PFOS)) and long-term exposure (Kurwadkar et al., 2022). Moreover, PFOS has higher sorption potential to soil than PFOA (Cai et al., 2019). Based on the measured ∑PFAS levels, soils from L5 and L3 (with ∑PFAS concentrations below 20 ng/g dw) were grouped as reference soil for the microbiome analysis. In contrast, L1 and L2 (∑PFAS concentrations between 54 and 186 ng/g dw) were classified as PFAS-exposed soil for further analysis.

Greenhouse soil was spiked with 15 PFAS and measured levels of individual PFAS ranged from 1.5 to 3.5 µg/g dw at the start of the experiment in the spiked soils (Fig. 1). Comparable levels were measured throughout 0–6 months (Table S1). Changes in soil levels of individual PFAS over 3–6 month exposure were previously reported (Nassazzi et al., 2025). The ∑PFAS in the non-spiked reference soil was 3.7 to 15 ng/g dw and was comparable to the ∑PFAS in the environmental reference soil (Table S1). However, the individual PFAS concentrations used in greenhouse were over 30-fold higher than the levels in contaminated environmental soil. As such, the effects of PFAS on soil microbial communities are not solely dependent on the concentration of PFAS, but also on other factors such as soil depth and organic carbon content (Bao et al., 2018). PFAS are known to be hydrophobic and can attach to organic carbon, which may influence their bioavailable concentrations in soil and levels leached in groundwater (Ahrens et al., 2015; Gobelius et al., 2017).

3.2. Bacterial community structure of PFAS exposed soil

The bacterial community of environmental and greenhouse soil exposed to PFAS for 6 months (greenhouse) to over 30 years (environment) was analyzed by 16S rRNA amplicon sequencing. After high-throughput sequencing, 2.2 million paired-end 300-bp reads were obtained and 99 % were retained after preprocessing for profiling in greenhouse soil. Almost all OTUs (99.8 %) were assigned to taxa showing that the microbiome in the soil samples were well-represented

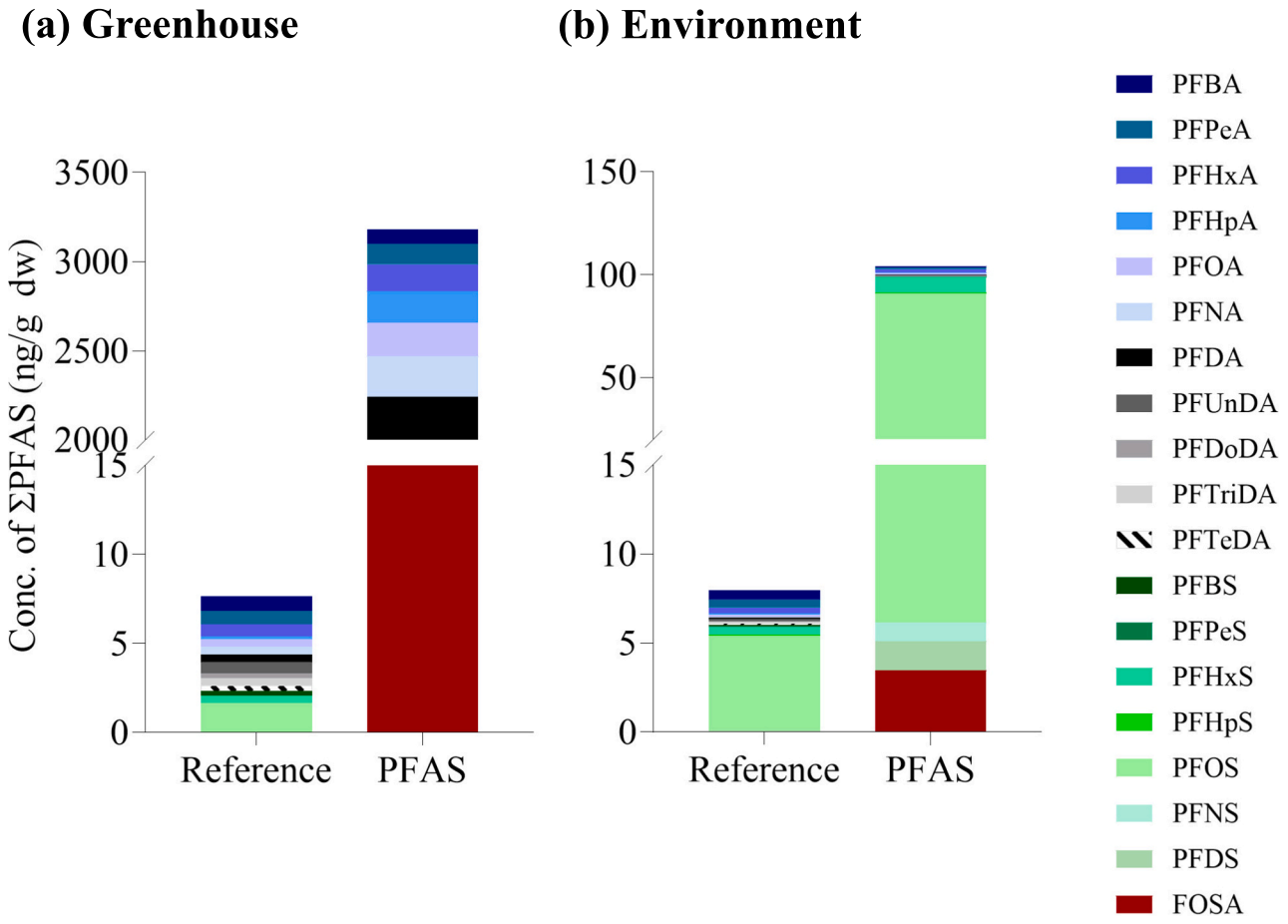

Fig. 1. ∑PFAS concentration (ng/g dw) in greenhouse and environmental soil groups. Concentrations of total PFAS measured in PFAS-exposed and reference soils in the a) greenhouse and b) environmental samples. Soil groups are described in Table S1.

in the reference database. In environmental soil, 743,807 reads were obtained and 99 % OTUs were retained and assigned taxa. After copy number correction with the marker gene, 619,525 and 185,957 sequences in greenhouse and environmental soil were assigned to 2424 and 1365 OTUs, respectively, which were then assigned to taxa. Sample rarefaction curves showed good coverage (Fig. S2), indicating that the sequencing depth and sampling was adequate in capturing richness and diversity of taxonomic groups in the soil groups (Cameron et al., 2021; Schloss, 2024). The results of read preprocessing, the OTU picking, and taxonomic assignment are described in Table S6.

Bacterial diversity in PFAS contaminated soil, as indicated by the Shannon and Simpson indices at the OTU level, significantly increased after both short-term (6-months) and long-term (over 30 years) PFAS exposure compared to reference soil ($p < 0.05$) (Fig. 2, Table S7). In greenhouse and environmental soil groups, the Simpson index suggests that species abundance remained balanced, however, species richness, particularly the number of rare-fraction OTUs (ACE), showed no significant change due to PFAS exposure, according to the abundance-based coverage estimator, ACE index ($p > 0.05$). The reported values are comparable to previous studies in soil exposed to individual PFAS for 3-months to 3-years (Huang et al., 2024; Li et al., 2017; Qiao et al., 2018; Xu et al., 2022). This suggests that PFAS contamination might cause a shift in dominance pattern by reducing certain communities and allowing fewer dominant ones to thrive, leading to a more evenly distributed community without a significant increase in species richness (Bao et al., 2018; Huang et al., 2024; Lai et al., 2018).

The effect of PFAS on microbial diversity observed through Shannon and Simpson indices is further reflected at the phyla level (Fig. 3). In greenhouse soil, a temporal trend with distinct shifts were observed during the 3-month period (Fig. S3). One month after PFAS spiking (Day 0), Actinobacteria and Gemmatimonadetes showed a significant decrease in relative abundance ($p < 0.05$), but both recovered by 3 months, with Gemmatimonadetes even exceeding initial levels ($p < 0.05$). In contrast, Acidobacteria decreased significantly over time ($p < 0.05$), whereas Bacteroidetes increased ($p < 0.05$). These findings suggest that PFAS effects on soil microbial communities are phylum-specific and can occur even during short-term exposure. In environmental soil, exposure to PFAS for over 30 years showed sustained decrease in Acidobacteria and Firmicutes ($p < 0.01$) and increase in Gemmatimonadetes and Bacteroidetes ($p < 0.01$) (Fig. S4). The changes in Acidobacteria, Gemmatimonadetes and Bacteroidetes parallels observations in the greenhouse soil and previous reports of short-term exposure to PFOS and PFOA (Bao et al., 2018; Xu et al., 2022), providing strong evidence of the adaptability of some groups to PFAS contamination, even after prolonged exposure. Given that Gemmatimonadetes and Bacteroidetes typically represent less dominant taxa in soil, their enrichment may indicate ecological niche shifts or competitive advantages under PFAS-induced stress, which can be revealed in future investigation into their potential roles in soil function and biogeochemical cycling (Cai et al., 2020; Xu et al., 2022). Conversely, the significant reduction in Acidobacteria, one of the most dominant and functionally diverse soil bacterial groups, in short-term and long-term exposures can have potential consequences for soil ecosystem health. (Fierer et al., 2012; Kielak et al., 2016). Acidobacteria are widely associated with oligotrophic conditions, contributing to organic matter decomposition, carbon cycling and nutrient turnover in soils (Fierer et al., 2007; Kielak et al., 2016). Taken together, PFAS induced abundance of Gemmatimonadetes and Bacteroidetes combined with suppression of functionally important groups like Acidobacteria suggest a shift toward a more copiotrophic microbial community (Fierer et al., 2012; Qiao et al., 2018; Xu et al., 2022). Such shifts may influence soil nutrient dynamics, organic matter stability and plant-microbe interactions, emphasizing the need for studies to assess the functional implications of microbial adaptation and loss of dominant taxa under long-term PFAS exposure (Cai et al., 2020; Xu et al., 2022). A shift toward a more copiotrophic community is associated with changes in functional gene abundances related to metabolism and cellular processes (Fierer et al., 2012; Qiao et al., 2018).

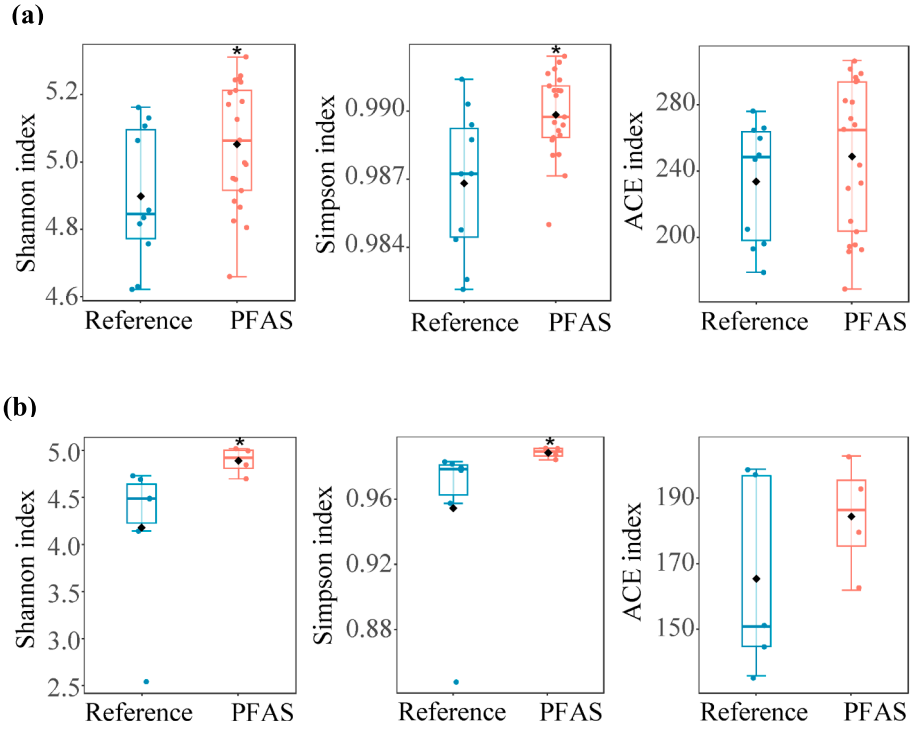

Fig. 2. Alpha diversity indices in greenhouse and environmental soils exposed to PFAS. Shannon, Simpson and ACE indices comparing microbial diversity between PFAS-exposed and reference soils in (a) greenhouse soil over 6 months and (b) environmental soil after 30 years. Asterisk (*) indicate significant differences at $p < 0.05$ based on Mann-Whitney tests (Wilcoxon Rank Sum Test) adjusted for multiple comparisons using the Benjamini-Hochberg procedure (FDR) procedure.

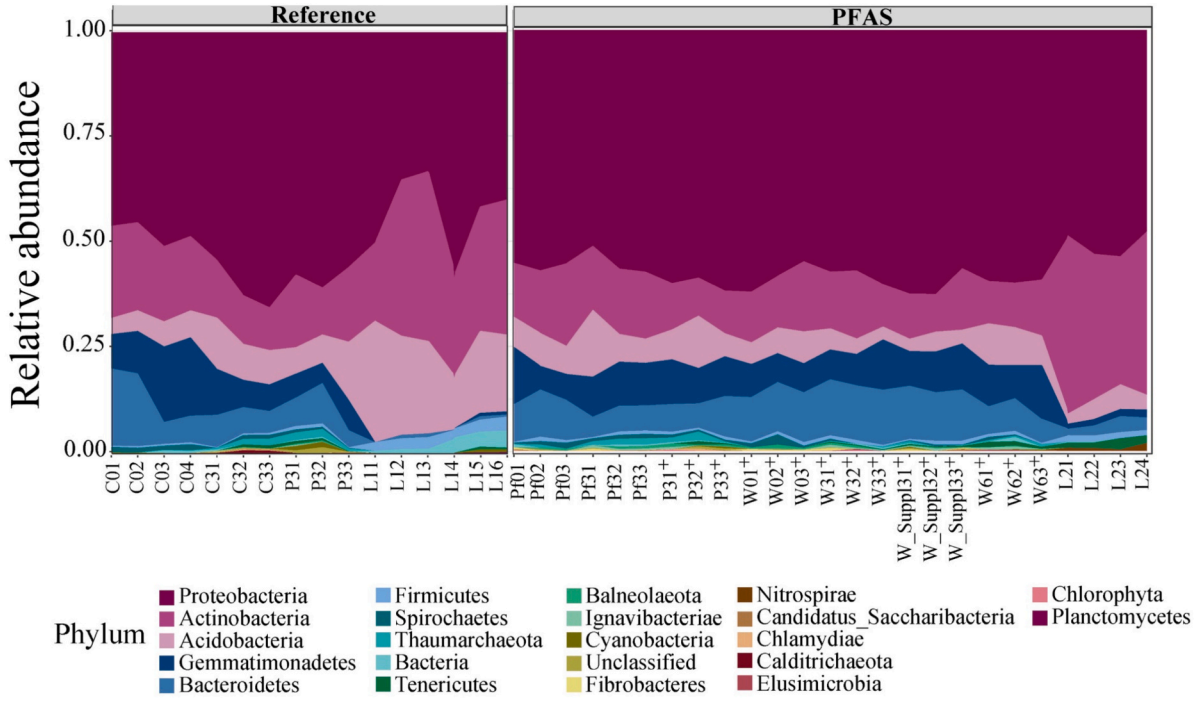

Fig. 3. Relative abundance of bacterial phyla in greenhouse and environmental soils. Stacked bar plots showing phyla-level compositions in PFAS-exposed and reference soils. Differences between groups highlight phyla-specific shifts associated with PFAS exposure and soil type. Samples are described in Table S1.

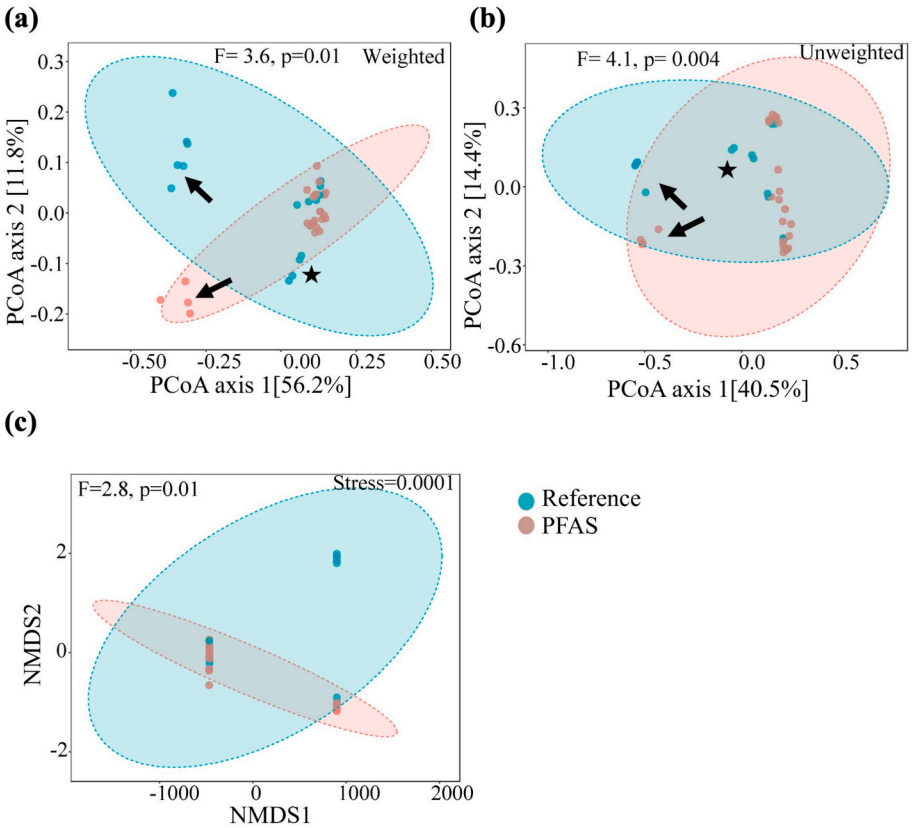

Fig. 4. Beta diversity analysis of bacterial communities in greenhouse and environmental soils. Principal coordinate analysis (PCoA) based on (a) weighted and (b) unweighted UniFrac distances, (c) nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distances. Environmental soil groups (arrows) cluster distinctly between PFAS-exposed and reference soils, while greenhouse samples show limited separation except for the Day 0 reference soil (★).

3.3. Variation in soil community structure between short-term and long-term PFAS exposure

Comparison of community structure between the greenhouse and environmental soil groups using beta diversity indicated that significant shifts in bacterial community after PFAS exposure occurred only in the environmental soils ($F = 3.6–3.4$, $p < 0.05$) (Fig. 4, Table S8). This demonstrates that long-term PFAS contamination in environmental soil induces distinct shifts in microbial community composition. The observed separation, particularly in weighted UniFrac, accounts for the relative abundance, and this suggests that PFAS-exposed environmental soils have diverged significantly from the respective reference soils, with notable shifts in dominant taxa. In contrast, the greenhouse soils (GH) showed no clear separation between PFAS-exposed and the respective reference groups, except for the day 0 reference sample, which clustered apart from other samples. This separation likely reflects the initial microbial response to PFAS spiking (30 days) and transplantation, which disrupted the baseline microbial composition. However, the lack of further separation between PFAS-exposed and reference soils after 90 days suggests microbial stabilization, where neither prolonged PFAS exposure nor transplantation induced substantial shifts compared to soil that remained untreated and without plants over the same period. Clustering analysis using the Ward algorithm further supported these trends, highlighting the pronounced divergence in long-term PFAS exposed soils compared to minimal changes in short-term exposures (Fig. S5). Together, these results support the conclusion that short-term exposure to PFAS (3 months) has subtle, transient effects on microbial communities, whereas long-term exposure (environmental soils) drives more pronounced and lasting shifts, potentially linked to adaptive process and ecological restructuring overtime.

Our findings align with previous studies, which also reported shifts in bacterial phyla under PFAS exposure, though the magnitude and specific phyla affected vary with the type and duration of PFAS exposure (Bao et al., 2018; Cai et al., 2020; Li et al., 2017; Qiao et al., 2018; Xu et al., 2022). Acidobacteria and Firmicutes have been shown to decrease in soils contaminated with PFOS and PFOA, while Bacteroidetes and Gemmatimonadetes increased (Xu et al., 2022; Qiao et al., 2018). However, we observed greater complexity in this study due to the mixed PFAS exposure (15 PFAS in the greenhouse and 19 in the environment), suggesting that cumulative PFAS contamination exerted a more complex selection pressure on soil microbiomes. By analyzing short-term spiked and long-term field-contaminated soils exposed to PFAS mixtures, we observed stronger divergence under chronic low-level exposure, extending both prior single-compound and short-duration studies. This design revealed duration-linked enrichment of specific orders/genera (e.g., Sphingomonadales, Nitrosomonas / Nitrosospira) that were not detected after short-term exposure, highlighting potential long-term biomarkers. This is consistent with studies showing that contamination with PFAS mixtures may lead to distinct microbial shifts compared to single compounds (Bao et al., 2018). Mechanistically, the alteration in microbial diversity may be tied to PFAS-induced changes in soil properties such as pH and nutrient cycling, which can influence microbial structure (Cai et al., 2019; Jiao et al., 2018; Xu et al., 2023). In this study, the pH and metal concentrations of PFAS exposed and reference environmental soil was comparable, with no statistically significant differences (Table S2). Both soil types had slightly acidic to neutral pH (6.52–7.12). Metal levels, including Ca, Mg, Fe, Al, Zn and Cu were similar and within typical background ranges. These aligns with findings of previous studies on PFAS-contaminated soils (Xu et al., 2023; Xu et al., 2022). The similarity suggests that the observed microbial community changes are less likely driven by altered geochemical conditions but linked to PFAS exposure. Previous studies have demonstrated that physicochemical properties- such as ionic strengths, organic matter content, clay content, PFAS chain-length and charge affect PFAS sorption and its effect on microbial communities (Abou-Khalil et al., 2022; Bao et al., 2018; Li et al., 2018). In the context of our findings, the lack of variation in pH and metal content suggests that these factors alone do not account for the microbial shifts we observed. Instead, localized soil-microbe interactions such as bioavailability, hydrophobicity, and charge-based binding dynamics are likely responsible for the changes (Huang et al., 2024; Xu et al., 2023; Xu et al., 2022). For instance, we noted an increase in Bacteroidetes and Gemmatimonadetes, which may indicate adaptation to PFAS-altered organic matter or surface properties. Conversely, Acidobacteria decreased, potentially reflecting sensitivity to nutrient shifts or disrupted carbon cycling (Fierer et al., 2007). These phylum-specific responses underscore the influence of PFAS on soil ecosystems (Abou-Khalil et al., 2022; Bao et al., 2018; Cai et al., 2019; Xu et al., 2023; Xu et al., 2022). As such, long-term, low-level PFAS exposure in environmental soils appears to drive significant microbial shifts, which could affect processes like carbon cycling and liter decomposition overtime (Huang et al., 2024; Xu et al., 2022). Our results emphasize the importance of considering exposure duration when assessing PFAS ecological impacts.

3.4. The influence of plants on the bacterial community of PFAS exposed soil

The influence of plants on modulating the effect of spiked PFAS was assessed by comparing the relative abundance of bacterial groups in transplanted soil in the greenhouse experiments (Fig. 5). Without PFAS exposure, all phyla had comparable abundance in poplar transplanted (P3) and reference soil (C3). Poplar transplantation significantly decreased abundance of Actinobacteria by an average of 5.5 % ($p < 0.05$) and willow increased the abundance of Bacteroidetes by over 5 %, while Acidobacteria decreased by 5.5 % ($p < 0.05$). As PFAS exposure alone did not affect the two phyla, the influence could be associated with the willow plant. Willow supplemented with beneficial microbial consortia exhibited similar effects to un-supplemented soil, likely because the introduced consortia failed to establish themselves. This is indicated by their absence in the amplicon sequencing results, where their abundance fell below the detection threshold of $\geq$1 %. This suggests that the consortia did not persist or proliferate to a detectable level in the soil environment. Moreover, Firmicute abundance remained unaffected by PFAS exposure or plant transfer, likely due to their low community representation.

Li et al. (Li et al., 2023) reported that poplar exudates influence the microbial community differently where nitrogen-fixing, phosphorous solubilizing groups are enriched in the rhizosphere. However, root exudates could also play a crucial role in the recruitment of beneficial microbiota that alleviates plant stress (Li et al., 2023; Vives-Peris et al., 2020). Willow plants were known to produce salicylic acid, a phenolic compound that can inhibit some soil microbes, but also stimulate others that can degrade it (Schmidt et al., 2000). Such plants also accumulate heavy metals in their above-ground tissues, reducing the metal availability and toxicity in the soil (Sandil and Gowala, 2022). Therefore, the differential enrichment mainly by willow could be related to the bacterial adaptability in the root exudates as Bacteroidetes can produce numerous carbohydrate-active enzymes covering a large spectrum of substrates that enrich them around the rhizosphere (Thomas et al., 2011). These groups are interesting candidates for phytoremediation of PFAS contaminated soil.

In previous work, we have shown that willow and poplar plants accumulated short-chain PFAS in leaves and stems, but long-chain ones remained mainly in the roots and soil (Nassazzi et al., 2025). Therefore, the observed differences could be attributed to the specific bacterial groups special metabolic function to plant exudates or adaptation mechanisms to the PFAS (Xu et al., 2023; Xu et al., 2022). These results suggest that poplar and willow affect bacterial groups differently and the observed subtle changes in abundance can affect functional redundancy within soil microbiomes, nutrient cycling, production of important microbial metabolites as specific bacterial taxa can play disproportionately large roles in key processes (Chen et al., 2020b). On the other hand,

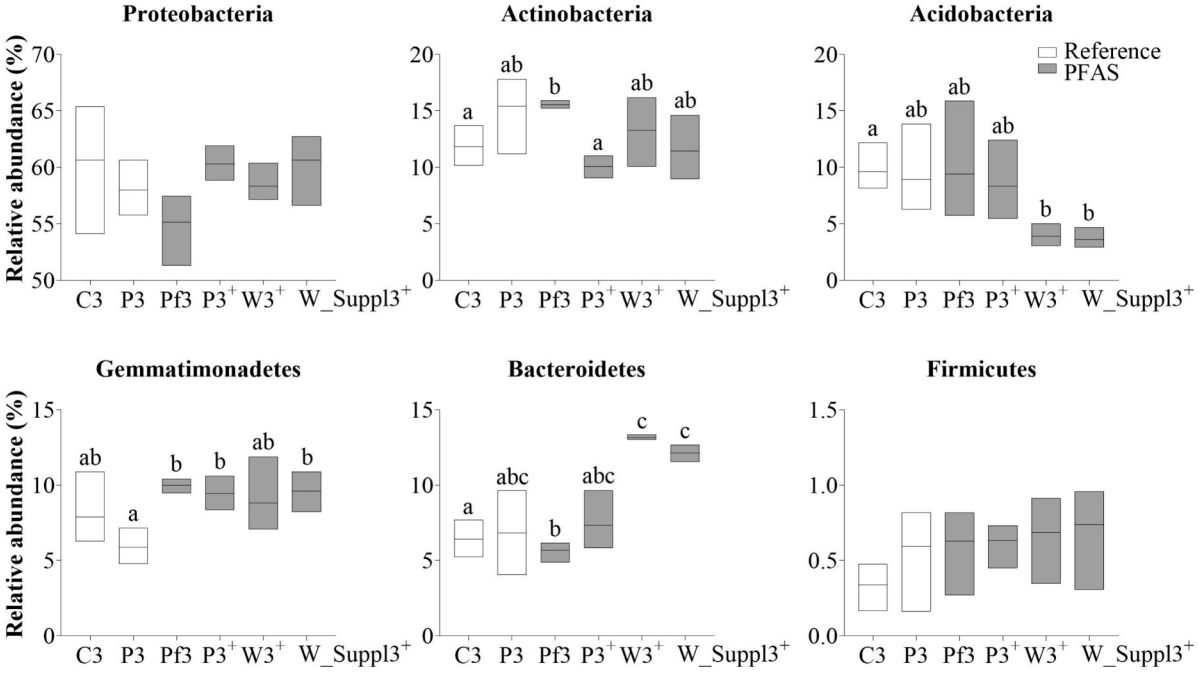

Fig. 5. Effect of plants and 3-month PFAS exposure on relative abundance of dominant phyla in greenhouse soils. Phyla-level relative abundances by sample groups are displayed as floating bars (min-max) with a line at the mean. Lowercase letters above the bars indicate multiple-comparison groupings. Bars with different letters are significantly different (Welch's *t*-tests, $\alpha = 0.05$). Pair-wise comparisons were made using CLR-transformed abundances. Sample groups: C3 (control soil), Pf3 (PFAS only), P3 (poplar), $P3^+$ (poplar+PFAS), $W3^+$ (willow+PFAS), W_Suppl3^+ (willow+PFAS with microbial supplement). Shading indicates PFAS-spiked treatments. Only phyla with mean relative abundance >1 % are shown. $n = 3$ per sample group.

abundant taxa exhibited a much broader niche width and environmental adaptivity than the rare taxa, contributing more to soil and plant function (Dong et al., 2021; Jiao et al., 2019). Taken together, poplar and willow associated community changes that were not observed with PFAS exposure alone highlight the potential of these plants to influence specific bacterial groups. Additionally, bacterial taxa enriched after long-term PFAS exposure, particularly those adapting to the contamination, offer an intriguing opportunity for effective phytoremediation in PFAS-contaminated soils. Although this study focused on bacteria communities, other rhizosphere microbes (especially fungi) may also modulate responses to PFAS. A recent study showed that, although PFOA negatively impacted arbuscular mycorrhizal fungi, it shifted resource allocation from roots to shoots and enhanced plant tolerance to PFAS stress (Yan et al., 2025). Such findings suggest potential plant–fungus–bacteria feedback under chronic PFAS exposure.

3.5. Identification of biomarker taxa for PFAS exposure

Building on the findings from diversity indices and phyla-level relative abundance comparisons, we further explored taxonomic shifts due to PFAS exposure using clustering analysis and LEfSe biomarker discovery model. Clustering with the heat tree analysis at the order level identified more taxonomic groups with higher abundance in PFAS-exposed environmental soils (19 order taxa) compared to greenhouse soils (7 order taxa) (Fig. 6a and b, Table S9 and S10). Notably, Clostridiales, Cytophagales, Micrococcales, Sphingobacteriales, and Sphingomonadales were enriched in both soil types. Some of these groups have been previously reported to be correlated with PFOS and PFOA exposures (Cai et al., 2020; Li et al., 2017; Qiao et al., 2018; Xu et al., 2022). However, direct comparisons cannot be made with single and mixed PFAS exposures.

The LEfSe model identified genera differentially enriched in response to PFAS exposure by integrating statistical significance with biological relevance. This is followed by Linear Discriminant Analysis (LDA), which quantified the magnitude of differential abundance and highlighted key taxa with potential functional impact based on their LDA scores. This two-step approach not only allowed the visualization of broader clustering trends but also provided mechanistic insight by pinpointing taxa significantly enriched in response to long-term PFAS contamination, and aiding in the identification of potential bio-indicators (Xu et al., 2022). Interestingly, no genera were identified in the greenhouse soil, suggesting that short-term PFAS exposure may not significantly alter the microbial community at the genera level, potentially due to the resilience of microbial communities or the insufficient time for observable shifts, lower bioavailability of PFAS, or the buffering capacity of the soil under greenhouse management practices (Khair Biek et al., 2024). In the environmental soil group, 33 genera were identified as taxa with significantly higher abundance in PFAS exposed soil with an LDA score of 0.1–0.5 (Fig. 7a, Table S11). *Nitrosospira*, *Nakamurella*, *Gemmatimonas*, *Nitrosomonas*, *Nordella* and *Pseudonocardia* were identified as the top biomarkers according to the LDS scores (Fig. 7b). This suggests that prolonged exposure to PFAS can result in significant microbial shift, possibly due to selective pressure favoring PFAS-tolerant genera (Xu et al., 2022). Studies have shown that chronic exposure to PFAS can lead to the enrichment of specific bacterial groups capable of PFAS degradation or resistance, which might explain the distinct microbial profiles in the environmental soil in the present work (Bao et al., 2018; Chen et al., 2020a; Han et al., 2011; Saifur and Gardner, 2021; Zubeldia-Varela et al., 2023). More diverse microbial groups were observed than previously reported, likely due to the inclusion of short- and long-term exposures and using PFAS mixtures in the greenhouse experiments in the range typically found in the environment, building on prior work that use short-term or single PFAS exposures. We refrained from genus-specific correlations with individual PFAS because the available sample size within PFAS chain-length classes and strong inter-PFAS collinearity reduce statistical power and inflate false positives after FDR control. Instead, we report robust duration-linked enrichment and propose confirmatory analyses with expanded cohorts and targeted meta-omics analysis.

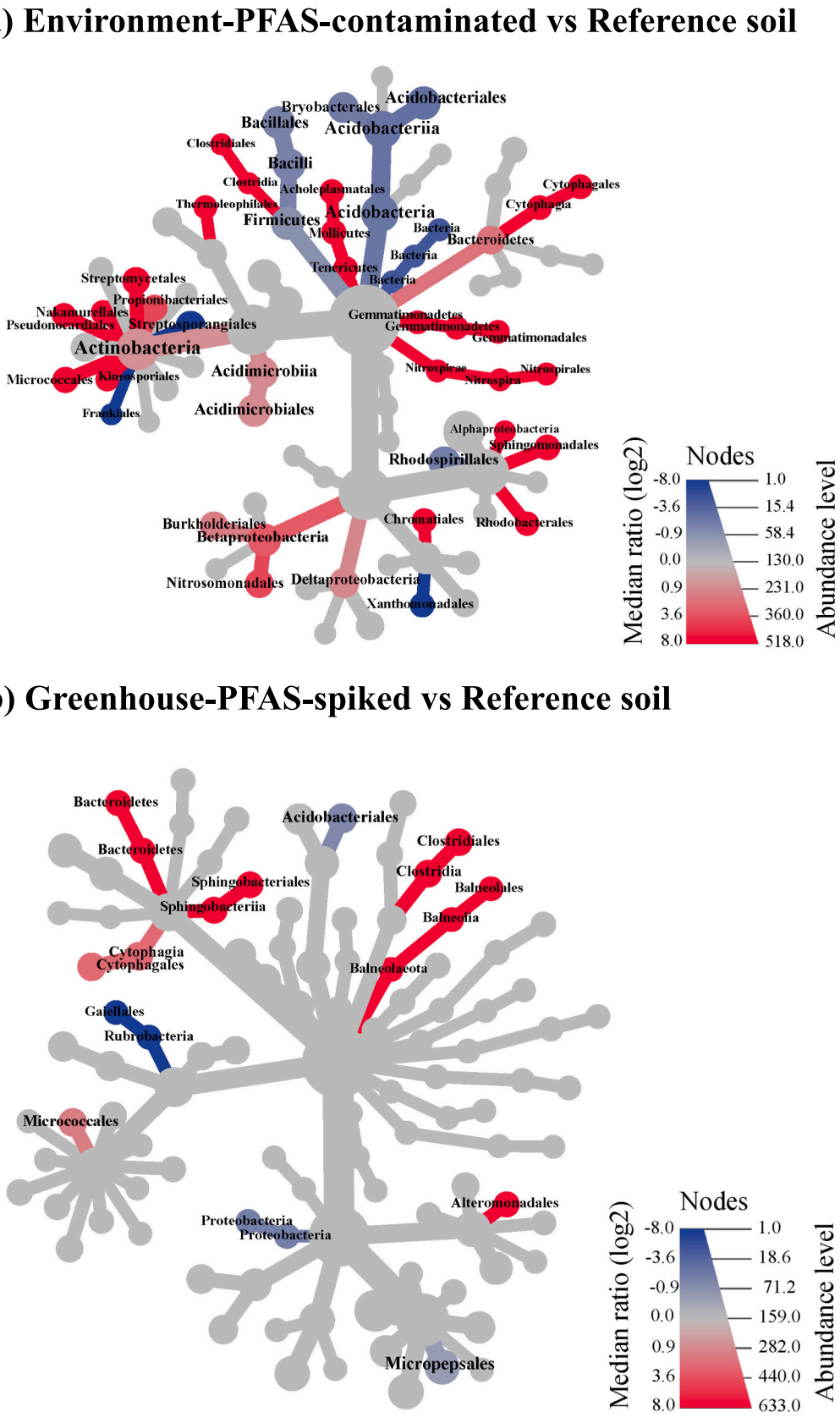

Fig. 6. Heat tree analysis showing taxonomic differences at the order level. a) Environmental and b) greenhouse soils highlighting orders significantly enriched ($p < 0.05$) in PFAS-exposed versus reference groups. Colors represent median abundance differences based on non-parametric Wilcoxon Rank Sum tests. The analysis provides an overview of taxonomic shifts in response to PFAS exposure across soil types.

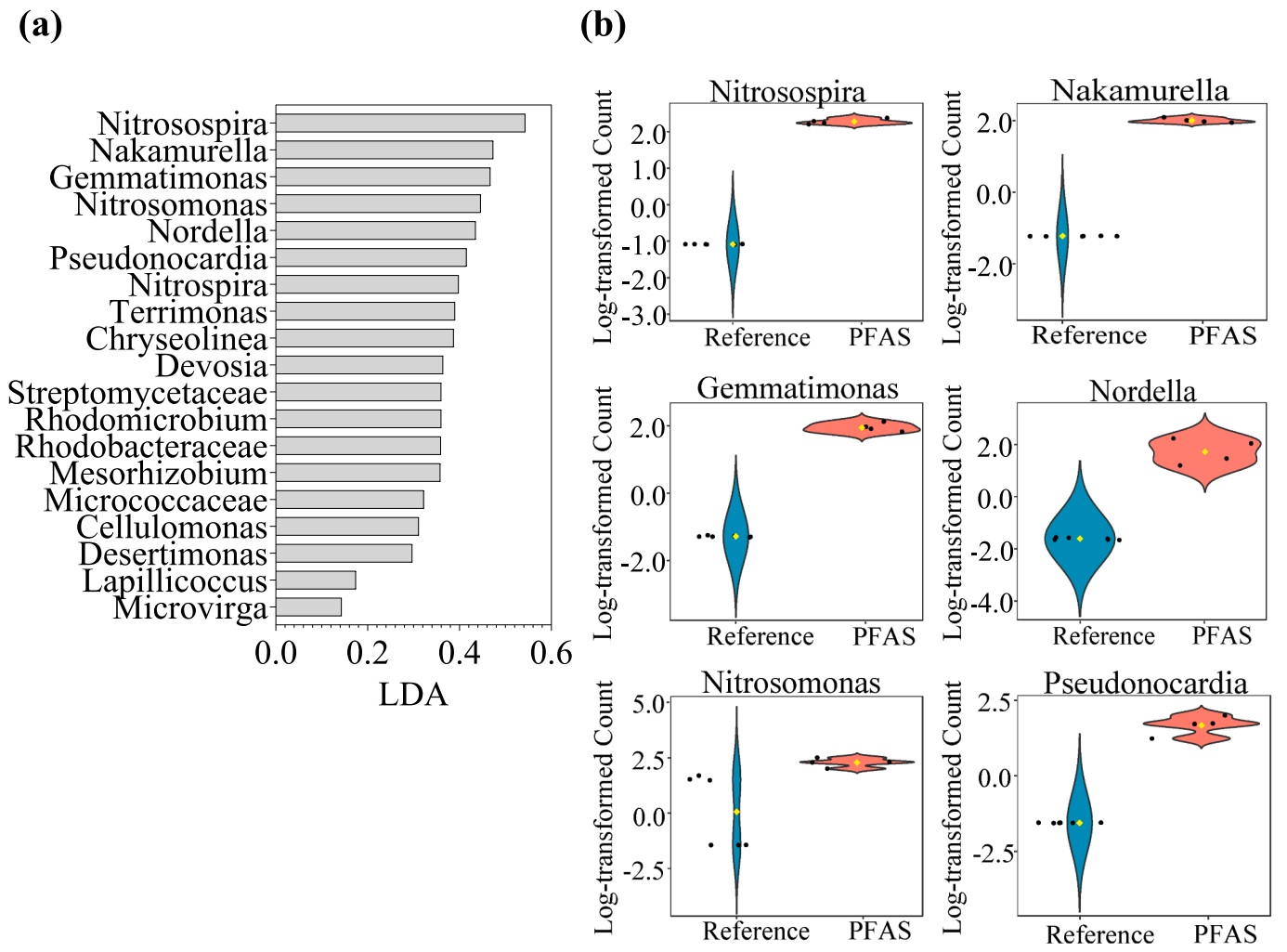

Fig. 7. Genera-level biomarkers for PFAS exposure identified using LEfSe analysis. a) Linear discriminant analysis (LDA) scores highlighting genera differentially enriched between PFAS-exposed and reference soils. b) Log-transformed counts for genera with LDA scores >0.4. Statistical significance was determined using Wilcoxon rank-sum tests adjusted with FDR ($p < 0.05$, FDR < 0.05).

3.6. *Effect of PFAS on soil microbial function*

To investigate the microbial mechanisms involved in PFAS adaptation, functional gene redundancy was done from the amplicon sequences and abundance data using Tax4Fun2 tool (Wemheuer et al., 2020). More than 4000 gene functions showed a higher functional redundancy index during PFAS exposure compared to 3476 in reference soil (Fig. S6). The top 10 gene functions with the highest redundancy in PFAS exposure were shown in Table 1. Nine have enzymatic functions, including oxidoreductases, transferases, NADH oxidoreductase and formate hydrogenlyase, involved in xenobiotics biodegradation and metabolism, lipid, carbohydrate, cofactors and vitamins metabolism. Among the genes, *bbsG* and *mcr* are involved in anaerobic toluene and propanoate metabolism, respectively. These are particularly interesting as these enzymes can also act on PFAS (LaFond et al., 2023; Sharma et al., 2018; Van Hamme et al., 2013). Patterns of the predicted KOs linked to xenobiotic metabolism and redox processes (e.g., bbsG, mcr) were strongest in long-term environmental soils, consistent with chronic

Table 1
Functional redundancy index (FRI) ratios of top 10 PFAS biased functions in bacterial communities enriched in PFAS exposed and Reference soil groups.

Gene category	Gene ID	KO name	Reference	PFAS	Enrichment*
Oxidoreductases	K19073	DVR	5.16E-06 ± 1.63E-06	2.08E-05 ± 2.96E-06	3.2
	K00112	glpB	5.03E-06 ± 1.66E-06	2.12E-05 ± 2.88E-06	3.1
	K07545	bbsG	5.08E-06 ± 1.82E-06	2.12E-05 ± 3.17E-06	2.7
	K14468	mcr	5.3E-06 ± 1.7E-06	2.06E-05 ± 3.25E-06	1.9
Transferases	K07281	ipct	4.82E-06 ± 1.69E-06	0,00002 ± 3.17E-06	2.1
	K07291	dipps	4.82E-06 ± 1.69E-06	0,00002 ± 3.17E-06	2.1
	K13522	nadM	6.45E-06 ± 1.81E-06	2.44E-05 ± 3.05E-06	3.6
Formate hydrogenlyase subunit 1	K15827	hycB	5.32E-06 ± 1.61E-06	0,00002 ± 3.08E-06	2.0
NADH oxidoreductase	K11933	hcr	3.53E-07 ± 2.35E-07	3.37E-06 ± 1.47E-06	0.0
Signaling and cellular processes	K18444	rrp1	1.79E-06 ± 8.98E-07	7.88E-06 ± 1.53E-06	0.7

* PFAS-biased KOs are the top 10 by PFAS/Reference log-ratio (Fig. S6). Group differences were tested on per-sample FRI using a two-sided Mann–Whitney U, with Holm–Šídák adjustment across the listed KOs. Enrichment = −log10(adjusted p-value); significant at adjusted $p < 0.05$.

selection of metabolic capacities (Table S12), aligning with prior reports of PFAS-impacted soils showing metabolic adjustments under sustained exposure (Cao et al., 2022). *bbsg* belong to the KEGG pathway module (M00418) responsible for anaerobic degradation of toluene to benzoyl-CoA by converting the methyl group at the aromatic ring to a carboxyl group (Kanehisa et al., 2023). *mcr* is part of the 3-hydroxypropionate bicycle carbon fixation pathway module involved in propanoate metabolism by converting acyl-CoA via dicarboxylate semialdehyde. As certain PFAS also contain of aromatic rings, *bbsg* could be involved in biodegradation of PFAS in the environment. Co-metabolism and enzyme versatility seen in *mcr* could also have an important role in the versatility and adaptability of certain bacterial communities in PFAS exposed soil. Microbial communities may in turn influence PFAS fate and transformation via co-metabolism and redox processes. Prior work implicates taxa/enzymes in FTSAs biotransformation and xenobiotic metabolism that could intersect with PFAS degradation (e.g., *Gordonia*, bbsG/*mcr*-linked routes) (Van Hamme et al., 2013). Future validation should pair biomarker tracking with PFAS speciation (including precursors) and metabolomics to resolve putative degradation networks. It's important to note that these functions are inferred from taxonomy and should be validated with shotgun metagenomics and/or metatranscriptomics in future studies.

Previous studies showed that different PFAS affect soil microbial groups differently (Bao et al., 2018). More groups are identified in the present study due to inclusion of soil containing a PFAS mixture over several years. Therefore, more comprehensive biomarker signatures are presented than previous reports of 80–90-day exposures with single PFAS (Bao et al., 2018; Cai et al., 2020; Xu et al., 2022). These groups might have unique adaptation mechanisms or special functions that enable them to become enriched during PFAS exposure (Xu et al., 2023; Xu et al., 2022). The specific mechanisms could be revealed with functional enrichment studies. Such methods may include differential gene regulation involved in xenobiotic degradation pathways between PFAS tolerant and susceptible groups of bacteria (Xu et al., 2022). Since functions are inferred, their verification should pair community profiling with shotgun metagenomics (KO/EC quantification), metatranscriptomics (pathway expression under PFAS), and targeted assays (qPCR of bbsG/mcr carriers, enrichment cultures). Recent PFAS metaomics and MAG-level work illustrate feasible routes to link predicted pathways to measured gene inventories and expression in PFAS-impacted matrices (Sorn et al., 2025).

In conclusion, we have shown that long-term PFAS exposure in the environment caused significant shifts in bacterial community than short-term high concentration exposures. This highlights the importance of considering both the duration and concentration of PFAS exposure when assessing their impact on soil ecosystems. While PFAS enriches some microbial groups and increases the overall diversity of the soil microbiome, it did not lead to a dominant microbial group. We have also identified a set of taxa that could be considered biomarkers of PFAS exposure and contribute to the discrimination between PFAS exposed and non-exposed soil, irrespective of exposure duration in environmentally relevant mixture. The microbial community metabolic adaptations to long-term PFAS exposure were also inferred from predicted genes. The present study provides a strong foundation for future metagenomic/metatranscriptomic profiling to confirm the abundance and functionality of the predicted pathways.

CRediT authorship contribution statement

Yared H. Bezabhe: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Winnie Nassazzi:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Savita Tapase:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Lutz Ahrens:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Jana Jass:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT/OPENAI to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106561.

Data availability

The datasets generated and used in the current study are available in the NCBI repository, Sequence Read Archive (SRA) under the accession SUB15005063(PRJNA1215315).

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