

Cross-kingdom Microbial Reassembly During Organic Amendment-Assisted Biodegradation of PAHs in Soil

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Research Article

Keywords: PAH-contaminated soil, organic amendment, microbial community assembly, cross-kingdom networks, hydrocarbon-degrading bacteria, fungal guilds, bioremediation

Posted Date: October 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11166271/v1>

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Additional Declarations: No competing interests reported.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are persistent soil contaminants whose biodegradation depends on microbial metabolic potential, community structure, and associations. This study evaluated how leaf organic amendment regulates bacterial–fungal reassembly during PAH bioremediation in soil microcosms. Treatments comprised unamended control, PAH-only soil, and PAH-contaminated soils amended with 10, 30, or 70% (w/w) leaf organic matter over 56 days. Bacterial 16S rRNA V3–V4 and fungal ITS1 amplicons were processed as amplicon sequence variants and analysed using Hellinger-transformed abundance data, ordination, differential abundance, functional inference, co-occurrence networks, neutral modelling and stability metrics. Principal component analysis showed treatment- and time-dependent restructuring, with PC1/PC2 explaining 50.0/16.8% of bacterial and 44.1/13.6% of fungal variance. Redundancy analysis identified residual PAH concentration, PAH removal and time point as major gradients, explaining 50.1/44.3% of constrained bacterial variance and 65.9/24.7% of fungal variance. Bacterial–fungal concordance was significant (Procrustes $r = 0.652$, $p = 0.001$). Treatment-responsive taxa included Enterobacterales, *Bradyrhizobium*, *Bacillus*, *Barnettozyma californica* and *Apiotrichum chiropterorum*. Functional inference showed stable hydrocarbon-degrading bacterial profiles (0.45–0.59) and strong sensitivity to amendment in yeast-like fungi. The co-occurrence network contained 46 nodes, 183 edges and six modules, with putative keystone taxa including Enterobacterales, Helotiales, *Trichoderma stromaticum*, *Rhodococcus* and *Bradyrhizobium*. Neutral modelling indicated stronger stochastic assembly in fungi ($R^2 = 0.772$) than bacteria ($R^2 = 0.377$), whereas beta-dispersion was not significant. High amendment caused greater disturbance, with the lowest recovery at 70% leaf amendment (0.10). Hence, leaf amendment reorganised biodegradation-associated bacterial–fungal communities, highlighting microbial associations, functional restructuring and assembly processes as key features of PAH bioremediation in soil.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic contaminants composed of two or more fused aromatic rings. They enter terrestrial environments through natural and anthropogenic sources, including incomplete combustion of fossil fuels and biomass, petroleum processing, industrial emissions, traffic-derived particles, and contaminated runoff. Because of their hydrophobicity, low aqueous solubility, and strong sorption to soil organic matter, PAHs often persist in soil and become poorly bioavailable, limiting natural attenuation and complicating remediation (Montano et al. 2025; Feng et al. 2025). Several PAHs are toxic, mutagenic, or carcinogenic, and their accumulation in soil poses risks to microbial functioning, food-chain transfer, and human health (Singh and Singh 2025; Ghosal et al. 2016). Therefore, improving biologically based approaches to PAH attenuation remains a priority in contaminated soil management.

Microbial biodegradation is a major route for PAH transformation and detoxification because naturally occurring microorganisms can use aromatic hydrocarbons as carbon and energy sources or transform them through co-metabolic processes (Peng et al. 2026). Bacterial PAH degradation has been widely

studied, particularly in genera such as *Pseudomonas*, *Rhodococcus*, *Mycobacterium*, *Sphingobium*, and related hydrocarbon-degrading taxa. These bacteria commonly initiate PAH oxidation via monooxygenases or dioxygenases, followed by ring cleavage and subsequent central metabolic pathways (Zhang et al. 2022; Cerniglia 1992; Seo et al. 2009). Fungi also contribute to PAH transformation, especially through extracellular oxidative enzymes such as laccases, lignin peroxidases and manganese peroxidases, as well as cytochrome P450-associated intracellular oxidation systems that can act on structurally complex and high-molecular-weight PAHs (Harms et al. 2011; Pozdnyakova 2012; Cerniglia and Sutherland 2010). However, PAH removal in soil is rarely determined by single taxa alone. Instead, biodegradation depends on microbial associations, substrate bioavailability, nutrient status, oxygen availability, metabolic complementarity, and ecological interactions within the resident soil microbiome.

Bacterial–fungal interactions are particularly relevant to biodegradative systems because bacteria and fungi differ in growth form, substrate acquisition, dispersal and enzymatic capacity. Fungi can extend through soil pores as hyphal networks, mobilize nutrients and modify access to hydrophobic contaminants, while bacteria may exploit fungal-derived metabolites or transformation products generated during partial PAH oxidation. Such cross-kingdom interactions may include facilitation, competition, antagonism, syntrophy and niche partitioning, thereby influencing both contaminant transformation and microbial community assembly (Zhang et al. 2025; de Boer et al. 2005; Kohlmeier et al. 2005). Despite this ecological relevance, many PAH bioremediation studies still emphasize bacterial or fungal responses in isolation. This limit understanding of how bacterial–fungal coupling contributes to biodegradation-associated community restructuring in response to organic amendments.

Organic amendment is a practical strategy for enhancing microbial PAH biodegradation. Plant-derived residues and other organic materials can increase carbon and nutrient availability, stimulate microbial activity, modify soil physicochemical properties, and provide co-substrates that support co-metabolic transformation (Akinseye and Charles 2026b). However, organic inputs may also alter PAH sorption and bioavailability, and their effects are likely to be dose dependent. Low to moderate amendment may stimulate biodegradative microbial activity, whereas excessive amendment can shift nutrient stoichiometry, increase oxygen competition, promote contaminant immobilisation through sorption, or drive persistent community disturbance (Akinseye and Charles 2026a; Cébron et al. 2011; Sayara and Sánchez 2020). Therefore, effective amendment-assisted bioremediation requires understanding not only whether organic inputs stimulate PAH attenuation, but also how amendment intensity restructures biodegradation-associated microbial communities and their functional potential.

High-throughput amplicon sequencing and ecological modelling provide useful tools for evaluating biodegradation-associated microbiomes under contaminant stress. Ordination approaches, including principal component analysis and redundancy analysis, can identify treatment- and contaminant-associated shifts in community composition. Differential abundance analysis can reveal taxa responsive to amendment or PAH gradients, while functional inference and fungal guild classification can identify putative hydrocarbon-degrading, nutrient-cycling, or substrate-responsive groups (Douglas et al. 2020;

Nguyen et al. 2016). Co-occurrence network analysis can further identify microbial associations and putative hub taxa, although correlation-based networks should be interpreted as ecological association patterns rather than direct evidence of biological interaction (Faust and Raes 2012; Röttgers and Faust 2018). In addition, neutral community models and dissimilarity-based assembly metrics can provide insight into the balance between stochastic processes and deterministic filtering during microbiome restructuring (Sloan et al. 2006; Zhou and Ning 2017).

A key unresolved question is how organic amendment intensity links PAH-remediation gradients with bacterial–fungal reassembly, microbial association networks, biodegradation-associated functional potential, and community stability. It remains unclear whether amendment-assisted PAH attenuation is associated primarily with enrichment of specific degradative taxa, coordinated bacterial–fungal responses, altered community assembly processes, or changes in resilience following disturbance. Addressing this gap is important for improving bioremediation strategies because contaminant removal may depend not only on microbial diversity, but also on the organization of functionally relevant microbial associations.

In this study, we investigated how graded leaf organic amendment regulates bacterial and fungal community dynamics during PAH bioremediation in soil microcosms. Treatments included an unamended control, PAH-only soil, and PAH-contaminated soils amended with 10%, 30%, or 70% (w/w) leaf organic matter over a 56-day incubation. Bacterial 16S rRNA V3–V4 and fungal ITS1 amplicon sequencing were combined with ordination, differential abundance analysis, functional inference, Procrustes analysis, cross-kingdom co-occurrence network analysis, neutral community modelling and stability assessment. We hypothesized that leaf amendment would induce treatment- and time-dependent bacterial–fungal reassembly; that residual PAH concentration, PAH removal, and time would structure microbial composition; that bacterial and fungal communities would show coordinated but kingdom-specific responses; and that assembly processes and resilience would differ between bacteria and fungi as amendment intensity increased.

By linking PAH-remediation gradients with bacterial–fungal community structure, microbial associations, functional potential and assembly processes, this study provides a biodegradation-focused ecological framework for understanding organic amendment-assisted PAH bioremediation. The findings are expected to clarify how leaf-derived organic inputs reshape biodegradation-associated soil microbiomes and inform strategies for optimizing microbial community function while minimising persistent ecological disturbance.

Materials and Methods

Soil collection, PAH contamination, and amendment preparation

Soil microcosms were established using a silt-loam soil previously described by Akinseye and Knapp (2026a). Briefly, topsoil was collected from the 0–20 cm layer of a residential garden located on a reclaimed former mine site in South Lanarkshire, Glasgow, UK. Based on site history, texture and

diagnostic characteristics, the soil was classified as a *Spolic Technosol*. After collection, soil was air-dried, manually cleaned to remove stones, root debris, and soil fauna, sieved through a 2-mm mesh, and stored at room temperature before the experimental setup. Baseline soil properties included pH 7.24 ± 0.18 , electrical conductivity of $2500 \pm 315 \mu\text{S cm}^{-1}$, water-holding capacity of $73.2 \pm 0.84\%$, and silt-loam texture comprising 45.0% sand, 54.2% silt and 0.8% clay. The soil contained $2.9 \pm 0.31\%$ corrected organic carbon, $0.12 \pm 0.08\%$ total nitrogen, and a C/N ratio of 43.3 ± 0.55 , indicating moderate nitrogen limitation.

Phenanthrene and pyrene were selected as representative PAHs. Analytical grade phenanthrene and pyrene standards ($\geq 98\%$ purity; Thermo Fisher Scientific) were prepared as a 1:1 (v/v) mixture in hexane. Soil was rewetted to 15% moisture on a dry-weight basis and pre-incubated at 30°C for five days to stabilise microbial activity prior to contamination. PAH spiking was performed using a partial-treatment approach. Briefly, 7.5 mL of the phenanthrene–pyrene solvent mixture (1:10 solvent-to-soil ratio) was added to 75 g of soil, representing 25% of the total soil mass. The flask was sealed for 5 min to enhance solvent dispersion, after which the solvent was evaporated for 16 h under a fume hood. The spiked soil fraction was then thoroughly homogenised with the remaining unspiked soil to obtain a uniform PAH-contaminated matrix with a final phenanthrene/pyrene concentration of 150 mg kg^{-1} dry soil.

Leaf organic amendment was prepared from naturally senesced *Pinus sylvestris* and *Populus nigra* litter collected in mid-August 2024 from park and roadside stands in the Royston district of North Glasgow, UK. Recently fallen leaf material was collected under dry weather conditions, transported to the laboratory within 1 h, air-dried, oven-dried at $40\text{--}50^\circ\text{C}$, and ground to a homogeneous powder. Pine and poplar leaf powders were mixed at a 1:1 ratio and stored at room temperature until use. The pine–poplar amendment was characterised by high organic carbon content ($51.36 \pm 0.17\%$) and total phenolic content of $26.71 \pm 0.34 \text{ mg L}^{-1}$, providing lignocellulosic material and potential co-metabolic substrates for microbial PAH transformation.

Microcosm setup and incubation

PAH-contaminated soil was amended with powdered pine–poplar leaf litter at 10%, 30%, and 70% (w/w, dry-weight basis). The experimental design for the microbiome study included an unamended control, PAH-only soil, and PAH-contaminated soils amended with 10%, 30% or 70% leaf organic matter. Where included, the leaf-only treatment was treated as an amendment control and analysed separately from PAH-spiked treatments. Each treatment was established in triplicate microcosms.

Microcosms were assembled in 1-L amber glass bottles containing 500 g soil. Bottle openings were covered with perforated aluminium foil to permit gas exchange while limiting external contamination. All microcosms were manually agitated daily to maintain homogeneity and aerobic conditions. The incubation was continued for 56 days, with destructive or subsampling collections on days 1, 7, 14, 28, and 56 for microbial community analysis. In the original PAH degradation experiment, samples were collected at days 0, 7, 14, 28, and 56; the present sequencing design used the available microbiome sampling points after quality filtering.

Throughout incubation, environmental conditions were monitored to support interpretation of PAH dissipation and microbial community shifts. Soil moisture was maintained within 20–35% by periodic measurement and adjustment. Moisture content was determined every 3–4 days using a Sartorius moisture analyser by drying 5–7 g soil at 105°C to constant weight. Soil temperature was measured at a depth of 3 cm using a handheld digital soil thermometer with a precision of $\pm 0.1^\circ\text{C}$ and remained within approximately 15–21°C during incubation. Soil pH was measured periodically using a calibrated Seven Excellence meter in a 1:2.5 soil-to-water suspension. These environmental variables were later used as explanatory factors for PAH-remediation gradients and microbial community restructuring.

PAH extraction, quantification and removal calculation

Residual phenanthrene and pyrene concentrations were determined over the 56-day incubation to quantify PAH dissipation and derive biodegradation-associated kinetic parameters. PAHs were extracted using a Dionex Accelerated Solvent Extractor (ASE 350). Soil samples were mixed with diatomaceous earth as a dispersant and extracted at 100°C and 1500 psi using 5 min static extraction cycles. Extracts were concentrated to a final volume of 1.5 mL prior to instrumental analysis.

PAH quantification was performed using a Finnigan Focus gas chromatograph equipped with a flame ionisation detector (GC-FID). The carrier gas pressure was maintained at 20 psi. The oven programme was initiated at 50°C, increased to 250°C at $10^\circ\text{C min}^{-1}$, and held at 250°C for 10 min. Phenanthrene and pyrene concentrations were quantified using external calibration curves prepared from analytical standards. Residual PAH concentrations were expressed as mg kg^{-1} dry soil.

Percentage PAH removal was calculated using:

$$\text{PAH removal (\%)} = [(C_0 - C_t) / C_0] \times 100$$

where C_0 is the initial PAH concentration, and C_t is the residual PAH concentration at each sampling time point. PAH dissipation was further modelled using a one-phase exponential decay model, and the degradation rate constant (k) was used to calculate half-life according to:

$$t_{1/2} = \ln(2) / k$$

PAH concentration and removal data used as environmental/remediation variables were obtained from the corresponding degradation experiment described by Akinseye and Knapp (2026a). Residual phenanthrene and pyrene concentrations were quantified over the 56-day incubation and used to calculate percentage PAH removal and first-order kinetic parameters. In the present study, residual PAH concentration, PAH removal percentage, time point and treatment were used as explanatory variables in downstream microbial community analyses, including ordination, redundancy analysis and community restructuring assessments.

DNA extraction and quality assessment

Total genomic DNA was extracted from approximately 0.25 g of soil using the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's instructions. Mechanical lysis was performed using bead beating on an MP Biomedicals homogeniser for 30 s to improve disruption of bacterial and fungal cells. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), based on A260/A280 and A260/A230 ratios. Extracted DNA was stored at – 20°C until PCR amplification.

Amplicon library preparation and sequencing

Purified DNA extracts were submitted to the NU-OMICS sequencing facility, Northumbria University, Newcastle upon Tyne, UK, for amplicon library preparation and sequencing. Bacterial community composition was characterised by amplification of the V4 region of the 16S rRNA gene using the Earth Microbiome Project 515F–806R primer set: 515F, 5'-GTGYCAGCMGCCGCGGTAA-3', and 806R, 5'-GGACTACNVGGGTWTCTAAT-3'. Fungal community composition was characterised by amplification of the ITS region using the Earth Microbiome Project ITS1F–ITS2 primer set: ITS1F, 5'-CTTGGTCATTTAGAGGAAGTAA-3', and ITS2, 5'-GCTGCGTTCTTCATCGATGC-3'. The Earth Microbiome Project 16S protocol targets the V4 region with 515F–806R primers, while the ITS protocol uses ITS1f–ITS2 primers for fungal microbial eukaryotic lineages.

Amplicon library preparation was performed by the sequencing facility using its Illumina amplicon-sequencing workflow. PCR reactions contained 1× KAPA 2G Robust polymerase mix, 0.5 µM of each primer and 5 µL template DNA to support amplification from potentially low-biomass samples. Positive and negative controls were included during amplification; the positive control was the ZymoBIOMICS microbial community DNA standard, and the negative control contained no sample template. Amplicons were pooled and sequenced using paired-end 2 × 250 bp chemistry. Bacterial 16S rRNA amplicons were sequenced on an Illumina MiSeq i100, while fungal ITS amplicons were sequenced on an Illumina MiSeq platform. Adapter sequences were trimmed by the platform software before FASTQ delivery, whereas primer sequences were retained and removed during downstream bioinformatic processing. The sequencing runs were prepared with a target PhiX concentration of 15–25% and loaded at 4–4.5 pM.

Sequence processing and ASV taxonomic assignment

Raw demultiplexed reads were processed using QIIME2 v2023.5 (Bolyen et al. 2019). Sequence quality filtering, denoising, paired end read merging and chimera removal were performed using DADA2, which infers amplicon sequence variants (ASVs) after error correction (Callahan et al. 2016). Bacterial and fungal datasets were processed separately. Taxonomic classification was performed using naïve Bayes classifiers trained on the SILVA v138 database for bacterial 16S rRNA sequences and the UNITE v8.3 database for fungal ITS sequences.

For the bacterial dataset, chloroplast, mitochondrial and unassigned sequences were removed. For the fungal dataset, non-fungal, unassigned and low-confidence taxonomic assignments were excluded. Rare ASVs representing < 0.01% relative abundance across the dataset were removed to reduce low-

abundance noise. The resulting bacterial and fungal feature tables were converted to relative abundance and Hellinger-transformed prior to multivariate analyses, as Hellinger transformation improves the suitability of community-composition data for Euclidean-based ordination (Legendre and Gallagher 2001). After quality-control filtering, 26 samples were retained for downstream ecological analyses.

Diversity metrics, ordination and environmental fitting

Alpha diversity was assessed using the Shannon diversity index to describe within-sample bacterial and fungal diversity. Beta-diversity patterns were evaluated using Bray–Curtis dissimilarity calculated from transformed community abundance data. Prior to ordination, bacterial and fungal feature tables were converted to relative abundance and Hellinger-transformed to reduce the influence of highly abundant taxa and improve suitability for Euclidean-based multivariate analysis.

Principal component analysis (PCA) was performed separately for bacterial and fungal communities using Hellinger-transformed abundance matrices. PCA was used to visualise treatment- and time-dependent variation in community composition across control, PAH-only and leaf-amended treatments. Samples were plotted according to treatment and time point, and treatment-level confidence ellipses were used to summarise clustering patterns and within-treatment dispersion in ordination space.

Redundancy analysis (RDA) was performed using the *vegan* package in R to assess relationships between microbial community composition and biodegradation-associated variables (Oksanen et al. 2022). Separate RDA models were constructed for bacterial and fungal communities and constrained by residual PAH concentration, percentage PAH removal and time point. These variables represented the major contamination, remediation and temporal gradients in the microcosm system. Environmental vectors were fitted to the ordination space to evaluate the direction and relative strength of PAH-related gradients associated with bacterial and fungal community restructuring.

Variation partitioning was used to estimate the relative contribution of treatment, PAH-related variables and temporal effects to microbial community variation. This approach enabled assessment of whether bacterial and fungal community patterns were primarily associated with amendment treatment, residual PAH burden, PAH removal efficiency or incubation time.

Differential abundance analysis

Treatment-responsive bacterial and fungal taxa were identified separately using non-parametric Kruskal–Wallis tests applied to relative-abundance data. This approach was selected because microbial abundance data are typically non-normally distributed, zero-inflated and unevenly dispersed across treatment groups. Multiple testing correction was performed using the Benjamini–Hochberg false-discovery rate (FDR) method (Benjamini and Hochberg 1995), and taxa with FDR-adjusted p values < 0.05 were considered significantly treatment-responsive. Effect size was calculated as the difference between the maximum and minimum median relative abundance of each taxon across treatments. Taxa showing both statistical significance and high effect-size values were prioritised as the strongest

bacterial or fungal indicators of amendment-associated community restructuring. Where possible, taxa were reported at the lowest reliable taxonomic rank; otherwise, higher-level assignments were retained.

Functional inference and fungal guild classification

Bacterial functional potential was inferred using PICRUSt2 (Douglas et al. 2020) from bacterial ASV representative sequences and abundance tables. Predicted functional profiles were screened for pathways and functional categories associated with hydrocarbon degradation, aromatic compound metabolism and nitrogen cycling, as these processes are relevant to PAH transformation and amendment-driven nutrient turnover. Functional profiles were summarised by treatment and time point to evaluate whether leaf amendment altered predicted biodegradation-associated bacterial potential.

Fungal ecological guilds were assigned using FUNGuild (Nguyen et al. 2016) based on taxonomic assignments from the fungal ITS dataset. Guild assignments were grouped into major ecological categories relevant to organic-matter turnover and contaminant-associated community restructuring, including saprotrophs and yeast-like fungi. Where confidence scores were available, only assignments with acceptable confidence levels were retained, while ambiguous or unassigned taxa were excluded from guild-level summaries. Relative abundances of fungal guilds were calculated for each sample and summarised across treatments and time points.

Because PICRUSt2 and FUNGuild provide predicted functional potential rather than direct measurements of gene expression, enzyme activity or PAH mineralisation, outputs were interpreted cautiously as indicators of putative bacterial functional capacity and fungal ecological roles. These analyses were used to support interpretation of community restructuring along PAH-remediation gradients, rather than to confirm active biodegradation pathways.

Bacterial–fungal concordance analysis

Bacterial–fungal community concordance was assessed using Procrustes analysis implemented in the *vegan* package in R. Separate bacterial and fungal ordinations were first generated from Hellinger-transformed abundance matrices using the same set of matched samples. The bacterial and fungal ordination configurations were then superimposed by Procrustes rotation to evaluate whether both kingdoms showed similar compositional structuring across PAH and leaf-amendment treatments.

The Procrustes correlation coefficient was used to quantify the strength of concordance between bacterial and fungal community configurations. Statistical significance was assessed using a permutation test with 999 permutations. Sample-wise Procrustes residuals were calculated to estimate the distance between paired bacterial and fungal ordination positions for each sample. Lower residual distances were interpreted as stronger bacterial–fungal community concordance, whereas higher residual distances indicated greater divergence between bacterial and fungal responses. Procrustes outputs were used to evaluate coordinated community-level responses to PAH contamination, leaf amendment and incubation time, rather than to infer direct biological interactions.

Cross-kingdom co-occurrence network analysis

Cross-kingdom co-occurrence network analysis was performed to identify bacterial and fungal taxa showing significant association patterns across PAH and leaf-amendment treatments. Network construction was based on the filtered bacterial and fungal taxonomic abundance tables generated after removal of rare ASVs. Dominant and treatment-informative bacterial and fungal taxa were retained for network analysis to reduce the influence of low-abundance taxa and improve interpretability.

Pairwise associations among bacterial and fungal taxa were calculated using Spearman rank correlation based on relative-abundance profiles across samples. Correlations with $|\rho| > 0.6$ and $p < 0.05$ were retained as significant edges. Positive correlations were interpreted as taxa showing similar abundance patterns across samples, whereas negative correlations indicated inverse abundance patterns.

Networks were generated and visualised using the *igraph* package in R and further refined in Gephi. Nodes represented bacterial or fungal taxa, while edges represented significant positive or negative co-occurrence associations. Network topology was evaluated using degree centrality, network density, modularity and module membership. Candidate hub taxa were identified based on high degree centrality and central positions within network modules.

Because the network was inferred from correlation-based abundance patterns, edges were interpreted as co-occurrence associations rather than direct biological interactions. Therefore, the network analysis was used to identify putative bacterial–fungal association patterns linked to PAH contamination and leaf amendment, not to confirm physical interaction, syntrophy or causal relationships among taxa.

Community assembly analysis

Community assembly patterns were evaluated separately for bacterial and fungal communities using a neutral community model based on Sloan et al. (2006). The model relates taxon occurrence frequency to mean relative abundance across samples and was applied to the filtered ASV abundance tables. Model fit, expressed as R^2 , was used to estimate the degree to which bacterial and fungal occurrence patterns conformed to neutral expectations. Higher model fit was interpreted as stronger consistency with neutral or stochastic assembly, whereas lower model fit indicated greater deviation from neutral expectations and possible influence of deterministic processes such as environmental filtering.

Beta-dispersion analysis was performed to assess differences in within-treatment community variability by calculating the distance of samples to their treatment centroid. Differences in dispersion among treatments were tested using permutation-based analysis. Raup–Crick-style dissimilarity and Bray–Curtis dissimilarity summaries were also calculated as additional indicators of community turnover. These metrics were used as assembly-related proxies to compare bacterial and fungal community structuring under PAH contamination and leaf amendment. Because phylogeny-based β NTI and RCbray partitioning were not applied, assembly outcomes were interpreted cautiously as evidence of relative

conformity to neutral expectations and community turnover, rather than as formal partitioning of selection, dispersal limitation, homogenising dispersal and ecological drift.

Machine learning and exploratory feature ranking

Random Forest regression was used as an exploratory feature-ranking approach to identify bacterial and fungal taxa associated with PAH removal. Percentage PAH removal was used as the response variable, and bacterial-only, fungal-only and combined bacterial–fungal feature sets were evaluated separately. Prior to modelling, low-abundance and non-informative features were filtered from the taxonomic abundance tables. Random Forest models were constructed using 1000 trees, and model performance was assessed using mean squared error and percentage variance explained. Feature importance was estimated using percentage increase in mean squared error (%IncMSE), where higher values indicated greater contribution of a feature to model performance.

Because the dataset contained a limited number of quality-filtered samples, Random Forest outputs were interpreted as exploratory indicators of taxa associated with PAH-remediation gradients rather than as validated predictive biomarkers. Machine-learning outputs were therefore used to support ecological interpretation alongside ordination, differential abundance, functional inference and network analyses.

Stability and recovery analysis

Microbial community stability was assessed using temporal distance from baseline community composition. For each treatment, the earliest sequenced time point was used as the baseline community state, and Bray–Curtis dissimilarity was calculated between baseline samples and subsequent time points. Separate distance-to-baseline trajectories were first generated for bacterial and fungal communities to quantify the magnitude and persistence of community restructuring over the 56-day incubation.

An integrated bacterial–fungal community distance was then calculated by averaging the bacterial and fungal Bray–Curtis distance values for each treatment and time point. Because Bray–Curtis dissimilarity ranges from 0 to 1, this approach allowed bacterial and fungal community shifts to be combined into a single cross-kingdom stability metric. Treatment-level distance trajectories were used to compare the extent of microbial disturbance across control, PAH-only and leaf-amended treatments.

Community recovery at the final time point was estimated using a recovery index calculated as:

Recovery index = $1 - \text{integrated community distance}$

where higher values indicate greater return toward the baseline community state and lower values indicate more persistent community restructuring. Recovery values were compared among treatments to evaluate whether increasing leaf-amendment intensity altered microbial resilience during PAH bioremediation.

All statistical analyses were performed in R v4.3.0 unless otherwise stated. Data visualisation was conducted using *ggplot2* and related R packages. Community ecology analyses, including Bray–Curtis dissimilarity, ordination, redundancy analysis, beta-dispersion and variation partitioning, were performed using the *vegan* package. Network analyses were conducted using *igraph* in R, with final network visualisation and layout refinement performed in Gephi. Functional inference and fungal guild classification were conducted using PICRUSt2 and FUNGuild, respectively. Unless otherwise stated, statistical significance was assessed at $p < 0.05$. Where multiple comparisons were performed, p values were adjusted using the Benjamini–Hochberg false-discovery rate method. Processed ASV tables, taxonomic assignments, sample metadata, PAH-removal data, network files and analysis outputs are available in the accompanying Zenodo repository [**Record ID: 22960951**].

Results

Principal component analysis (PCA) of Hellinger-transformed relative abundance data showed treatment- and time-dependent structuring of both bacterial and fungal communities. For bacteria, PC1 and PC2 explained 50.0% and 16.8% of the total variance, respectively, with samples separating mainly along PC1 according to amendment status. Leaf-amended and leaf-dominated samples, including PAH_70%-Leaf clustered toward the positive PC1 axis, indicating marked divergence from control and PAH-only samples, whereas PAH_10%-Leaf and PAH_30%-Leaf showed partial overlap, indicating transitional community restructuring at lower amendment levels. Fungal communities also showed compositional differentiation, with PC1 and PC2 explaining 44.1% and 13.6% of the variance, respectively. Compared with bacteria, fungal samples displayed tighter treatment-level clustering and reduced overlap among groups, suggesting a more coherent response to leaf amendment. Across both kingdoms, temporal shifts indicated progressive microbial community reassembly during the 56-day incubation. Therefore, PCA indicated that leaf amendment substantially altered bacterial and fungal community composition, with fungi showing stronger treatment-level coherence and bacteria showing greater heterogeneity across amendment gradients.

Taxonomic restructuring and differential abundance of bacterial and fungal communities

Hierarchical clustering of the top 24 bacterial and fungal taxa showed treatment- and time-associated community restructuring across the PAH and leaf-amendment gradients (Fig. 2A, 2B). Heatmaps based on Hellinger-transformed and z-score-scaled relative abundance data showed distinct bacterial and fungal taxonomic patterns, with metadata annotations indicating correspondence between microbial composition, residual PAH concentration, PAH removal percentage, treatment and incubation time. In bacterial communities, taxa including *Bradyrhizobium*, *Rhodococcus*, *Pseudomonas*, Enterobacteriaceae and *Sphingobium* varied across treatments, with several showing increased relative abundance under leaf-amended conditions. Bacterial clustering separated PAH-only and leaf-amended samples, although the pattern was relatively diffuse, indicating greater within-treatment heterogeneity. In contrast, fungal

communities showed more cohesive treatment-level clustering, with Sordariales, *Mortierella*, Agaricales and Chaetomiaceae increasing in relative abundance under leaf amendment, particularly at higher amendment levels.

Differential abundance analysis further identified treatment-responsive taxa in both kingdoms (Fig. 2C, 2D). Among bacteria, Enterobacterales, *Bradyrhizobium* and *Escherichia–Shigella* showed marked treatment-associated shifts, with effect sizes up to approximately 0.4 and strong statistical support ($-\log_{10}$ FDR > 2). Ranked significant taxa showed that fungal taxa had the largest effect sizes, particularly *Barnettozyma californica* (effect size $\approx$ 0.85; FDR $\approx$ 0.0447) and *Apiotrichum chiropterorum* (effect size $\approx$ 0.65; FDR $\approx$ 0.0447). Among bacteria, Enterobacterales (effect size $\approx$ 0.35; FDR $\approx$ 0.00322) and KD4-96 (effect size $\approx$ 0.30; FDR $\approx$ 0.00449) showed the strongest treatment-associated responses, with additional significant shifts observed for *Bacillus*, *Bradyrhizobium* and Enterobacteriaceae (FDR < 0.01). Overall, fungal taxa showed larger treatment-response effect sizes, whereas bacterial taxa showed stronger statistical significance, indicating distinct but complementary bacterial and fungal responses to PAH contamination and leaf-amendment intensity.

Redundancy analysis (RDA) showed that residual PAH concentration, PAH removal percentage and incubation time were major explanatory gradients associated with bacterial and fungal community structure (Fig. 3A, B). For bacteria, RDA1 and RDA2 explained 50.1% and 44.3% of the constrained variance, respectively. Bacterial samples showed clear treatment-level separation, with higher leaf-amendment treatments, particularly 30–70% leaf, separating from control and PAH-only samples. Residual PAH concentration was positively associated with RDA2, whereas PAH removal percentage was negatively associated with RDA2, indicating opposing contamination and remediation gradients. Time point aligned mainly with RDA1, suggesting a temporal component in bacterial community succession.

For fungi, RDA1 and RDA2 explained 65.9% and 24.7% of the constrained variance, respectively. Fungal samples showed a more compressed ordination pattern with greater treatment overlap than bacteria, indicating weaker treatment-specific separation but clear responsiveness to PAH-related gradients. Residual PAH concentration was strongly associated with fungal distribution along RDA1, whereas PAH removal percentage and time point were associated with shifts along RDA2. Hence, RDA indicated that bacterial communities showed stronger treatment-specific differentiation, while fungal communities displayed more overlapping but PAH-responsive compositional patterns.

Procrustes analysis of Hellinger-transformed bacterial and fungal ordinations showed significant cross-kingdom community concordance across treatments (Fig. 4A-B). The superimposed ordination revealed coordinated bacterial and fungal compositional structuring, with a significant Procrustes correlation of $r = 0.652$ ($p = 0.001$). Shorter distances between paired bacterial and fungal points indicated stronger concordance between kingdoms, whereas residual displacement showed that bacterial and fungal communities retained distinct compositional responses. Treatment-level patterns were preserved in Procrustes space, with leaf-amended and leaf-dominated samples separating from control and PAH-only samples. Sample-wise residuals were generally low but varied among treatments; control samples

showed relatively greater dispersion, whereas intermediate leaf-amended treatments, including PAH_30%-Leaf and PAH_70%-Leaf, showed lower displacement. Hence, Procrustes analysis indicated significant but incomplete bacterial–fungal concordance, suggesting that both kingdoms responded to shared PAH-remediation and amendment gradients while maintaining kingdom-specific community trajectories.

Cross-kingdom co-occurrence network analysis revealed a modular association structure between bacterial and fungal taxa across PAH and leaf-amendment treatments (Fig. 5). The final network comprised 46 nodes and 183 significant edges, including 86 positive and 97 negative correlations. Network topology indicated moderate connectivity, with a density of 0.128 and modularity of 0.170, and resolved six modules together with eight isolated taxa. The network contained bacteria-enriched, fungi-enriched and mixed modules, indicating structured within- and cross-kingdom association patterns. Module 1 comprised 12 nodes equally distributed between bacteria and fungi, whereas Module 2 contained 11 nodes and was dominated by fungal taxa, representing the largest interconnected modules. Candidate hub taxa included Enterobacterales, a Helotiales-affiliated taxon, *Trichoderma stromaticum*, *Rhodococcus* and *Bradyrhizobium*, which showed high connectivity and central positions within modules. Overall, the network indicated that PAH contamination and leaf amendment were associated with structured bacterial–fungal co-occurrence patterns, but these correlations should be interpreted as ecological associations rather than direct biological interactions.

Functional metabolic inference

Functional inference and fungal guild classification showed contrasting bacterial and fungal responses to leaf-amendment intensity (Fig. 6). Predicted hydrocarbon-degrading bacterial profiles remained relatively abundant and stable across treatments ($\approx 0.45\text{--}0.59$), with a slight increase under 10% leaf amendment, indicating sustained putative functional potential for PAH degradation. In contrast, nitrogen-associated bacterial groups were more treatment-sensitive, increasing under low to moderate amendment levels (10–30% leaf; ≈ 0.30), declining sharply at 70% leaf amendment (≈ 0.00), and showing partial recovery in the PAH-only treatment (≈ 0.056). Fungal guild analysis showed that yeast-like fungi had the strongest amendment-associated response, increasing markedly under 10% leaf amendment (median ≈ 1.0) before declining at higher amendment levels and in PAH-only soil (≈ 0.15). Saprotrophic fungi remained consistently low across treatments (≤ 0.02). Therefore, fungal guild composition showed a stronger non-linear response to amendment intensity, whereas predicted bacterial hydrocarbon-degradation potential remained comparatively stable, suggesting putative functional redundancy within bacterial PAH-associated communities.

Community assembly analysis

Community assembly analysis showed contrasting bacterial and fungal assembly patterns (Fig. 8). Fungal communities conformed more closely to neutral model expectations ($R^2 = 0.772$; 305 taxa) than bacterial communities ($R^2 = 0.377$; 24 taxa), with a lower unexplained fraction in fungi (0.228) than bacteria (0.623). This pattern indicates stronger consistency with neutral or stochastic structuring in

fungi, whereas bacterial communities showed greater deviation from neutral expectations, suggesting a stronger influence of deterministic filtering. Beta-dispersion analysis showed no significant treatment-associated differences in community dispersion for bacteria ($F = 1.366, p = 0.279$) or fungi ($F = 2.143, p = 0.102$), although fungi showed a stronger non-significant trend toward dispersion differences. Community turnover metrics further supported kingdom-specific structuring, with a higher mean Raup–Crick Jaccard-based proxy in fungi (0.758) than bacteria (0.411), while mean Bray–Curtis dissimilarity was comparable but slightly higher in fungi (0.715) than bacteria (0.691). Hence, fungal communities showed stronger conformity to neutral expectations and greater compositional turnover, whereas bacterial communities were less well explained by neutral processes and showed patterns more consistent with deterministic environmental filtering or niche-based selection. These assembly metrics were interpreted as ecological proxies rather than formal partitioning of selection, dispersal limitation, homogenising dispersal and drift.

Machine learning and exploratory feature ranking

Random Forest regression was applied as an exploratory feature-ranking approach to identify microbial taxa associated with PAH removal. The model constructed with 1000 trees explained a modest proportion of variance in PAH removal (17.23%; MSE = 832.02), indicating limited predictive strength but useful ecological ranking of PAH-removal-associated features. Model comparison showed that the bacterial-only feature set explained more variance (17.18%) than the fungal-only model (7.51%) and the combined bacterial–fungal model (14.50%), suggesting that bacterial taxa contributed more strongly to PAH-removal gradients in this dataset. After excluding non-informative features such as chloroplast and unresolved kingdom-level labels, the highest-ranked interpretable features included *Sordariales* sp., *Pseudeurotium*, *Pseudomonas*, *Rhizobiaceae*, *Filimonas lacunae*, *Apiotrichum chiropterorum*, *Bacillus*, *Schwanniomyces polymorphus*, *Brevundimonas*, *Enterobacterales*, *Pseudoxanthomonas* and *Barnettozyma californica*. These features were interpreted as taxa associated with PAH-remediation gradients rather than validated predictive biomarkers, given the modest variance explained and limited sample size.

Stability / resilience modelling

Integrated microbial stability and recovery analysis showed treatment-dependent differences in community trajectories over the 56-day incubation (Fig. 9). Distance-to-baseline profiles indicated that all treatments diverged from their initial community composition during early incubation, followed by partial stabilisation. The greatest compositional deviation occurred in PAH_70%-Leaf, which showed the highest mean distance from baseline (≈ 0.9) and remained distant throughout incubation, indicating persistent community restructuring. In contrast, control and PAH-only treatments showed smaller deviations (≤ 0.4), while PAH_10%-Leaf and PAH_30%-Leaf showed intermediate responses consistent with amendment-dose effects.

Final recovery indices, calculated as $1 - \text{integrated community distance}$, were highest in PAH-only (0.66) and control (0.63) treatments, indicating closer return toward baseline community composition. Leaf-amended treatments showed lower recovery, with PAH_70%-Leaf having the lowest index (0.10),

followed by PAH_30%-Leaf (0.18) and PAH_10%-Leaf (0.24). Hence, increasing leaf-amendment intensity was associated with stronger and more persistent bacterial–fungal community restructuring, suggesting a trade-off between amendment-induced microbiome reorganisation and recovery toward the baseline state.

Discussion

This study shows that leaf organic amendment substantially restructured bacterial and fungal communities in PAH-contaminated soil microcosms, but the responses differed between kingdoms. The main value of the work is that it links PAH-remediation gradients with bacterial–fungal community reassembly, cross-kingdom concordance, co-occurrence network structure, predicted functional potential, community assembly patterns and recovery dynamics within a single experimental framework. This integrated approach moves beyond a bacterial-only or taxonomic survey perspective and provides a community-level interpretation of organic amendment-assisted PAH bioremediation.

Ordination analysis showed clear treatment- and time-associated restructuring of both bacterial and fungal communities. The first two PCA axes explained 66.8% of bacterial variation and 57.7% of fungal variation, indicating that amendment status and incubation time were major sources of compositional variation. Leaf-amended treatments diverged from control and PAH-only samples, suggesting that organic inputs altered the ecological conditions of the contaminated soil. This agrees with previous work showing that organic amendments can influence microbial activity, nutrient availability, contaminant bioavailability and the abundance of hydrocarbon-associated microorganisms (Iturbe-Espinoza et al., 2023; Haritash and Kaushik, 2009). However, the bacterial and fungal ordination patterns were not identical. Bacterial communities displayed broader overlap and greater within-treatment heterogeneity, whereas fungal communities showed more coherent treatment-level clustering. This contrast suggests that bacterial communities may have undergone more flexible transitional turnover, while fungi were more consistently structured by amendment-derived organic substrates.

The heatmap and differential abundance results further support this interpretation. Several bacterial taxa, including *Bradyrhizobium*, *Rhodococcus*, *Pseudomonas*, Enterobacteriaceae, *Sphingobium*, Enterobacterales, KD4-96 and *Bacillus*, showed treatment-associated shifts. Some of these taxa, or closely related lineages, have been associated with hydrocarbon degradation, aromatic compound transformation, nitrogen cycling or tolerance to contaminated environments (Torres-Beltrán et al. 2025; Adebayo et al. 2024; Ghosal et al. 2016). Their increased relative abundance under leaf amendment suggests that plant-derived organic matter may have favoured bacterial groups ecologically relevant to PAH attenuation. However, because amplicon sequencing does not measure metabolic activity directly, these taxa should be interpreted as amendment-responsive and biodegradation-associated, rather than confirmed active degraders. Fungal taxa, including *Barnettozyma californica* and *Apiotrichum chiropterorum*, showed some of the largest treatment-response effect sizes, indicating strong fungal sensitivity to amendment gradients. Other fungal groups, including *Sordariales*, *Mortierella*, *Agaricales* and *Chaetomiaceae*, also varied across treatments. These patterns are consistent with the capacity of

fungi to respond to plant-derived substrates and participate in pollutant transformation through extracellular oxidative enzymes, hyphal growth and co-metabolic activity (Harms et al. 2011; Pozdnyakova 2012).

The RDA strengthened the link between microbial composition and remediation gradients. For bacteria, RDA1 and RDA2 explained 50.1% and 44.3% of constrained variance, while fungal RDA1 and RDA2 explained 65.9% and 24.7%, respectively. The environmental vectors showed that residual PAH concentration, percentage PAH removal and timepoint were important structuring variables. The opposite orientation of residual PAH concentration and PAH removal indicates that microbial communities were organized along a contamination–remediation gradient. This pattern is consistent with the dual role of PAHs as both toxic selective pressures and potential carbon substrates for specialized microorganisms (Ghosal et al., 2016; Akinseye and Knapp 2026b). Bacteria showed stronger treatment-specific separation in constrained ordination space, suggesting stronger deterministic filtering by PAH concentration, amendment intensity and temporal succession. Fungi also responded to PAH-related gradients, but their clustering was more compressed, suggesting coordinated but less sharply separated community restructuring.

RDA strengthened the link between microbial composition and PAH-remediation gradients. For bacteria, RDA1 and RDA2 explained 50.1% and 44.3% of the constrained variance, respectively, whereas fungal RDA1 and RDA2 explained 65.9% and 24.7%. Residual PAH concentration, PAH removal percentage and incubation time were important explanatory gradients, with residual PAH concentration and PAH removal oriented in opposite directions. This pattern indicates that microbial communities were structured along a contamination–remediation gradient (Ghosal et al. 2016; Akinseye and Knapp 2026a). Bacterial communities showed stronger treatment-level differentiation in constrained ordination space, suggesting greater sensitivity to amendment intensity, residual PAH burden and temporal succession. Fungal communities also responded to PAH-related gradients, but their ordination pattern was more compressed and overlapping, suggesting PAH-responsive but less sharply separated community restructuring.

The significant Procrustes result provides evidence of bacterial–fungal community concordance across the microcosms. The Procrustes correlation was significant ($r = 0.652$, $p = 0.001$), indicating that bacterial and fungal community configurations were aligned across samples. This suggests that both kingdoms responded to shared environmental gradients, including PAH contamination, amendment regime and incubation time. However, the residual displacement between paired bacterial and fungal ordination points shows that the responses were not identical. This incomplete concordance is ecologically meaningful because bacteria and fungi differ in growth form, dispersal strategy, substrate acquisition and enzymatic capacity (de Boer et al. 2005; Rousk and Bååth 2011). Therefore, the results support coordinated but kingdom-specific microbial reassembly rather than full functional equivalence between bacteria and fungi.

Cross-kingdom co-occurrence network analysis provided additional evidence of structured bacterial–fungal association patterns. The network contained 46 nodes and 183 significant edges, including 86 positive and 97 negative correlations, organised into six modules. This mixture of positive and negative associations suggests that taxa responded to amendment and PAH gradients in both similar and opposing ways. Positive associations may reflect shared ecological preferences, co-response to amendment-derived substrates or compatible niche distributions, whereas negative associations may reflect divergent environmental preferences or competitive exclusion (Turnbull et al. 2012; Faust and Raes 2012; Röttgers and Faust 2018). Candidate hub taxa included Enterobacterales, a Helotiales-affiliated taxon, *Trichoderma stromaticum*, *Rhodococcus* and *Bradyrhizobium*. The presence of both bacterial and fungal candidate hubs suggests that network structure was not dominated by one kingdom alone. Nevertheless, these results should be interpreted cautiously because correlation-based networks do not demonstrate direct physical interaction, syntrophy or causality (Faust and Raes 2012; Röttgers and Faust 2018). The network therefore provides evidence of putative ecological association patterns rather than confirmed microbial interactions.

Functional inference and guild classification indicated contrasting bacterial and fungal responses to amendment intensity. Predicted bacterial hydrocarbon-degrading profiles remained relatively stable across treatments, with values of approximately 0.45–0.59. This suggests that putative PAH-associated functional potential was maintained despite taxonomic turnover, possibly reflecting functional redundancy among bacterial groups. Such redundancy may help sustain ecosystem processes under disturbance (Krucoń et al. 2026; Allison and Martiny 2008). In contrast, nitrogen-associated bacterial groups were more variable, increasing under low to moderate amendment but declining strongly at 70% leaf amendment. This may reflect shifts in nutrient stoichiometry, oxygen availability, microbial competition or altered C balance under high organic input. Fungal guilds showed a stronger non-linear response, particularly yeast-like fungi, which increased under 10% leaf amendment and declined at higher amendment levels and in PAH-only soil. Saprotrophic fungi remained consistently low, suggesting that fungal functional restructuring was guild-specific rather than a general increase in decomposer fungi (Shi et al. 2025; Moreira et al. 2025). Because PICRUST2 and FUNGuild provide inferred functional potential rather than direct measurements of gene expression, enzyme activity or PAH mineralisation, these outputs should be interpreted as supportive ecological indicators.

Community assembly analysis provided further insight into the contrasting responses of bacteria and fungi. The neutral model showed a stronger fit for fungi ($R^2 = 0.772$; 305 taxa) than bacteria ($R^2 = 0.377$; 24 taxa), with a lower unexplained fraction in fungi (0.228) than bacteria (0.623). This indicates that fungal occurrence patterns were more consistent with neutral or stochastic expectations, whereas bacterial communities showed greater deviation from neutral structure. The weaker neutral model fit in bacteria may reflect stronger deterministic filtering by residual PAH concentration, amendment intensity, nutrient availability or substrate specialisation (Zhu et al. 2024). This interpretation is consistent with the stronger bacterial separation observed in RDA and with the enrichment of several amendment-responsive bacterial taxa. In contrast, the higher fungal neutral-model fit suggests that stochastic

processes, dispersal-related dynamics or demographic effects may have contributed more strongly to fungal assembly under the microcosm conditions (Sloan et al. 2006; Zhou and Ning 2017).

Beta-dispersion analysis showed no significant treatment-associated differences in community dispersion for bacteria ($F = 1.366$, $p = 0.279$) or fungi ($F = 2.143$, $p = 0.102$). This suggests that observed community differences were more likely associated with directional compositional shifts than with unequal within-treatment dispersion. Community turnover metrics nevertheless showed kingdom-level contrasts (Yuan et al. 2022). The mean Raup–Crick Jaccard-based proxy was higher in fungi (0.758) than bacteria (0.411), indicating greater fungal compositional turnover, whereas mean Bray–Curtis dissimilarity was comparable between fungi (0.715) and bacteria (0.691). Together, these patterns support the interpretation that fungal communities showed stronger neutral or turnover-related signals, while bacterial communities showed greater deviation from neutral expectations. However, these metrics should be treated as assembly-related proxies. Future work using phylogeny-based β NTI and RCbray partitioning would be required to distinguish selection, dispersal limitation, homogenising dispersal and ecological drift more rigorously (Stegen et al. 2013; Larsen et al. 2022; Zhou and Ning 2017).

The exploratory Random Forest analysis further supported the stronger contribution of bacterial features to PAH-removal gradients. The bacterial-only model explained more variance than the fungal-only model, and several highly ranked bacterial features, including *Pseudomonas*, Rhizobiaceae, *Bacillus*, Enterobacterales and *Pseudoxanthomonas*, overlapped with taxa identified in ordination and differential abundance analyses. This agreement strengthens the interpretation that bacterial community restructuring was closely associated with PAH-remediation gradients (Carvalho et al. 2026). However, the modest variance explained, and small sample size indicate that the model should be viewed as exploratory feature ranking rather than a validated predictive tool.

The stability and recovery analysis revealed an important amendment-dose effect on microbial community trajectories. All treatments diverged from baseline during early incubation, followed by partial stabilisation. The strongest compositional deviation occurred under 70% leaf amendment, where mean distance from baseline approached approximately 0.9 and the final recovery index was lowest (0.10). In contrast, PAH-only and control treatments showed higher final recovery indices of 0.66 and 0.63, respectively, while 10% and 30% leaf amendments showed intermediate but reduced recovery values of 0.24 and 0.18. These findings indicate that leaf amendment strongly redirected microbial community composition, but higher amendment intensity produced more persistent community restructuring. Therefore, greater organic amendment input is not necessarily ecologically optimal. Excessive amendment may create alternative microbial states through substrate oversupply, altered nutrient balance, oxygen limitation, increased sorption of hydrophobic contaminants or intensified microbial competition (Li et al. 2023; Akinseye and Charles 2026a).

Several limitations should be considered when interpreting these findings. First, the study was conducted under controlled microcosm conditions, and field soils may show greater spatial

heterogeneity, fluctuating temperature and moisture regimes, plant-root effects and variable contaminant exposure. Second, PAH dissipation was assessed using residual PAH concentrations, but direct mineralisation, metabolite formation and isotope-based carbon flow were not measured. Therefore, PAH removal should be interpreted as biodegradation-associated dissipation rather than definitive mineralisation. Third, amplicon sequencing provides relative community profiles and does not directly quantify absolute microbial abundance, gene expression or enzymatic activity. Fourth, PICRUSt2 and FUNGuild infer functional potential from taxonomic and marker-gene data and should not be treated as direct evidence of active biodegradation pathways. Fifth, co-occurrence networks identify statistical associations, not confirmed biological interactions. Finally, neutral model and Raup–Crick-based outputs provide useful assembly proxies but do not formally partition ecological processes in the way phylogeny-based β NTI and RCbray approaches can.

Hence, this study supports a systems-level model of amendment-assisted PAH bioremediation in which bacterial and fungal communities respond jointly but differently to leaf-derived organic inputs. Leaf amendment acted as a strong ecological modifier, restructuring community composition, enriching treatment-responsive taxa, altering predicted functional profiles, generating modular cross-kingdom association networks and modifying community assembly trajectories. Bacteria appeared more strongly associated with deterministic filtering and maintained stable predicted hydrocarbon-degradation potential, whereas fungi showed stronger conformity to neutral expectations, greater compositional turnover and stronger guild-level sensitivity to amendment intensity. The findings suggest that amendment-based PAH bioremediation should not focus only on increasing organic input or microbial diversity, but on promoting functionally relevant and recoverable bacterial–fungal community states capable of supporting contaminant attenuation while avoiding persistent community disruption.

Conclusion

This study shows that leaf organic amendment substantially reorganised bacterial and fungal communities in PAH-contaminated soil microcosms, with community structure associated with amendment intensity, incubation time, residual PAH concentration and PAH removal. By integrating bacterial 16S rRNA and fungal ITS amplicon profiling with ordination, differential abundance, Procrustes analysis, co-occurrence networks, functional inference, community assembly modelling and recovery metrics, the study provides a cross-kingdom ecological perspective on amendment-assisted PAH bioremediation.

The findings indicate that bacterial and fungal communities responded to shared PAH-remediation gradients but followed distinct compositional and assembly trajectories. Bacterial communities showed greater deviation from neutral expectations and maintained relatively stable predicted hydrocarbon-degradation potential, suggesting stronger association with deterministic filtering and putative functional redundancy. In contrast, fungal communities showed stronger conformity to neutral expectations, greater compositional turnover and higher guild-level sensitivity to amendment intensity. Cross-kingdom concordance and modular co-occurrence patterns further suggest that PAH-

contaminated soil microbiomes are structured by coordinated community-level responses rather than by individual degradative taxa alone.

Leaf amendment promoted clear microbial restructuring, but high amendment intensity was associated with reduced recovery toward the baseline community state. This indicates a potential trade-off between organic-matter-driven biostimulation and microbiome recovery. Therefore, amendment-based PAH bioremediation strategies should consider not only contaminant removal, but also amendment dose, bacterial–fungal community structure, predicted functional redundancy and post-disturbance recovery.

The study is limited by its microcosm design and reliance on amplicon-based community profiling, predictive functional inference and correlation-based network analysis. Direct PAH mineralisation, metabolite formation, gene expression, enzyme activity and field-scale validation were not assessed. Future work combining metagenomics, metatranscriptomics, enzyme assays, absolute abundance measurements and isotope-based PAH mineralisation analyses would help confirm the active mechanisms underlying bacterial–fungal contributions to PAH attenuation.

Declarations

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the Petroleum Technology Development Fund (PTDF), Nigeria, through doctoral scholarship support awarded to Olanrewaju Roland Akinseye.

Author Contribution

O.R.A.: Conceptualization, methodology, investigation, data curation, formal analysis, bioinformatics, software, visualization, validation, and writing—original draft. C.W.K.: Supervision, conceptual guidance, methodology oversight, resources, project administration, interpretation of results, and writing—review and editing. Both authors reviewed and approved the final manuscript.

Acknowledgement

The authors acknowledge the Petroleum Technology Development Fund (PTDF), Nigeria, for supporting the doctoral research associated with this study. Technical and laboratory support provided by the

Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, UK, is also gratefully acknowledged.

Data Availability

The datasets supporting the findings of this study are publicly available in Zenodo at [Record ID: 22960951]. The repository contains raw bacterial 16S rRNA and fungal ITS sequencing reads, sample metadata, processed ASV and taxonomic abundance tables, PAH concentration and removal data, differential abundance results, functional inference outputs, bacterial–fungal concordance and network data, community assembly metrics, stability and recovery analyses, Random Forest outputs, supplementary tables and accompanying documentation.

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Figures

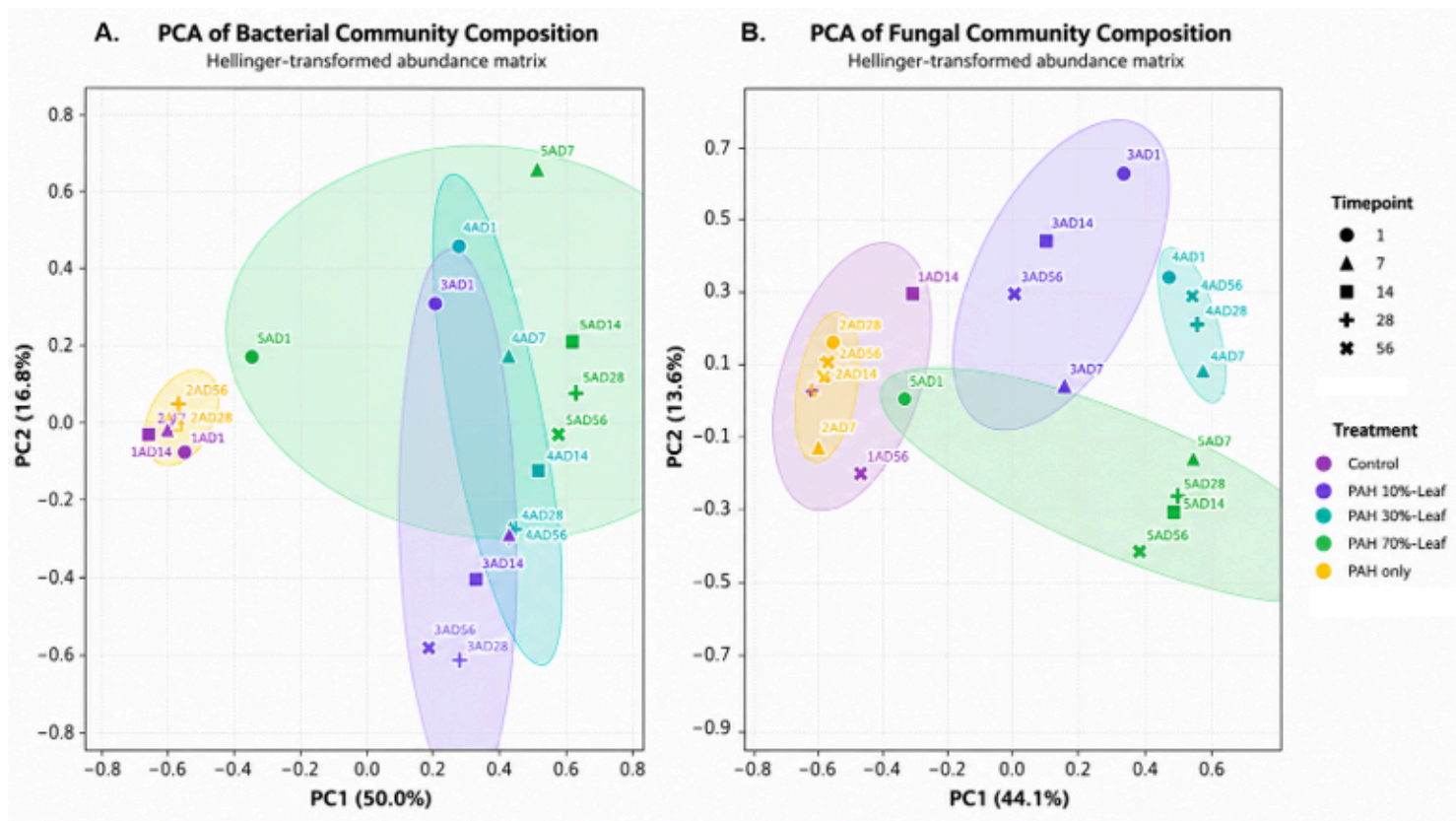


Figure 1

Principal component analysis (PCA) of bacterial and fungal community composition

Principal component analysis (PCA) of Hellinger-transformed relative abundance data showing the compositional structure of (A) bacterial and (B) fungal communities across treatments and timepoints. The first two principal components explained 50.0% and 16.8% of the variance for bacteria, and 44.1% and 13.6% for fungi, respectively. Points represent individual samples, coloured by treatment and shaped by timepoint. Ellipses denote the 95% confidence intervals for each treatment group. Both bacterial and fungal communities exhibited clear treatment-driven separation, with leaf-amended treatments diverging from control and PAH-only groups, and temporal progression indicating dynamic community shifts over the course of the experiment.

stronger statistical significance, indicating distinct bacterial and fungal responses to PAH contamination and organic amendment.

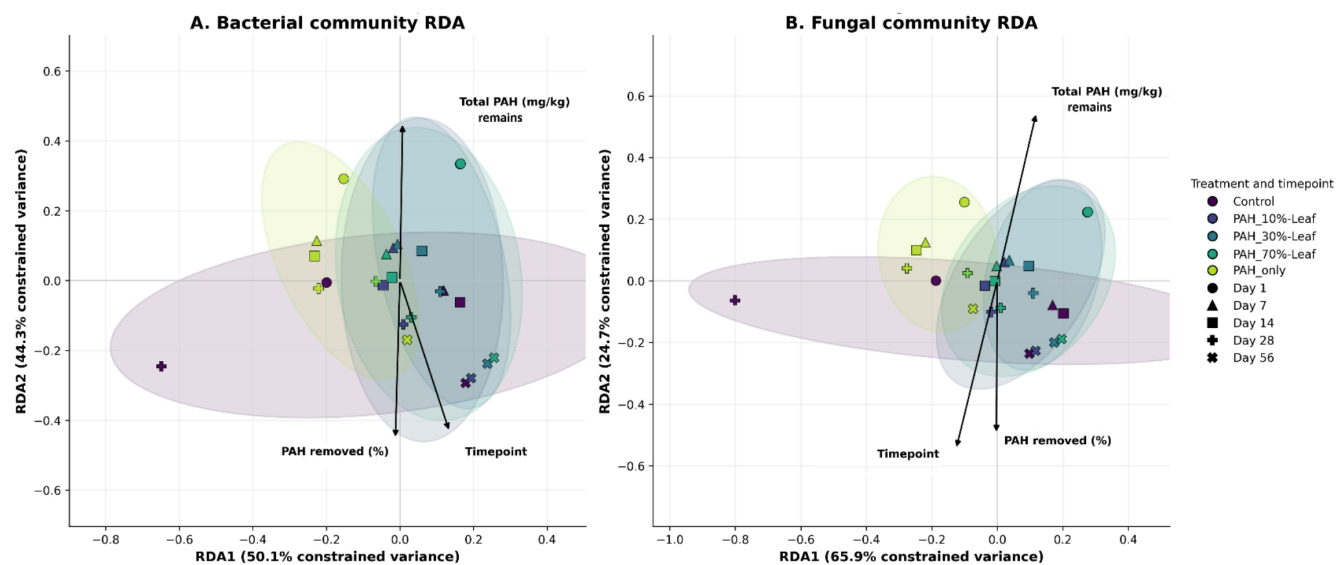


Figure 3

Redundancy analysis (RDA) of bacterial and fungal community composition constrained by environmental variables.

(A) Bacterial communities show clear treatment-driven separation, with RDA1 and RDA2 explaining 50.1% and 44.3% of constrained variance, respectively. (B) Fungal communities exhibit weaker separation, with RDA1 and RDA2 explaining 65.9% and 24.7% of variance. Arrows represent environmental gradients, indicating that total PAH concentration, PAH removal, and timepoint are key drivers of community structure, while ellipses denote treatment-level clustering.

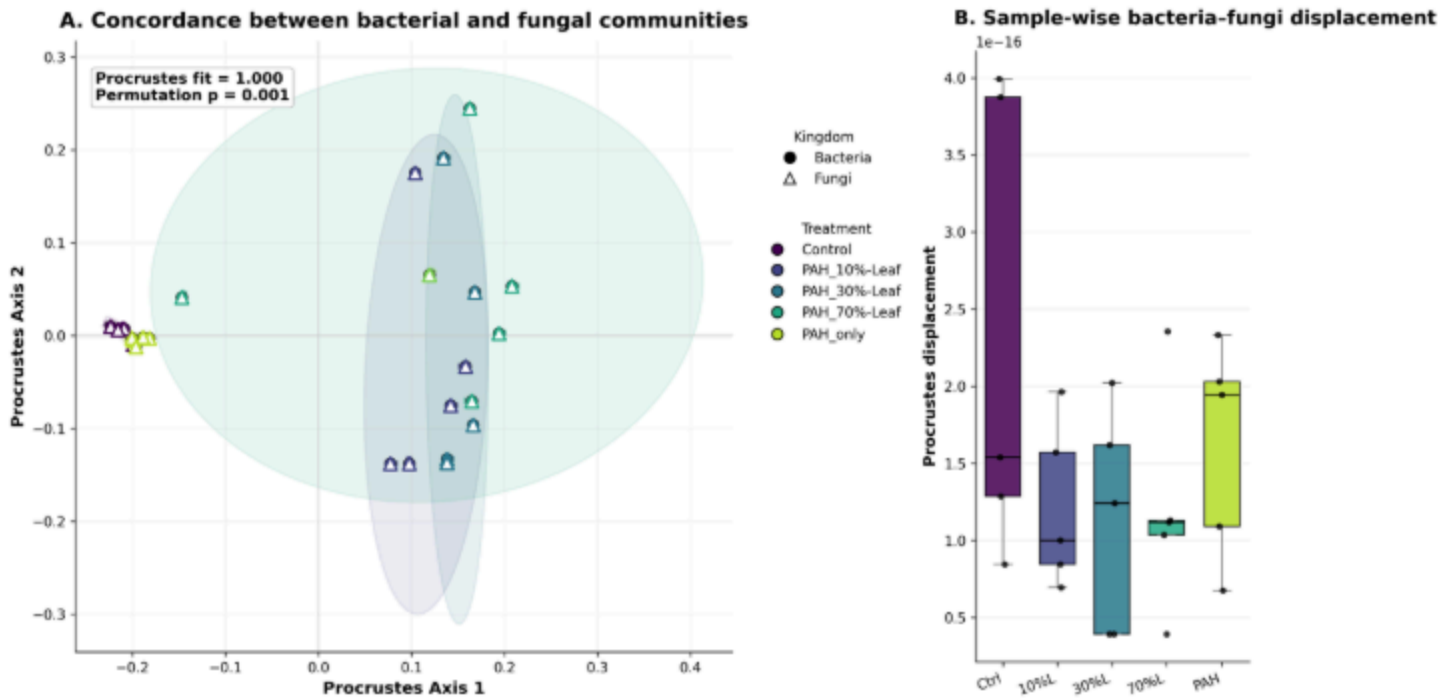


Figure 4

Procrustes analysis of bacterial–fungal community concordance

(A) Procrustes ordination showing alignment between bacterial (filled circles) and fungal (open triangles) communities, with arrows indicating sample-level displacement after rotation. A significant concordance was observed ($r = 0.652$, $p = 0.001$). (B) Boxplots of Procrustes displacement across treatments, indicating variability in the degree of coupling between bacterial and fungal community responses.

A. Cross-kingdom co-occurrence network

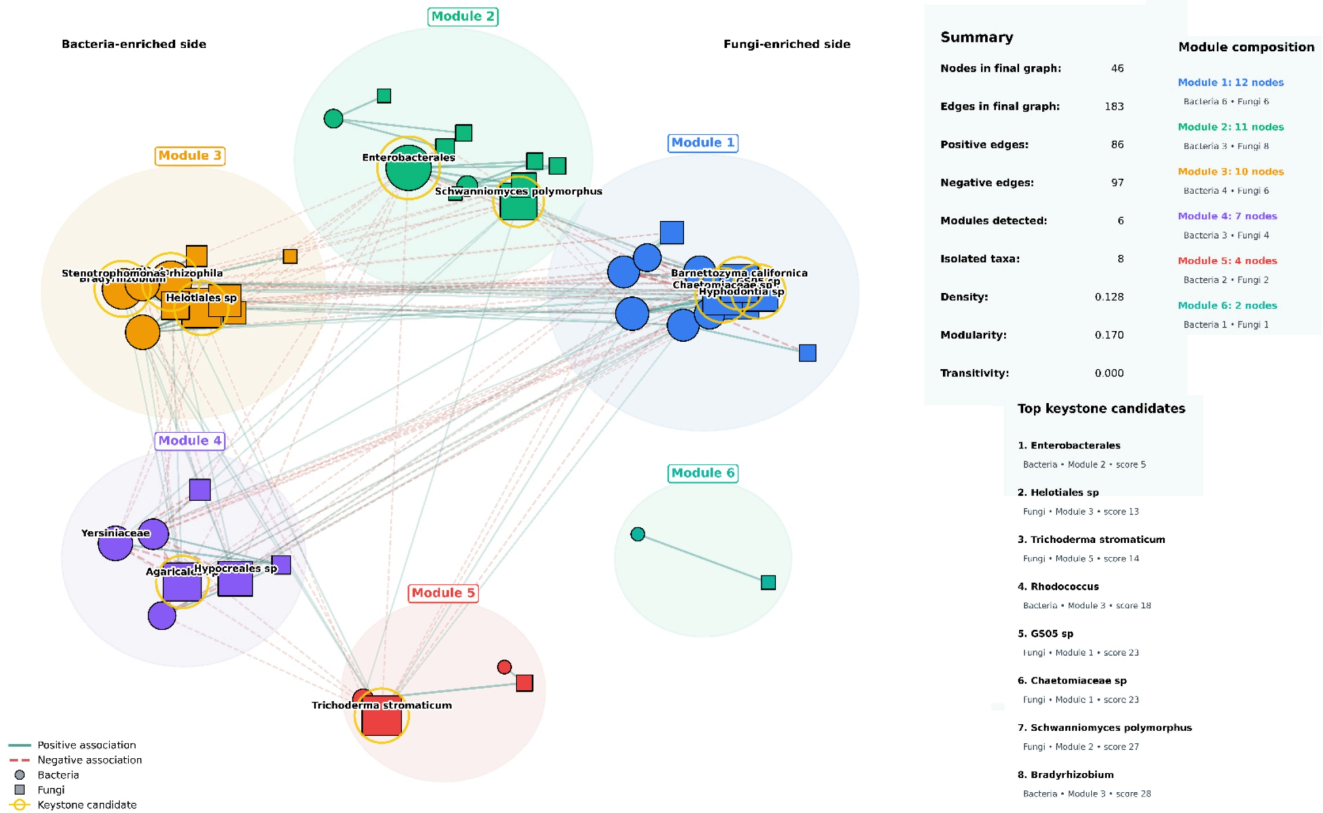


Figure 5

Cross-kingdom co-occurrence network of bacterial and fungal communities

Network showing interactions between bacterial (circles) and fungal (squares) taxa based on significant correlations. Edges represent positive (solid) and negative (dashed) associations. The network comprises 46 nodes and 183 edges, organized into six modules, with highlighted nodes indicating keystone taxa driving cross-kingdom interactions.

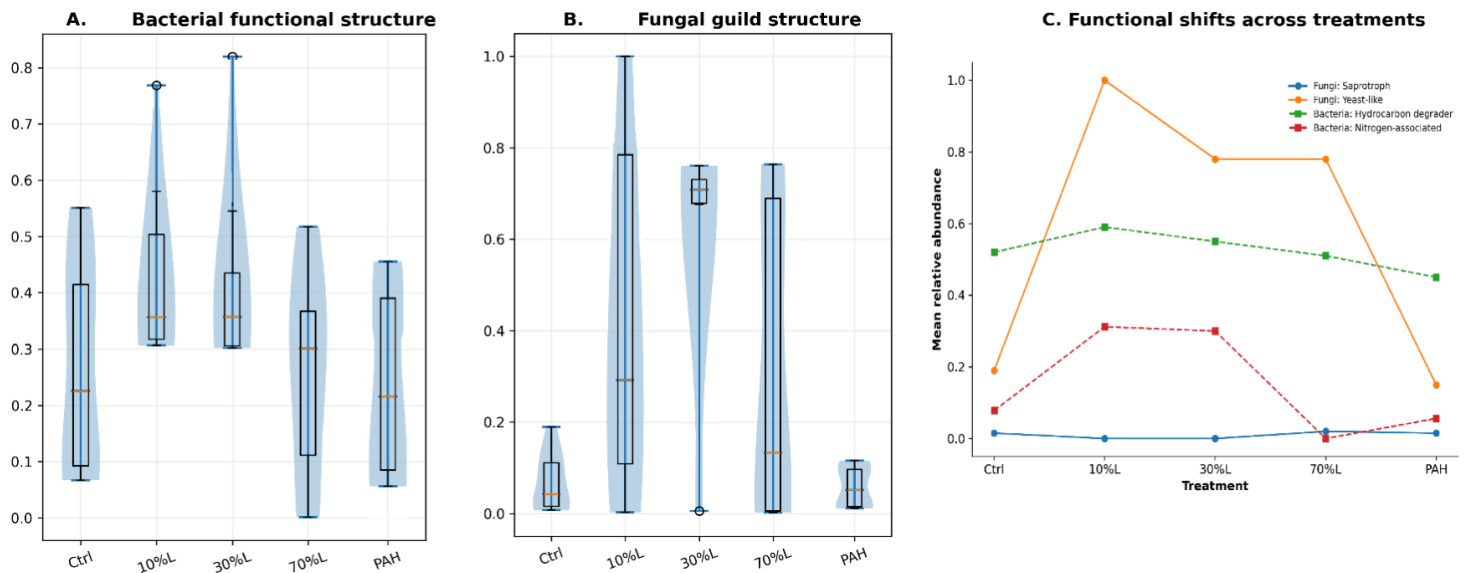


Figure 6

Guild and functional restructuring of microbial communities across treatments.

(A) Bacterial functional categories indicate relatively stable hydrocarbon-degrading communities across treatments, with moderate variation in nitrogen-associated taxa, which peak at 10–30%L and decline at higher amendment levels. (B) Distribution of dominant fungal guilds shows a strong increase in yeast-like fungi under moderate leaf amendment (10%L), with lower abundances in control and PAH-only treatments, while saprotrophs remain consistently low across all treatments. (C) Integrated trends highlight divergent responses, with fungi exhibiting stronger treatment sensitivity and bacteria maintaining functional stability, suggesting distinct ecological roles in PAH degradation.

Community assembly patterns in bacterial and fungal communities

Summary of neutral model fit, beta-dispersion, and dissimilarity metrics.

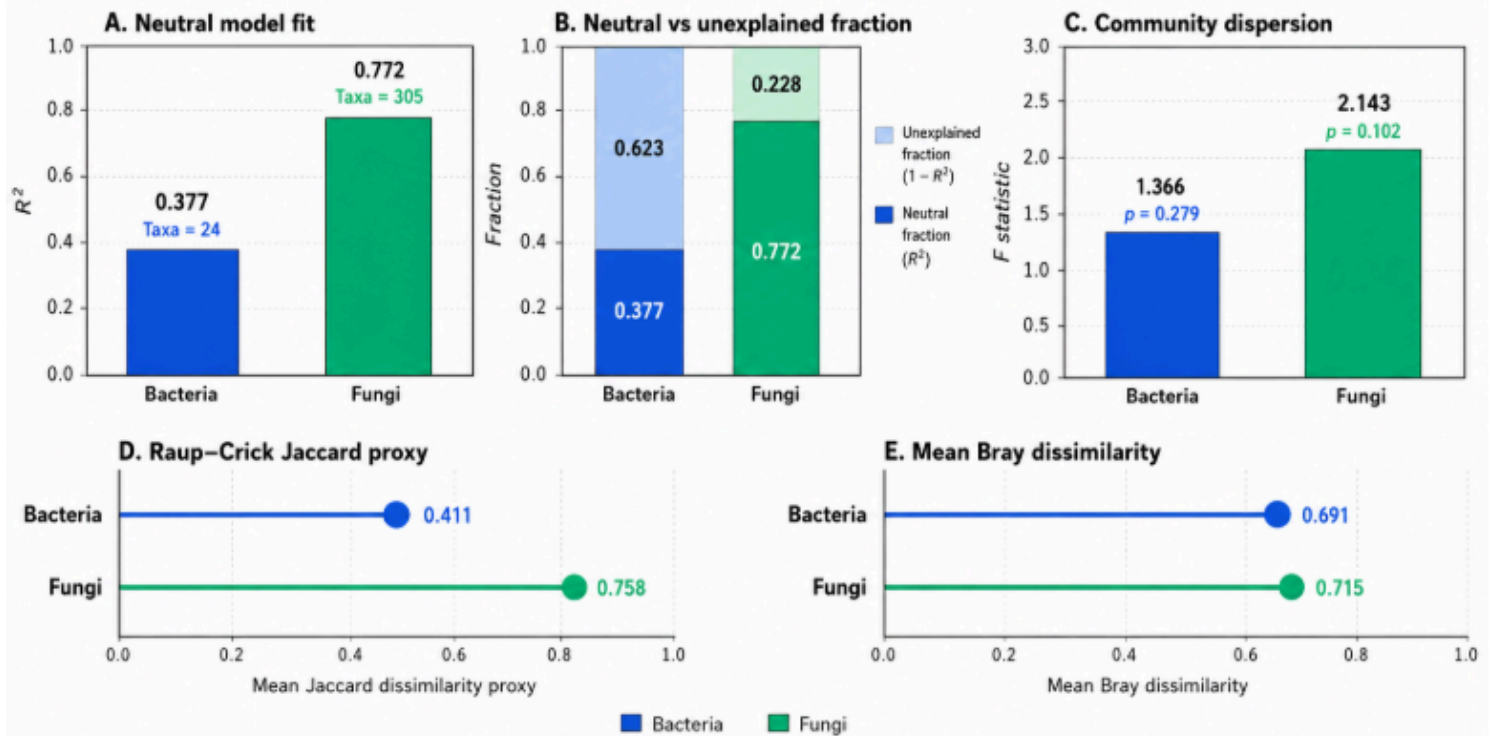


Figure 7

Community assembly patterns in bacterial and fungal communities

Neutral-model analysis indicated a stronger neutral assembly signal in fungal communities ($R^2 = 0.772$; 305 taxa) than in bacterial communities ($R^2 = 0.377$; 24 taxa). The neutral fraction was therefore higher in fungi (0.772) than bacteria (0.377), while the unexplained fraction was higher in bacteria (0.623) than fungi (0.228). Beta-dispersion analysis showed no significant treatment-associated differences in community dispersion for bacteria ($F = 1.366$, $p = 0.279$) or fungi ($F = 2.143$, $p = 0.102$). Raup–Crick Jaccard proxy values were higher for fungi (0.758) than bacteria (0.411), indicating greater fungal compositional turnover, whereas mean Bray dissimilarity was comparable between fungi (0.715) and bacteria (0.691). Together, these results suggest stronger stochastic or neutral structuring in fungi and greater non-neutral deviation in bacteria, although phylogeny-based analyses such as β NTI would be required to formally partition deterministic and stochastic assembly processes.

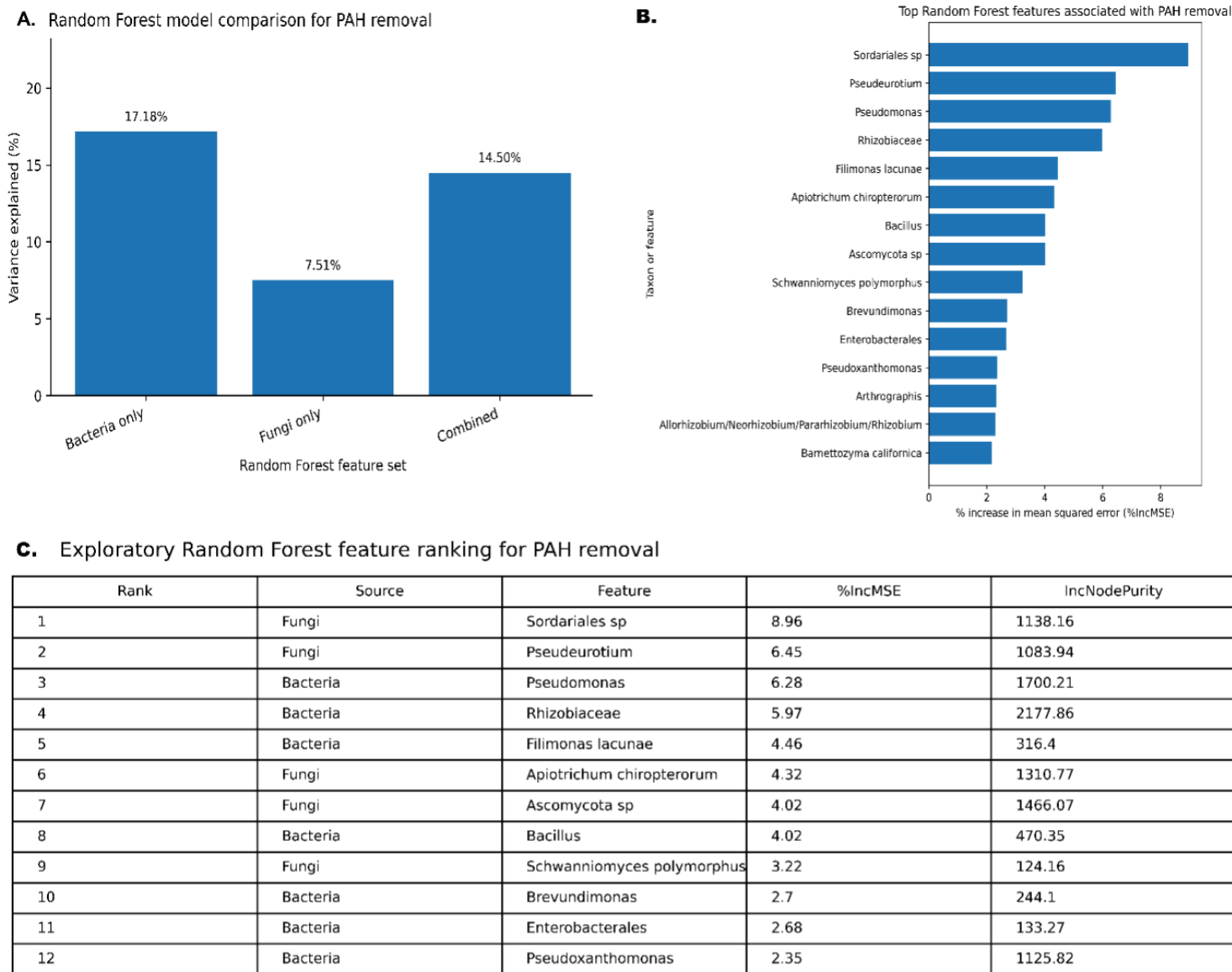


Figure 8

Exploratory Random Forest feature ranking of microbial taxa associated with PAH removal.

(A) Comparison of Random Forest model performance using bacterial-only, fungal-only and combined bacterial–fungal feature sets. Percentage variance explained was highest for the bacterial-only model, followed by the combined and fungal-only models. (B–C) Top interpretable microbial features ranked by percentage increase in mean squared error (%IncMSE), after excluding chloroplast and unresolved kingdom-level labels. Higher %IncMSE values indicate stronger contribution to model performance. Random Forest outputs were interpreted as exploratory feature-ranking results rather than validated predictive biomarkers because of the limited sample size and modest variance explained.

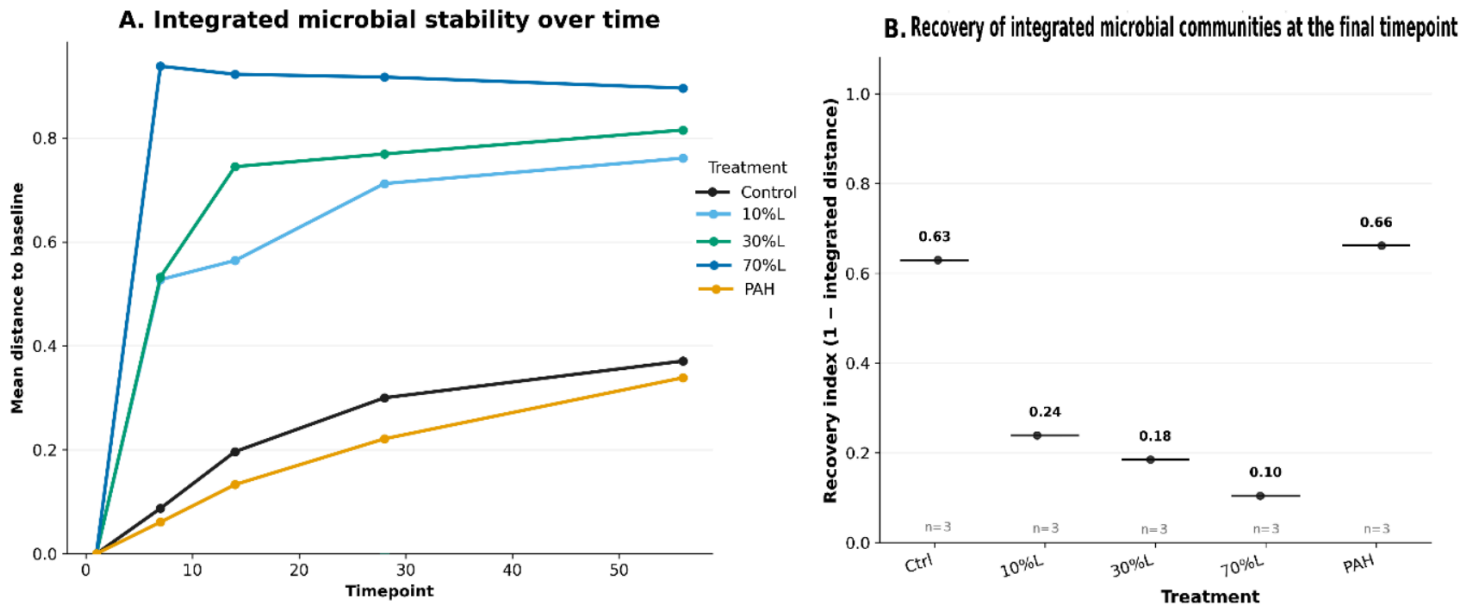


Figure 9

Stability and resilience of integrated bacterial-fungal communities across treatments

A) Temporal trajectories of mean distance to baseline showing community stability across treatments. (B) Recovery index (1 - integrated distance) at the final timepoint, indicating resilience differences among treatments, with higher values reflecting greater recovery toward baseline.

Supplementary Files

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- [Supplementaryfile.xlsx](#)