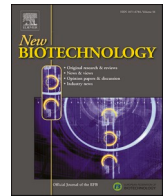




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Lavandula angustifolia and microbial bioaugmentation synergistically reshape rhizosphere microbiome and enhance heavy metals removal in historically contaminated soils

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ABSTRACT

Heavy metal contamination poses a serious threat to soil ecosystems and requires sustainable remediation approaches capable of restoring both chemical quality and microbial functionality. This study evaluates the effectiveness of plant-assisted bioremediation (*Lavandula angustifolia*) and bioaugmentation with a selected bacterial consortium of four strains (*Gordonia amicalis*, *Rhodococcus erythropolis*, *Acinetobacter puyangensis*, and *A. tibetensis*) in soils that have been historically contaminated with multiple pollutants - such as heavy metals (HMs) and polychlorinated biphenyls (PCBs). Microcosms were created with four treatments, i.e. Historically Contaminated Soil (HCS), Plant-assisted bioremediation (PLANT), microbial bioaugmentation (BIOAUG) and the combination of plant-assisted bioremediation and bioaugmentation (PLANT+BIOAUG) and monitored over a 90-days period through chemical analyses, 16S rDNA sequencing, diversity metrics, differential abundance tests and functional prediction. The PLANT+BIOAUG combination demonstrated the highest removal efficiency of Pb (44.75%) and Sn (66.87%), suggesting a robust synergistic interaction between plant and microbial inoculum. Microbial α -diversity remained stable across treatments, while β -diversity analyses (Bray–Curtis, PERMANOVA $p = 0.001$) revealed significant community restructuring. Taxonomic analyses highlighted shifts in key genera and an enrichment of bacterial families associated with metal transformation, redox processes, and stress tolerance. The functional prediction identified 7959 KEGG functions, with the combined treatment showing the highest functional redundancy in metal efflux systems, siderophore production, electron transport pathways, and EPS/biofilm formation. Overall, integrating *L. angustifolia* with a metal-resistant microbial consortium could improve both contaminant removal and microbial functional potential, supporting a robust and sustainable strategy for the remediation of multi-contaminated soils. These results provide valuable insights into synergistic plant-microbe processes and offer practical guidelines for *in situ* bioremediation within the framework of the circular economy and nature-based models.

1. Introduction

Rapid population growth, socioeconomic progress, and intense

urbanisation and industrialisation are causing an exponential increase in soil contamination, generating a serious global environmental crisis [1]. Among the most critical contaminants are persistent organic pollutants

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(POPs) and inorganic pollutants, particularly heavy metals (HMs). Some heavy metals play essential roles as enzyme cofactors, while others are considered non-essential [2]; the latter, which have no essential biological functions, are highly toxic at low concentrations and pose a threat to living organisms [3]. Their non-biodegradability and tendency to bioaccumulate result in ongoing ecological damage [4,5], contributing to a decline in soil quality through alterations to the composition and functionality of the microbiome [6–8], as well as through toxic effects on plants [9], and causing serious damage to vital organs, cognitive deficits and cancer-causing diseases even at low levels of exposure or chronic exposure [10,11].

The development of effective strategies to mitigate heavy metal contamination is a key objective in the field of contemporary environmental protection [12]. This process involves the removal of various classes of metal contaminants, as well as the selection of the most suitable remediation technologies in relation to the specific characteristics of the contaminated site [13]. Traditionally, soil remediation has relied on physical methods, such as soil replacement and thermal desorption, and chemical methods, such as solidification/stabilisation, soil washing, oxidation-reduction and electrokinetic remediation. While these approaches are rapid and effective, they have several drawbacks, including high energy costs, invasiveness, the potential to alter soil properties and the risk of generating secondary pollution [13–15]. In recent years, bioremediation methods have gained increasing attention due to their economic and environmental sustainability [16,17]. These approaches align with international policies, including those championed by the Environmental Protection Agency (EPA) and the European Commission through Nature-Based Solutions (NbS) [18]. In the context of heavy metal pollution, NbS are adapted to leverage natural ecosystem processes - such as plant root uptake, exudation of chelating agents, and microbial metabolic transformations - to immobilise, detoxify, or extract inorganic contaminants [19]. This provides a self-sustaining and cost-effective alternative to highly invasive physicochemical treatments, with the aim of enhancing the homeostatic capacities of ecosystems while providing social and economic benefits. Microbial bioremediation of heavy metals is a complex process involving several biochemical mechanisms, such as chelation and the transformation of toxic forms into non-toxic intermediates. Other mechanisms include ion exchange, precipitation and metal efflux [20]. Furthermore, it is crucial to understand the interactions between metals, soil, microbiota and plants [21], as these systems modulate adaptation to contaminated environments and influence the effectiveness of remediation strategies [3]. Plant-assisted bioremediation (PABR) strategies exploit the synergistic interactions between plants and rhizosphere microorganisms, combining the stabilising or phytoextractive action of plants with the ability of these microorganisms to transform, mobilise or immobilise heavy metals [22,23]. Plants have a significant impact on the bioavailability of metals [24]. They absorb metals through their roots, accumulate them in their tissues, precipitate them in the rhizosphere and exude organic compounds [25]. Meanwhile, the microbial community contributes to processes such as biosorption, bioaccumulation, bioprecipitation and redox transformations [26]. Aromatic plants are showing great promise as tools for PABR due to their high tolerance to heavy metals [27], extensive root systems [28], and production of secondary metabolites capable of modulating the microbial community [29]. *Lavandula angustifolia* Mill., belonging to the *Lamiaceae* family, is notable for its capacity to enhance the structure and functionality of the rhizosphere microbiome, tolerate metal-contaminated soils, promote metal stabilisation [30,31], and increase beneficial microbial activity [32]. This contributes to better soil quality in the long term. However, the cultivation of generally recognised safe aromatic plants, such as *L. angustifolia*, on contaminated soils introduces potential risks to public health. As the plant is widely perceived as harmless, unauthorised harvesting or direct contact with contaminated biomass by people passing by these fields could lead to exposure to heavy metals. Therefore, its cultivation must be closely combined with controlled biomass

management. Extraction of essential oils is an ideal strategy for mitigation and the promotion of a circular economy. This is because heavy metals are typically not detected in the volatile fractions, producing a safe and high-value product while containing any toxic risks [33].

The next step in the development of bioremediation strategies will be the use of microbial consortia [34]. These consist of different microbial species or strains combining complementary metabolic capabilities [35]. This results in synergistic activities and greater resilience to environmental stresses [19,36]. Concurrently, the development of omic technologies, notably metagenomics, has transformed the approach to studying bioremediation [37], overcoming the limitations of culture-based methods. Shotgun metagenomic sequencing and 16S rRNA gene sequencing are culture-independent approaches that facilitate exploration of the complete taxonomic and functional diversity of microbial communities [38]. This technology facilitates the quantification of changes in microbial composition in response to remediation treatments [39], the identification of genes putatively involved in pollutant metabolism, and the analysis of ecological dynamics and functional capacities of soil microbial communities in an integrated manner [16].

The aim of this study is to evaluate the synergistic responses of soil microbial communities and *L. angustifolia* during the remediation of a historically heavy metal contaminated soil. To investigate this, a specific bacterial consortium of four bacterial strains (*Gordonia amicalis*, *Rhodococcus erythropolis*, *Acinetobacter puyangensis*, and *Acinetobacter tibetensis*) was selected and assembled and preliminarily applied in the laboratory to a soil historically contaminated by heavy metals. These have been recently isolated and characterized in an environment naturally and chronically contaminated by petroleum hydrocarbons [40]. Although initially selected for their robust biodegradation capabilities against organic pollutants, these taxa possess a rich repertoire of genes conferring extreme resilience to environmental stress and high tolerance to heavy metals. Specifically, their genomes harbor key operons for metal resistance, including lead (*pbrT*, *pbrR*) and zinc/cadmium/cobalt (*czcABCD*) resistance systems (predominantly in *Acinetobacter* strains), along with widespread copper (*cop*) and arsenic (*ars*) resistance genes. Furthermore, all selected strains possess a robust oxidative stress response system (e.g., *katG*, *sodA*, *ahpC*) and biosynthetic gene clusters for metal-chelating siderophores and protective osmolytes such as ectoine. These synergistic characteristics make them ideal candidates for bioaugmentation technology in multi-contaminated matrices. The assessment analyses the plant's ability to tolerate and accumulate heavy metals, along with structural changes (e.g. metal efflux systems, siderophore production) in the microbial community. No studies have investigated the synergy between this specific microbial consortium and *L. angustifolia*. The key novelty of this work lies in the integration of high-throughput sequencing of the 16S rDNA gene, predictive functional profiling, and plant biomass assessment. This combined approach allows us to understand the specific ecological and molecular mechanisms that drive plant-microbe synergy.

The results obtained contribute to clarifying the mechanisms underlying advanced bioremediation strategies and offer new operational guidelines for *in situ* interventions with NbS on degraded soils.

2. Materials and methods

2.1. Sample collection and microcosm set up

Soil applied in microcosms were collected from a historically contaminated area [23], located close to Taranto (Apulia Region, Southern Italy; GPS coordinates: 40. 28 N, 17. 18 E). This area was recognised by the Italian government as a Site of National Interest (SIN) contaminated by heavy metals due to long-term emissions from the metallurgical and petrochemical industries. Sampling was carried out in November 2023, and a composite sample (0–20 cm depth) was taken, air-dried at room temperature, sieved through a 2 mm mesh, and

homogenised prior to its use in experimental microcosms.

The soil characterisation was performed in accordance with the Italian official methods for soil analysis, as stipulated in the DM 13/09/1999. The following methods should be used: Method III.1 for pH, Method IV.1 for EC, Method VII.3 (Walkey-Black method) for organic carbon, Method XV.3 (Olsen method) for assimilable phosphorus, and Method XIV.1 (Kjeldahl method) for total nitrogen.

Specifically, the levels of organic carbon, nitrogen, and phosphorus were found to be 15.02 ± 3.21 g/kg, 0.17 ± 0.07 g/kg, and 8.12 ± 1.81 mg/kg, respectively. Furthermore, the pH was recorded at 6.64 and the electrical conductivity (EC) was 0.99 dS/cm.

Regarding the contamination profile, the initial total concentrations of heavy metals in the sampled soil were determined by ICP-MS (as detailed in 2.2). The soil is predominantly characterized by high concentrations of total Zn (600.09 ± 74.79 mg/kg), total Pb (1647.34 ± 857.77 mg/kg), and total Sn (133.35 ± 60.45 mg/kg). The complete profile of initial heavy metal concentrations is reported in [Supplementary Table S1](#). Polychlorinated biphenyls (PCBs) were not assessed in this study, as previous characterizations of this specific study area demonstrated that their concentrations were within legal limits [23,41].

The microcosms were composed in pot (size 40 × 37 cm) containing 500 g of the homogenised contaminated soil. Four experimental treatments (three replicate pots per treatment) were established: (i) historically contaminated soil (HCS), (ii) soil inoculated with a hydrocarbon-degrading bacterial consortium (BIOAUG), (iii) soil planted with *L. angustifolia* (PLANT), and (iv) combined treatment consisting in bacterial consortium and *L. angustifolia* plants (PLANT+BIOAUG). The overall experimental design is illustrated in [Fig. 1](#) below.

The *L. angustifolia* plants were obtained from a Mondo Piante plant shop in Terlizzi, Puglia. The plants were approximately two weeks old, with an average height of 7–9 cm, and were in the juvenile vegetative growth stage at the time of acquisition and transplantation into the microcosms.

Bacterial isolates were grown from single colonies on LB agar plates in 10 mL of sterile LB medium (consisting of 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl). After 24 h of incubation at 28°C, a new inoculation was performed (1 mL of bacterial culture and 9 mL of LB

medium). The colony-forming units of this suspension were quantified using the dilution plate counting technique. The bacterial consortium, composed of *Gordonia amicalis*, *Rhodococcus erythropolis*, *Acinetobacter puyangensis*, and *Acinetobacter tibetensis*, was prepared from exponentially growing cultures as reported by Cavone et al., 2025 [40]; the cultures were then centrifugated and washed twice with sterile water and adjusted to an optical density (OD₆₀₀) of 1.0 before being mixed in equal volumes. The inoculum was incorporated into BIOAUG and PLANT+BIOAUG treatments at a final concentration of 2% (v/w), corresponding to 1×10^8 CFU g⁻¹ of soil.

All microcosms were tested in triplicate and kept in a growth chamber (Climate chamber Binder KBF 720, GmbH, Wetzlar, Germany) at 25 ± 1 °C, with a 16/8 h light/dark cycle and relative humidity of 65–80%. The experiment ran for a period of 90 days.

2.2. Heavy metal analyses in soil and plant biomass

Heavy metal analysis (Be, V, Cr, Co, Ni, Cu, Zn, As, Se, Cd, Sn, Tl, Pb) in the experimental microcosms was performed on soil samples at 0 and 90 days, roots, and leaves of lavender plants.

All samples, both soil and plant biomass, were ground in a ball mill until a particle size fraction smaller than 2 mm was achieved.

To extract the mineral elements, the samples were digested with acids, as indicated in the ISO 11466 (1995) method; that is, approximately 0.5 g of each sample was weighed with a precision balance and transferred to a vessel. In the vessel, the soils were treated with HCl and HNO₃ (3:1 ratio), while the plant organs (leaves and roots) were treated with 7 mL of HNO₃ and 1 mL of H₂O₂. The filled vessels were loaded into the mineralizer in a temperature-controlled microwave system (Ethos Touch Control, Milestone, Microwave Laboratory Systems, Sorisole (BG), Italy), running a two-step program according to DG-AG-02 Milestone Digestion Application Note: 10 min to reach 200 °C followed by 10 min at 200 °C. After cooling, the mineralized samples were transferred to a falcon, brought to a volume of 25 mL (For soils, a 1:50 dilution was performed; for plant organs, a 1:25 dilution was performed with MilliQ water) and left to decant for 24 h. After 24 h, the samples were diluted before being analyzed by ICP-MS.

Quantification of all mineral elements was performed with an

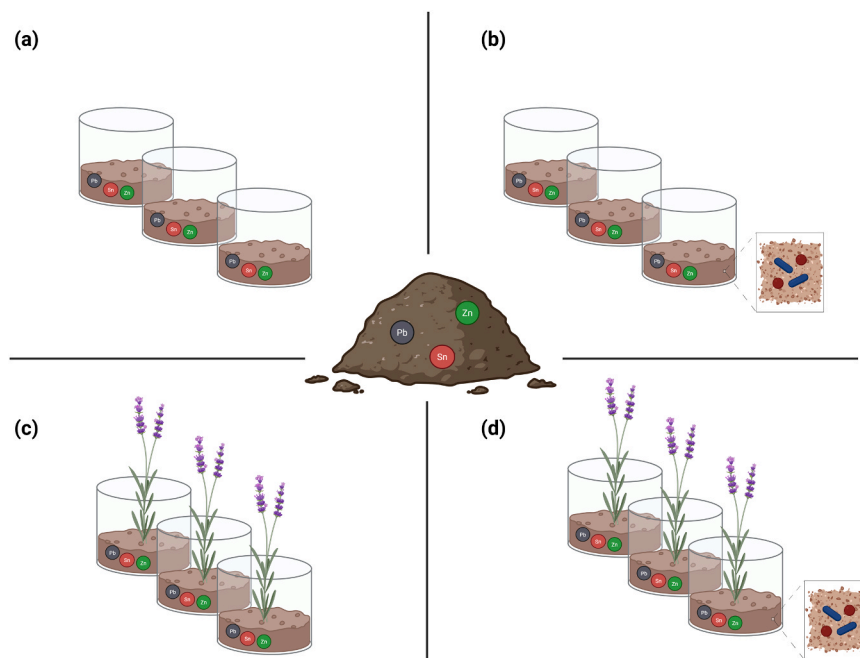


Fig. 1. Experimental design of soil microcosms. (a): historically contaminated soil (HCS), (b) soil inoculated with bacterial consortium (BIOAUG), (c) soil planted with *L. angustifolia* (PLANT), and (d) combined treatment (PLANT+BIOAUG).

inductively coupled plasma mass spectrometer (ICP-MS) model 7700x (Agilent Technologies, Tokyo, Japan). By completely atomizing and ionizing samples within the argon plasma, this analytical technique quantifies the total elemental concentration of each target metal, regardless of its original oxidation state or chemical speciation in the environmental matrix. Two six-point multilevel calibration curves were used to define two different working ranges covering concentrations of 0–10 ng/g and 0–2000 ng/g; final data were expressed in mg of element/kg of dry sample. Each value represents the mean of at least two replicates for each analysis. Quality control and assurance (QA/QC) included control samples (procedural blanks), duplicate digestions/measurements, and multi-level calibration verification. Control sample signals were monitored throughout the analytical sequence and used to verify the absence of significant contamination [22].

The percentage of heavy metal removal (R%) was calculated as follows:

$$R\% = \frac{C_i - C_f}{C_i} \cdot 100 \quad (1)$$

where C_i correspond to the initial concentration, while C_f is the concentration observed at 90 days.

To assess the plant's uptake capacity, the Bioaccumulation Factor (BAF) was calculated as the ratio of the metal concentration in the plant biomass to the initial metal concentration in the soil, while for the capacity of a plant to transfer heavy metals from the roots to the leaves, the Translocation Factors (TF) were calculated, as the ratio between the metal concentration in the leaves and that in the roots.

2.3. Soil DNA extraction and 16S rDNA gene sequencing

Soil samples collected from the microcosms were used for microbial community analyses

at 0 and 90 days. DNA extractions were performed using DNeasy PowerSoil kit (QIAGEN, Germantown, MD), in accordance with the manufacturer's instruction. The concentration of extracted genomic materials was measured using Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

Universal primers named Pro341F/Pro805R were used for the amplification of the V3-V4 hypervariable region of the prokaryotic 16S rDNA in accordance with [42]. PCR products were checked on 1.5% agarose gel. The amplicons were purified by enzymatic treatment with Thermolabile Exonuclease I (NEB), diluted 1:2, and amplified with a short cycle using Nextera XT Indexes (Illumina). PCR products were then normalised using SequalPrep (Thermo Fisher) and multiplexed. Pooled amplicons were purified and concentrated using magnetic beads Agencourt AMPure XP 1X (Beckman Coulter Inc.). High-throughput sequencing was performed, on illumina MiSeq platform (V3 chemistry - 300 bp paired-end), at BMR Genomics Srl, Padova, Italy. Raw sequence reads have been submitted in the Sequence Read Archive (SRA) under the BioProject accession number PRJNA1400311.

Bioinformatic processing was carried out using the QIIME2 software suite (v. 2023.7). Data were reported in [Supplementary Table 5](#).

The reads of the 21 samples (9 samples for the 0-day and 12 samples for the 90-day) were cleaned of primers using Cutadapt software (v. 2023.7) and then processed with the denoised-paired plugin of the DADA2 software [43]. Briefly, the sequences have been trimmed at the 3' end (Forward: 275 bp; Reverse: 200 bp) and filtered for quality and length. The sequences were then dereplicated and merged to obtain unique sequences. Finally, the chimeras were removed. The Amplicon Sequence Variants (ASVs) with a frequency < 0.005% and a length < 370 bp were filtered out to remove potential artifacts. To correct for uneven sequencing depth and allow for rigorous diversity comparisons, all samples were rarefied to an even depth of 14,363 reads per sample, which maintained an adequate Good's coverage. All reads from bacterial communities were classified to the lowest possible taxonomic rank using

a reference dataset from the SILVA database (v. 138) and GreenGenes2 database (v. 2022.10).

2.4. Data analysis

Data analyses were performed using R (version 4.4.2) with the packages phyloseq, microeco, and microbiome.

Alpha-diversity analyses were performed on all samples using a set of metrics, including Observed Features, Chao1, Shannon, Simpson, Pielou's evenness, and Faith's PD. For each index, the Kruskal–Wallis test was used to verify the significance of the comparisons between the experimental conditions.

Beta-diversity analyses were performed on all samples using Bray–Curtis metrics. Statistical analyses were performed to compare the experimental conditions using the PERMANOVA test (with 999 permutations), implemented via the *adonis2* function of the *vegan* R package. All statistical analyses were conducted considering three independent biological replicates ($n = 3$) for each experimental condition and time point).

Differential abundance between sample groups was tested with DESeq2 [44,45]. To identify significant and reliable taxonomic shifts, the output was filtered using stringent global thresholds: a corrected p-value (FDR) < 0.05, an absolute log2 fold change value greater than one ($|\log_2FC| > 1$), and a minimum mean abundance (baseMean) > 20. Heatmaps were generated using R package ComplexHeatmap [46].

The Prediction of functional capabilities in the soil microbial communities was carried out using TAX4FUN2 tool [47], an open-source R package that generate functionals profiles, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [48], and calculate the redundancy of specific functions by the functional redundancy index (FRI). KOs were grouped into broader functional categories relevant to bioremediation. Within each category, only KOs with the highest mean absolute functional redundancy index (aFRI) across samples were included.

The relative abundance table (738 ASVs, taxonomic level 7, SILVA classification v. 138) was pre-processed before LEfSe analysis by applying a specific prevalence filter for each comparison. For each experimental condition (HCS, PLANT, BIOAUG, PLANT_BIOAUG), only taxa present in at least 2 out of 3 samples ($\geq 66\%$) at least one of the two timepoints (T_0 or T_90) were retained, removing an average of 42% of low-prevalence features. The filtered files were formatted for LEfSe with normalization to 1,000,000 (parameter -o 1000000), necessary for the correct functioning of the LDA algorithm with relative abundance data. LEfSe analysis was performed separately for each condition comparing T_0 vs T_90, with LDA threshold > 2.0 and Kruskal–Wallis p-value < 0.05 (parameters: -l 5.0 -a 0.05).

3. Results

3.1. HMs monitoring in microcosms' soils and plant uptake capacity

ICP-MS analyses performed on microcosms soils at 0 and 90 days evidenced that Sn, Zn, and Pb total concentrations markedly exceeded the Italian legal limit (Italian Legislative Decree 152/06), set at 1 mg/kg, 150 mg/kg, and 100 mg/kg respectively ([Supplementary Figure S1](#)). Regarding European standards, Zn and Pb concentrations were found to be at the threshold or above the most restrictive limits defined by Council Directive 86/278/EEC (150 mg/kg for Zn and 50–300 mg/kg for Pb). For Sn currently no harmonized EU-wide limit for soil contamination exists.

Heavy metal concentrations are reported in [Supplementary Table S1](#), along with the percentage apparent reduction ([Supplementary Table S2](#)), calculated for each experimental treatment – as defined in [Eq. 1](#). After 90 days of incubation, metal removal efficiency varied considerably between the different treatments.

For zinc, the most significant apparent reduction was observed in the

PLANT treatment (8.27%), where the absolute concentration decreased from 600.09 ± 74.79 mg/kg to 550.49 ± 23.30 mg/kg. Treatments using the microbial consortium showed more modest reductions, with apparent reductions of 5.86% in BIOAUG (564.95 ± 29.48 mg/kg) and 5.12% in PLANT+BIOAUG (569.37 ± 25.63 mg/kg).

Conversely, reductions in lead concentration were consistently more stable in treatments using *L. angustifolia*. Specifically, apparent reductions reached values of 46.58% in BIOAUG, 50.37% in PLANT, and 55.42% in PLANT+BIOAUG. It is noteworthy that the PLANT+BIOAUG treatment demonstrated the most significant Pb reduction (reaching a final concentration of 734.31 ± 34.56 mg/kg) and showed the lowest level of variability, thus underscoring the reliability of this result. Tin exhibited a marginal decrease in the BIOAUG condition (30.65%) and a greater effect in the planted treatment (75.88% in PLANT and 76.28% in PLANT+BIOAUG). However, for both lead and tin in untreated soil at 0 days, the standard deviations are very high, highlighting the strong spatial heterogeneity typical of real, historically contaminated soils. This prevents the interpretation of these reductions as a consistent or reliable removal effect. Nevertheless, these results indicate that the simultaneous presence of *L. angustifolia* and the microbial consortium enhanced soil Pb and Sn mitigation synergistically.

The quantification of heavy metals in *L. angustifolia* biomass revealed distinct accumulation patterns, thus clarifying the remediation pathway. In the PLANT+BIOAUG treatment, concentrations of Pb, Sn, and Zn in plant tissues were 20.39 mg/kg, 0.51 mg/kg, and 20.33 mg/kg in roots, respectively, and 15.46 mg/kg, 0.10 mg/kg, and 11.26 mg/kg in leaves. The calculated bioaccumulation factors (BAF) for these key contaminants were consistently less than 1 (BAF < 0.1), suggesting that the primary role of *L. angustifolia* is phytostabilization.

3.2. Microbial communities' diversity analyses

The DNA concentration (ng/μl) in the soil samples of the microcosms showed an increasing trend from 0 to 90 days. This concentration increased from 7.16 ± 1.48 – 15.86 ± 3.30 ng/μl in PLANT. The BIOAUG condition revealed a moderate increase (from 13.38 ± 1.26 – 15.72 ± 3.78 ng/μl). In contrast, the PLANT+BIOAUG treatment maintained stable DNA levels over time (from 12.53 ± 2.46 – 12.50 ± 2.87 ng/μl), suggesting potential microbial competition or community stabilisation in the presence of the plant and the microbial consortium.

In order to study the effect of different bioremediation techniques on the bacterial community, 16S rDNA amplicon sequences were used to determine microbial diversity within and between treatments. ASVs were filtered at a frequency of 0.005%, and subsequent rarefaction analysis resulted in 710189 reads, corresponding to 2129 ASVs acquired from 21 samples (HCS, PLANT, BIOAUG, and PLANT+BIOAUG in triplicate at 0 days and at 90 days). The alpha rarefaction curves produced

on the rarefied sequences revealed that the sequencing depth was sufficient to study microbial diversity in all samples (Supplementary Figure S2.A and B).

The selected ASVs were then used to measure both α and β diversity, as well as for taxonomic assignment according to the SILVA database. Furthermore, analyses of the differential abundance of taxa were performed.

3.2.1. α -diversity indexes

The α -diversity was assessed using ecological indices (observed feature, Chao1, Shannon, Simpson, Evenness, and Faith's Phylogenetic Diversity) to evaluate the effects of designated conditions and time on microbial community diversity (the Shannon and Chao1 box plots are shown in Fig. 2). The Kruskal-Wallis's method was used to test for differences in diversity per sample across conditions. No significant differences were detected between the treatment conditions or sampling times for any of the diversity metrics (all $p > 0.05$; data available in Table 2). A marginally non-significant interaction effect ($p = 0.089$) was observed for Faith phylogenetic diversity, especially in PLANT+BIOAUG condition at 90 days, suggesting a potential time-dependent response in phylogenetic diversity across treatments. Overall, the results indicate that neither the treatment nor the incubation time had a significant impact on microbial alpha diversity overtime.

3.2.2. β -diversity indexes

A study of compositional changes (β -diversity) was conducted using the abundance-based Bray-Curtis metric. As illustrated in Fig. 3, the principal coordinate analysis (PCoA) was performed based on the distance matrix obtained with this index. The PCoA based on the Bray-Curtis matrix (Fig. 3.A) explained 39.85% of the total variance (PC1 22.7%, PC2 17.01%), PERMANOVA test demonstrated significant variations in microbial community structure ($p < 0.001^{**}$), both between treatments and over time. Further comparisons did not reach statistical significance after FDR correction (q values ~ 0.121), due to inherent mathematical limitations of the permutation test when used with small sample sizes, rather than a lack of biological effect. Indeed, several consistent trends were identified (p values < 0.1). The statistical trends and the spatial clustering of the PLANT+BIOAUG treatment in the PCoA visualization provide clear evidence of a progressive, time-dependent restructuring of the microbiome in the PLANT+BIOAUG combination process.

Venn diagram analysis, based on presence/absence of ASVs after merging replicates within each condition, highlighted a large persistent core microbiome shared among the baseline soil (HCS_T0) and all treatments at 90 days (381 ASVs, 56.6%), supporting the resilience of a substantial portion of the community. The PLANT+BIOAUG combination showed the highest number of condition-specific taxa (241 unique

Table 1

Heavy metals concentrations (average values and standard deviation) observed in plant biomass (leaves, roots). Bioaccumulation Factor - BAF and Translocation factor - TF calculated for the different experimental conditions (PLANT, PLANT+BIOAUG) after 90-days of incubation per treatment.

	PLANT				PLANT+BIOAUG			
	Roots	Leaves	BAF	TF	Roots	Leaves	BAF	TF
Be	0.11 ± 0.08	0.03 ± 0.03	0.04	0.32	0.16 ± 0.05	0.09 ± 0.02	0.07	0.54
V	2.93 ± 1.03	0.61 ± 0.30	0.03	0.21	2.04 ± 0.41	1.58 ± 0.58	0.02	0.77
Cr	3.85 ± 1.87	1.17 ± 0.48	0.03	0.30	3.10 ± 0.76	2.56 ± 0.78	0.02	0.83
Co	0.36 ± 0.15	0.10 ± 0.04	0.02	0.28	0.36 ± 0.07	0.34 ± 0.14	0.02	0.94
Ni	3.31 ± 1.20	1.42 ± 0.84	0.04	0.43	2.45 ± 0.42	1.45 ± 0.78	0.03	0.59
Cu	26.34 ± 3.01	84.60 ± 32.53	0.18	3.21	10.08 ± 3.58	60.66 ± 6.78	0.07	6.02
Zn	39.88 ± 5.38	37.15 ± 16.67	0.07	0.93	20.33 ± 2.96	11.26 ± 11.53	0.03	0.55
Cd	0.09 ± 0.05	0.01 ± 0.01	0.05	0.12	0.13 ± 0.07	0.06 ± 0.05	0.07	0.46
Se	0.14 ± 0.05	0.05 ± 0.03	0.02	0.36	0.13 ± 0.02	0.12 ± 0.04	0.02	0.87
As	0.38 ± 0.13	0.23 ± 0.05	0.02	0.61	0.38 ± 0.07	0.71 ± 0.30	0.02	1.89
Sn	0.50 ± 0.02	0.10 ± 0.03	0.00	0.20	0.51 ± 0.03	0.10 ± 0.04	0.00	0.20
Sb	0.11 ± 0.02	0.04 ± 0.01	0.01	0.33	0.08 ± 0.02	0.11 ± 0.04	0.01	1.41
Tl	0.04 ± 0.01	0.01 ± 0.00	0.10	0.17	0.10 ± 0.04	0.05 ± 0.01	0.23	0.47
Pb	26.49 ± 6.59	5.74 ± 2.51	0.02	0.22	20.39 ± 6.21	15.46 ± 5.20	0.01	0.76

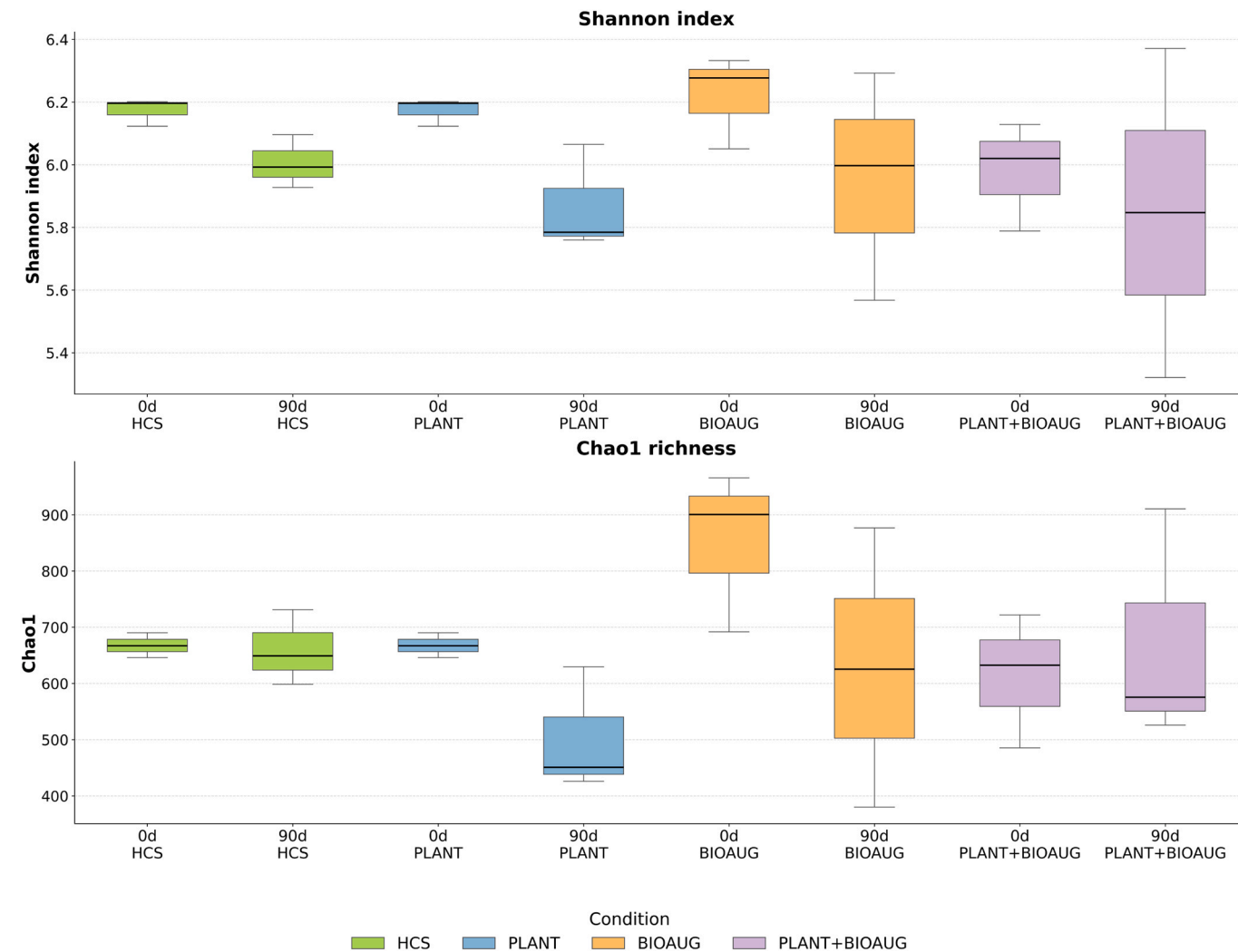


Fig. 2. α -diversity within treatments on the 0- and 90-day, according to the indexes Shannon and Chao1. No significant differences were detected according to the Kruskal-Wallis test.

Table 2
Alpha-diversity indices measured in microcosm soils across treatments (HCS, PLANT, BIOAUG, PLANT+BIOAUG) at 0 and 90 days. Values represent mean diversity estimates per treatment. No significant differences were detected across treatments or timepoints (Kruskal–Wallis test, $p > 0.05$).

	Time	Observed	Chao1	Shannon	Simpson	Evenness	Faith'sPD
HCS	0d	667.00	667.64	6.17	1.00	0.95	39.70
	90d	658.00	659.63	6.01	1.00	0.93	36.89
PLANT	0d	667.00	667.64	6.17	1.00	0.95	39.70
	90d	502.00	502.18	5.87	1.00	0.95	38.58
BIOAUG	0d	845.00	852.65	6.22	1.00	0.92	43.79
	90d	625.00	627.38	5.95	1.00	0.93	35.13
PLANT+BIOAUG	0d	612.67	613.33	5.98	1.00	0.93	36.34
	90d	667.67	670.71	5.85	0.99	0.90	42.74

ASVs, 7.1%), which exceeded the numbers observed in PLANT_T90 (72 unique ASVs, 1%) and BIOAUG_T90 (187 unique ASVs, 2.2%). This is consistent with the emergence of treatment-specific microbial niches driven by the combined effects of plant presence and bioaugmentation. Furthermore, the two conditions with *L. angustifolia* (PLANT_T90 and PLANT+BIOAUG_T90) shared 182 ASVs not detected in the other groups, indicating a consistent plant-associated component of the community at 90 days.

3.3. Analysis of the microbial community in soils

Taxonomy was assigned to 738 ASVs at 99% similarity using SILVA database (v.138), achieving the genus level for the most of ASVs with few exceptions for which family or order belonging to was annotated, providing detailed information on the composition of microbial communities in soil samples across treatments. At the phylum level (Fig. 4. A), microbial communities were consistently dominated by *Proteobacteria*. However, their relative abundance decreased over time in soils subjected to microbial inoculation, particularly in the combined PLANT+BIOAUG treatment.

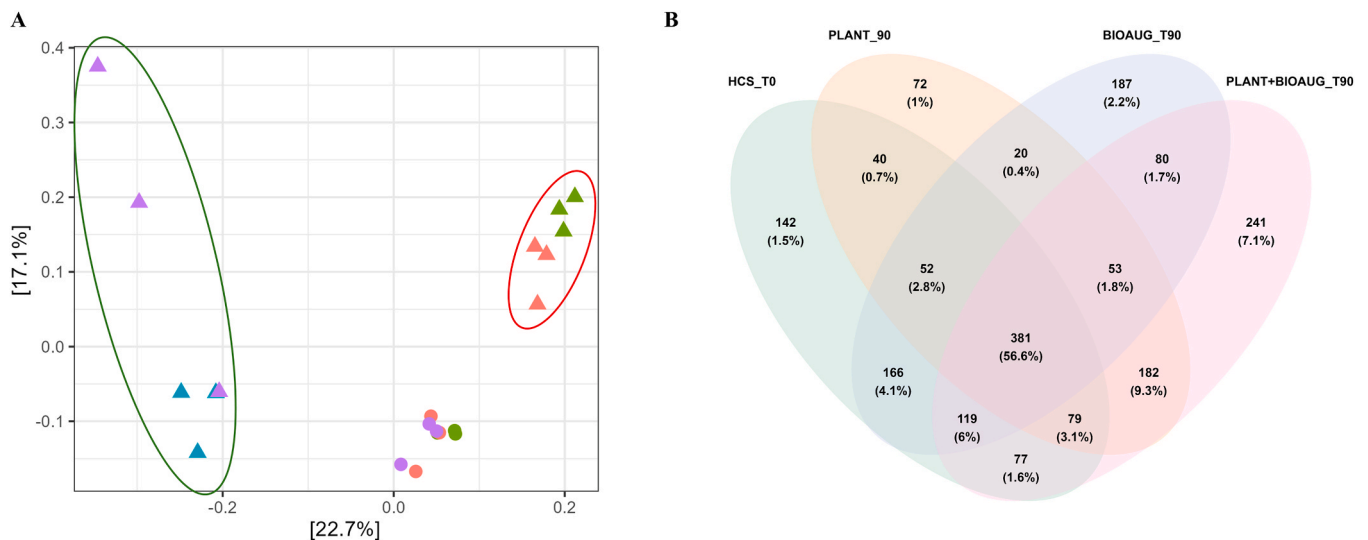


Fig. 3. (A) Principal Coordinate Analysis (PCoA) of soil bacterial communities based on the Bray–Curtis dissimilarity index, showing the distribution of samples at the beginning of the experiment (T0) and after 90 days (T90) under the different treatments. At T90, the green ellipse groups samples collected in the presence of *Lavandula angustifolia* (PLANT and PLANT+BIOAUG), whereas the red ellipse groups samples collected under plant-free conditions (HCS and BIOAUG). (B) Venn diagram illustrating the number of shared and unique amplicon sequence variants (ASVs) between the initial contaminated soil (HCS at T0) and the soils subjected to the different treatments after 90 days.

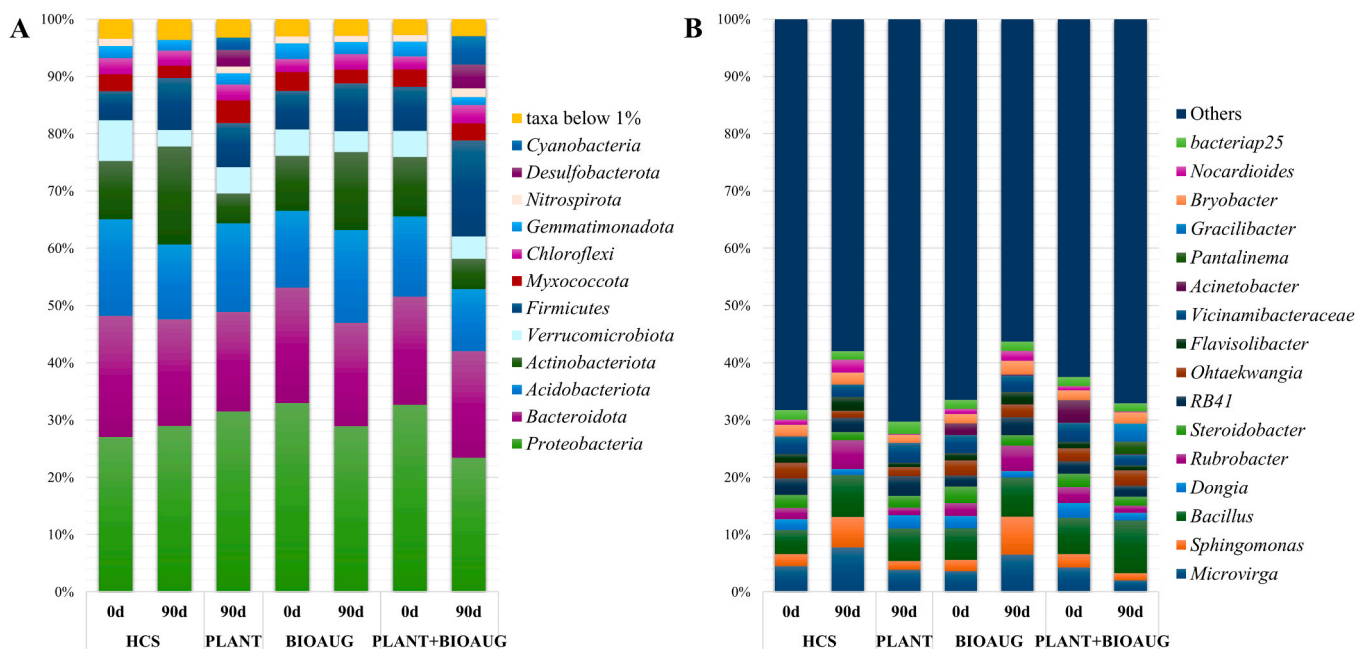


Fig. 4. Community composition at (A) phylum level and (B) genus level of different treatments at 0- and 90-days.

Bacteroidota and *Acidobacteriota*, which were initially among the most abundant phyla, generally declined across treatments. This suggests that there was selective pressure imposed by both the presence of plants and bioaugmentation strategies. *Actinobacteria* demonstrated contrasting trends, with an increase observed under bioaugmentation conditions and a decrease in planted systems. This highlights differential responses of major phyla to plant- and microbial-mediated factors. Minor phyla, including *Cyanobacteria* and *Desulfobacteriota*, increased primarily in the combined treatment, suggesting the establishment of synergistic interactions capable of promoting specific microbial consortia.

At a lower taxonomic level (Fig. 4.B), community composition showed greater variability and treatment-specific patterns.

Bioaugmentation promoted the persistence and enrichment of several introduced or functionally relevant taxa, while planted treatments showed a more selective pattern. In particular, *Microvirga* shows an increase over time in BIOAUG, indicating resilience and the ability to compete in both native and bioaugmentation conditions. In the PLANT+BIOAUG combination, however, its abundance decreases after 90 days, suggesting unfavourable competition with other genera or adaptation to the microenvironment. *Bacillus* levels increased significantly in all treatments, peaking in PLANT+BIOAUG after 90 days, highlighting the ability of this spore-forming genus to colonize disturbed and enriched environments and provide key functions for bioremediation. *Vicinamibacteraceae* is stable in PLANT and BIOAUG, while it disappears in PLANT+BIOAUG at 90 days, indicating reduced

competitiveness in systems with high microbial diversity. *Steroidobacter* and *Sphingomonas* are generally absent or present in low numbers in most treatments, except for BIOAUG, where they emerge following microbial inoculation, indicating strong artificial selection towards genera with specific functions (degradation of organic contaminants/metals). *Bryobacter*, *Rubrobacter*, and *Flavisolibacter* only emerge after 90 days in unplanted BIOAUG systems. These results underscore the adaptability of the community in response to bioaugmentation stimuli. Research findings indicate that *Comamonas* and *Acinetobacter* are

present in BIOAUG and PLANT+BIOAUG at 0 days. This observation underscores the integration of functional taxa that are imported through the microbial consortium. Genera such as *WCHB1–32*, *Gracilibacter*, and *Pantalinema* appeared only in the combined PLANT+BIOAUG treatment after 90 days, suggesting the possible creation of selective ecological niches.

Across treatments, a significant proportion of the community consisted of “uncultured”, “unclassified” and “taxa less than 2%”, grouped together in the “others” category. The significant contribution of low-

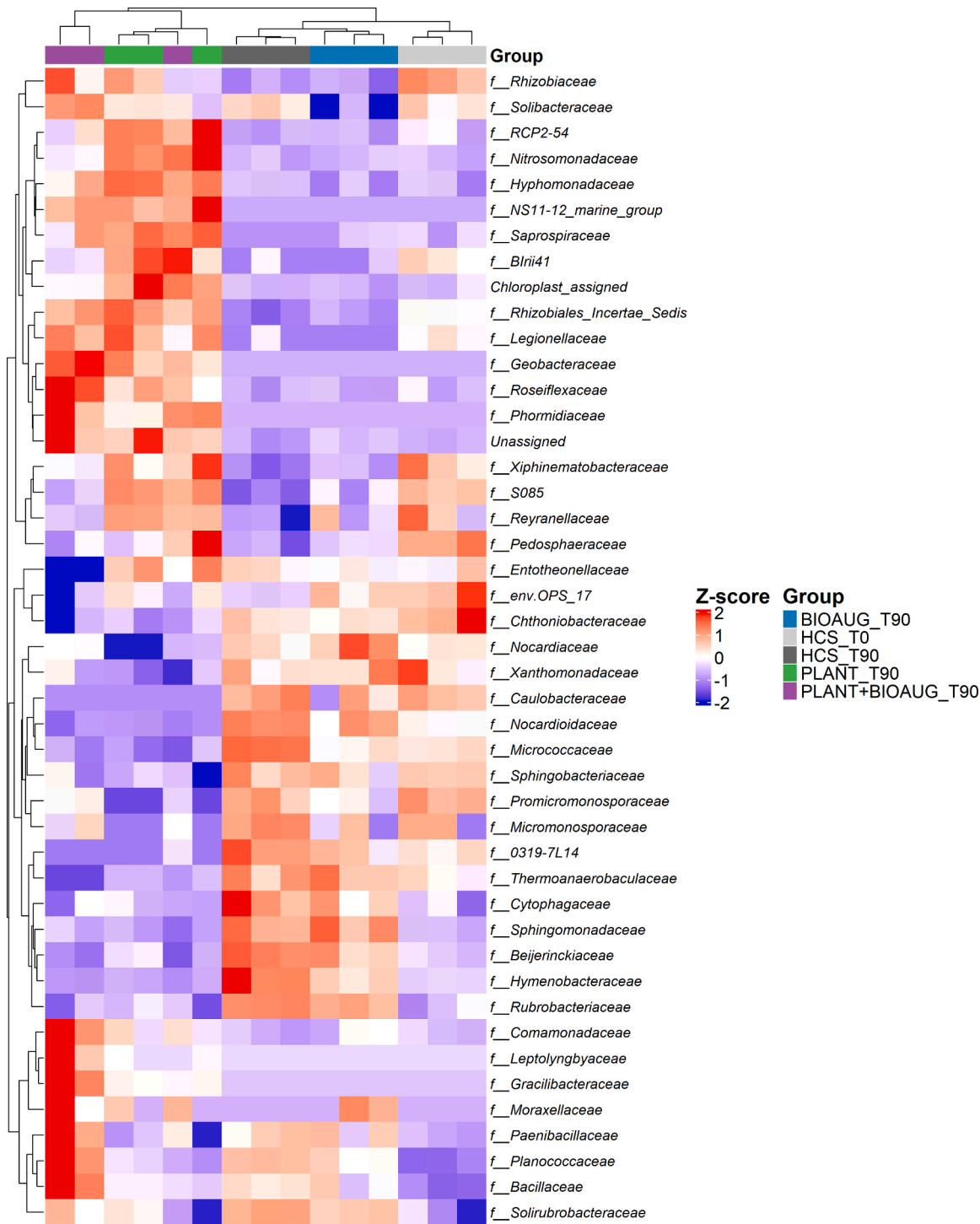


Fig. 5. Heatmap representing the normalized abundance (Z-score) of the significantly differentially abundant bacterial families across treatments (PLANT, BIOAUG and PLANT+BIOAUG treatments after 90 days with the soil (HCS) at the initial time point). To highlight biologically robust taxonomic changes, only families with strongly differential abundance are shown, filtered according to the following thresholds: p-value (FDR) < 0.05, |log2FC| > 1, and baseMean > 20.

abundance taxa suggests that the microbial community has diversified and developed into a more complex assemblage, observed especially in the planted treatments—PLANT and PLANT+BIOAUG at 90 days. It is important to note that the transition from *phylum* taxonomic levels to *genus* levels resulted in a reduction in apparent variability, enabling the identification of more stable and treatment-specific microbial patterns.

3.4. Taxa differential abundance by DESeq2 and LEfSe analysis

Differential taxa abundance analyses were performed using DESeq2. Treatment comparisons 90 days with the HCS control (0-day) are shown in Fig. 5 (Data were reported in Supplementary Table S3).

Not all ASVs were annotated to the *genus* level, and in the reported analysis, the taxa were decoded at *family* taxonomic level.

In the BIOAUG treatment at 90-days, an enrichment of families typical of aerobic and stress-resistant environments was observed, including *Sphingomonadaceae* ($\log_2FC = +1.86$), *Hymenobacteraceae* (+1.42), *Moraxellaceae* (+1.84) and *Rubrobacteriaceae* (+1.42), and *Beijerinckiaceae* (+0.76). Concurrently, families such as *Parachlamydiaceae* (−3.92) and *Legionellaceae* (−2.68) are significantly reduced, delineating a community oriented towards degraders and residual phototrophs, with a loss of groups not characteristic of the inoculum. The PLANT treatment determines the largest shift, with significant increases in families associated with rhizospheric micro-niches or biogeochemical processes, such as *Nitrosomonadaceae* (+1.75) *Saprospiraceae* (+3.29) and *Geobacteraceae* (+2.15). The heatmap confirms a high representation of these groups in the plant samples. Concurrently, the treatment has been shown to induce a substantial reduction in various *Actinobacteria*, including *Sphingomonadaceae* (−0.17), *Micrococcaceae* (−1.04) and *Nocardiaceae* (−2.93), as well as a decrease in *Hymenobacteraceae* (−0.79). Taken together, these changes suggest the establishment of a structured rhizosphere community, with a strong contribution from taxa involved in redox processes and carbon metabolism. The combined treatment (PLANT+BIOAUG) shows a blended profile, characterised by the simultaneous enrichment of taxa associated with both the rhizosphere and microbial degradation induced by inoculation. Specifically, there are notable increases in *Comamonadaceae* (+3.03), *Geobacteraceae* (+2.91), *Bacillaceae* (+1.83) and *Gracilibacteraceae* (+2.80), which the heatmap highlights as distinctive of the combined group. Concurrently, the presence of taxa characteristic of oligotrophic environments, including *Blastocatellaceae* (−0.38), *Caulobacteraceae* (−3.09) and *Nocardioidaceae* (−2.07), was identified.

The results show that the three treatments shape the microbial community differently: BIOAUG favours aerobic and degrading taxa, PLANT induces a restructuring linked to the rhizosphere and biogeochemical cycles, while PLANT+BIOAUG produces a synergistic profile integrating both factors.

LEfSe analysis was performed to identify the main microbial taxa that are structurally determining the differences between initial contaminated soil (HCS T0) and the different treatments after 90 days (Fig. 6). Please refer to Table 3 for a summary of the differential abundance analysis, including the total number of significant biomarkers identified for each comparison. The PLANT condition showed the highest number of discriminatory features (294 out of 335 tested), indicating that the lavender rhizosphere induced the most extensive and consistent reorganization of the soil microbial community over the course of the experiment. In contrast, BIOAUG showed the most marked reduction after the LDA filter (91 out of 269), suggesting that microbial inoculum alone produced more heterogeneous effects across biological replicates.

Natural temporal succession in the unplanted control (HCS T90) was characterised by an enrichment of *Sphingomonadaceae* and *Nocardioidaceae*. The introduction of the plant (PLANT T90) had a distinct effect on the rhizosphere, specifically selecting taxa such as *Geobacteraceae* (LDA score > 3.9). Specifically, the bioaugmentation treatment (BIOAUG T90) led to a substantial and significant enrichment of

Sphingomonadaceae (maximum LDA = 4.65), a taxon that is widely recognised for its toxic compound degradation capabilities. Furthermore, the combined treatment (PLANT+BIOAUG T90) revealed a synergistic remodelling of the microbiome, promoting the proliferation of potential specialized degraders, with *Comamonadaceae* (LDA > 4.0) and *Gracilibacteraceae* (which includes the genus *Lutispora*) emerging as the main general biomarkers of this experimental condition.

3.5. Prediction of the native microbes' functional capabilities

Functional prediction analysis was performed on the ASVs with more than ten counts per sample in at least two technical replicates, using the TAX4FUN2 tool; overall, 7959 KEGG functions were predicted in the microcosms [49]. The information related to KO terms were screened and 190 functional annotations of genes related to reduction and resistance to heavy metals, degradation of organic pollutants, redox metabolism, the electron transport system (ETS), response to oxidative stress, DNA repair mechanisms, organic acid metabolism, and the production of EPS, biosurfactants, and siderophores were selected (Supplementary Table S4). The most representative absolute functional redundancy index (FRI) calculated by treatment at baseline and final time points is reported in Table 4 and Fig. 7. However, it should be noted that functional profiles inferred from 16S rRNA gene sequencing represent indirect predictions based on reference genomes and may not fully capture the actual metabolic potential of the microbial community, due to database limitations and reduced sensitivity in detecting functional differences in complex environmental systems [50].

In the control (HCS), both at 0- and 90-days, the distribution of KOs was relatively homogeneous and not characterised by particularly dominant groups, suggesting a functional state typical of an untreated soil. In BIOAUG at 0 days, the introduction of the microbial consortium generated an immediate enrichment of functions related to flavoprotein-based energy metabolism and metal efflux, indicating a direct contribution of the inoculated strains to cellular resistance mechanisms and intracellular metal management. After 90 days (BIOAUG_T90), these groups were further consolidated and distributed across generally higher FRI values. The PLANT_T90 treatment showed a different functional profile, with a more marked increase in functions associated with chelation via siderophores, consistent with the potential of *L. angustifolia* plants to modulate metal availability through the release of root exudates and the selection of siderophore-producing bacterial genera. In these treatments (PLANT and BIOAUG at 90 days) consistent values were also observed for genes associated with the response to oxidative stress, in line with the selective pressure exerted by heavy metals and the need to maintain an efficient redox balance.

The combined treatment (PLANT+BIOAUG_T90) showed the most structured and redundant functional pattern, with concomitant high values of electron transport system (ETS) annotations, metal efflux and siderophore chelation, suggesting a synergistic effect between plants and microbial consortium. This combination highlighted not only an enhancement of the functions directly involved in detoxification (efflux, siderophores), but also a stable contribution of the biofilm and EPS group, which can facilitate the immobilization of metals and cellular protection in localized micro-niches of the rhizosphere. The results indicate that the functional redundancy associated with key processes for heavy metal resistance and mitigation is not only modulated by the type of treatment but is significantly amplified by the inoculated microbial consortium and further enhanced by plant–microbe interaction. These findings are consistent with a combined improvement in the capacity of the soil–rhizosphere system to support functions relevant to bioremediation under conditions of persistent contamination.

4. Discussions

The presence of heavy metals in soil systems alters the chemical and physical characteristics of the soil, resulting in direct toxic pressure on

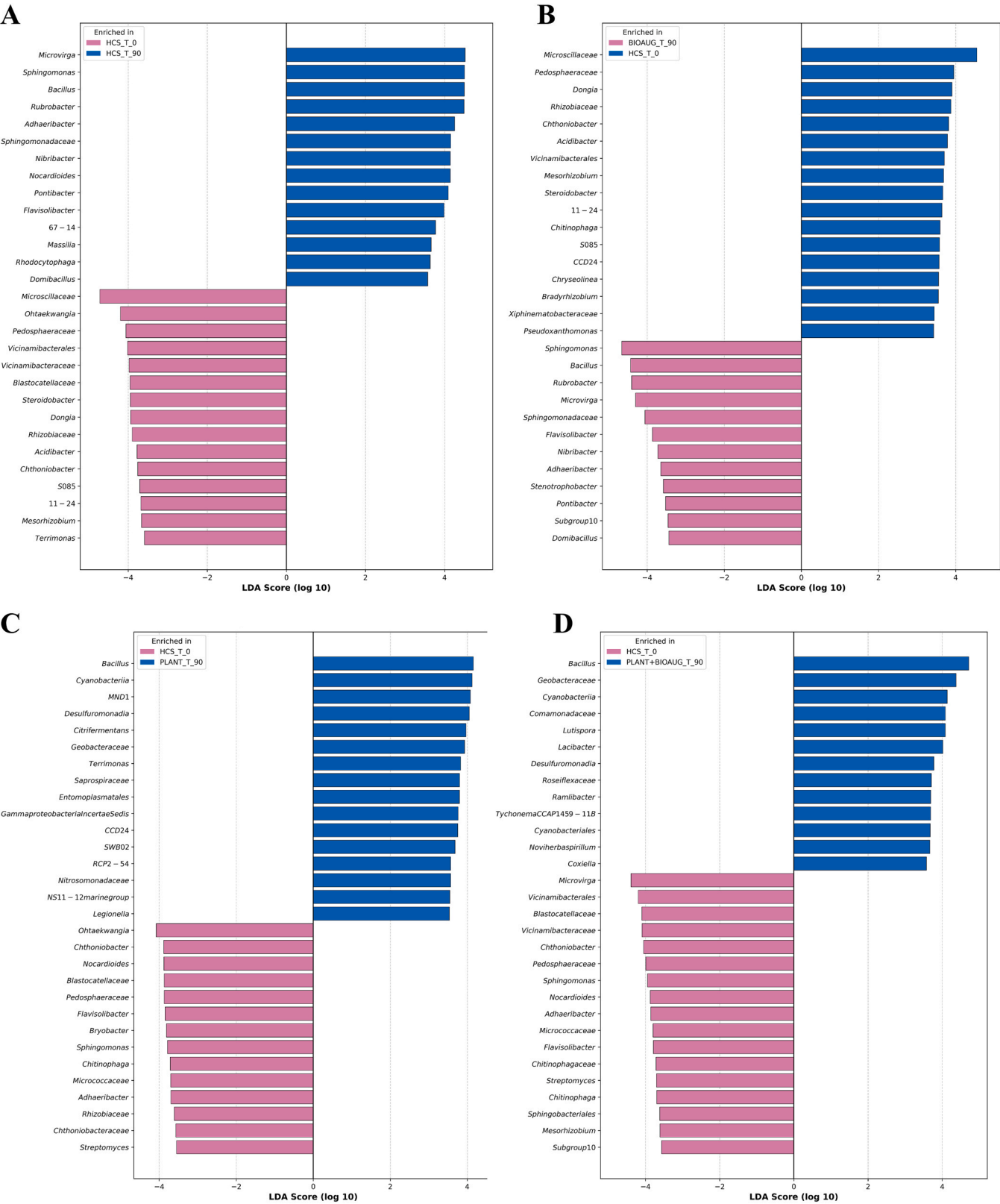


Fig. 6. Linear discriminant effect size (LEfSe) bar graphs showing the 30 most abundant bacterial taxa driving textural changes between the initial contaminated soil (HCS T0) and experimental treatments after 90 days of incubation: (A) unplanted control (HCS T90), (B) bioaugmentation (BIOAUG T90), (C) *L. angustifolia* presence (PLANT T90), and (D) combined treatment (PLANT+BIOAUG T90). Only traits meeting an absolute LDA score threshold > 2.0 and a Kruskal-Wallis significance of $p < 0.05$ are shown.

Table 3

Summary of the LEfSe analysis comparing taxa abundance at T0 versus T90 in the different treatments. Only parameters with an LDA score > 5.0 were considered significant biomarkers.

Condition	Feature after prevalence filter	Feature LDA > 2.0	Reduction
HCS	206	109	47%
PLANT	335	294	12%
BIOAUG	269	91	66%
PLANT_BIOAUG	218	113	48%

Table 4

Functional groups selected for the bioremediation of heavy metals, with reference to lead (Pb), zinc (Zn) and tin (Sn).

Group	KEGG	Functional annotation
Electron transfer	K00239	Succinate dehydrogenase / fumarate reductase, flavoprotein subunit
	K00240	Succinate dehydrogenase / fumarate reductase, iron-sulfur subunit
	K00330-K00331; K00342	NADH-quinone oxidoreductase (subunit A-B and M)
	K00425	Cytochrome bd ubiquinol oxidase subunit I
	K02300	Cytochrome o ubiquinol oxidase subunit IV
	K03612-K03617	Rnf electron transport complex (A–G subunits)
Pb resistance	K01533	Cu ²⁺ -exporting ATPase
	K01534	Cd ²⁺ /Zn ²⁺ -exporting ATPase
	K02654	leader peptidase (prepilin peptidase) / N-methyltransferase
Zn/Cd/Co resistance	K02074	zinc/manganese transport system ATP-binding protein
	K07263	zinc protease
	K09817	zinc transport system ATP-binding protein
	K15726	cobalt-zinc-cadmium resistance protein CzcA
	K16264	cobalt-zinc-cadmium efflux system protein
Sn resistance	K02115	F-type H ⁺ -transporting ATPase
	K00027	malate dehydrogenase (oxaloacetate-decarboxylating)
	K00029	malate dehydrogenase (oxaloacetate-decarboxylating) (NADP ⁺)
	K00767	nicotinate-nucleotide pyrophosphorylase (carboxylating)
	K00969	nicotinate-nucleotide adenyltransferase
	K17686	Cu ⁺ -exporting ATPase
	K03386	Peroxioredoxin (alkyl hydroperoxide reductase C)
	K03387	Alkyl hydroperoxide reductase F
Oxidative stress response	K03671- K03672	Thioredoxin 1 and Thioredoxin 2
	K03781	Catalase
	K03782	Catalase-Peroxidase
	K04564	Superoxide dismutase (Fe–Mn family)
	K04565	Superoxide dismutase (Cu–Zn family)
	K00381	Sulfite reductase (β-component); Fe-S metabolism
Biofilm, EPS and Siderophores	K00806	Undecaprenyl diphosphate synthase
	K00627	Pyruvate dehydrogenase E2 component
	K00658	2-oxoglutarate dehydrogenase E2 component
	K01993	HlyD secretion protein
	K01000	Phospho-N-acetylmuramoyl-pentapeptide-transferase
	K20997	PsIA polysaccharide biosynthesis protein

flora and fauna and even causing a lack of development [51]. Heavy metals can act as strong ecological filters, significantly impacting soil microbial communities by selecting for metal-tolerant taxa and suppressing sensitive groups. This, in turn, can lead to alterations in key soil functions [52,53]. This study provides a deeper understanding of the

ecological and functional mechanisms underlying the effectiveness of bioremediation treatments against heavy metals (especially Zn, Pb, and Sn) from historically contaminated soil. The combination of *L. angustifolia* and the selected hydrocarbon-oxidising bacterial consortium produced the most effective outcome in terms of contaminant removal and potential microbial functional enhancement. After 90 days, the PLANT+BIOAUG treatment achieved the highest reductions in Pb (44.75%) and Sn (66.87%) concentrations. The calculated bio-accumulation factors for Pb, Sn, and Zn indicate that *L. angustifolia* does not behave as a hyperaccumulator in this historically contaminated soil. Instead, the plant acts as an exclusion agent, limiting the translocation of toxic metals into its tissues. This exclusion behaviour has also been observed in controlled studies where lavender maintained transfer indices below unity, particularly for Zn, confirming that its accumulation strategy is strongly dependent on contamination level and environmental conditions [54]. The significant reductions in soil metal concentrations observed in the PLANT+BIOAUG treatment are not primarily due to phytoextraction. The primary mechanism is phytostabilization, which is strongly influenced by interactions in the rhizosphere. In fact, under realistic field conditions, metal immobilization processes often prevail over phytoextraction as the primary remediation technique. The roots, supported by the functional capabilities of the bio-augmented consortium (e.g. EPS production, biofilm formation and siderophore secretion), effectively immobilise and precipitate metals in the root zone, reducing their bioavailability, where microbial EPS, siderophores, and redox processes contribute to metal complexation and stabilization [52].

From an applicative perspective, this exclusion behaviour is extremely advantageous for NbS: it limits the entry of toxic metals into harvestable aerial biomass, thus minimising ecological risks and making the plant perfectly suited to circular economy applications, such as essential oil extraction. This aspect is particularly relevant for aromatic plants, as previous studies demonstrated that heavy metals accumulated in plant tissues are not transferred into essential oils, ensuring product safety [30,31,55].

The metagenomic data, resulted from 16S rDNA Next-Generation Sequencing, extend and refine those findings, showing that the observed contaminant removal corresponds to a shift in microbial community structure and function. The stability of α-diversity across treatments indicates that remediation did not compromise species richness [56]. Heavy metal stress does not necessarily reduce microbial richness but rather induces compositional shifts within the community [53]. Instead, it appears to have reorganised the existing community towards functionally relevant taxa adapted to the new rhizospheric environment. Variations in β-diversity demonstrate a structural change in the microbial communities in response to the different bioremediation approaches [16]. This combined stability in α-diversity and the significant shift in β-diversity indicate robust community turnover: rather than depleting overall microbial richness, treatments led to the replacement of sensitive taxa with specialized populations able to tolerate heavy metals and degrade organic co-contaminants [26].

A divergence in β-diversity is commonly associated with deterministic selection processes driven by metal toxicity and plant–microbe interactions [53,57]. The analysis of samples in PCoA plots demonstrated a clear distinction between the planted and unplanted cluster and the other conditions, indicating that the synergistic effect of plant–microbe interaction acted as the predominant ecological factor [8].

Taxonomic analysis showed that *Proteobacteria*, *Bacteroidota* and *Acidobacteriota* were dominant in all treatments, as reported in previous studies [58,59]. These phyla are widely recognised as dominant groups in contaminated soils due to their metabolic versatility and tolerance to environmental stress [52]. Bioaugmented soils showed a reduction in *Proteobacteria*, suggesting a selection effect [60]. The phylum *Actinobacteria* increased in BIOAUG conditions and was also dominant in other Pb and Zn polluted soils [61]. *Actinobacteria* are frequently associated with heavy metal resistance and organic matter degradation, reinforcing



Fig. 7. FRI of the functional activity of genes related to the reduction and resistance to heavy metals, degradation of organic pollutants, redox metabolism, electron transport system (ETS), response to oxidative stress, DNA repair mechanisms, organic acid metabolism and production of EPS, biosurfactants and siderophores in the 0- and 90-day treatments. The five highlighted functional groups are marked as follows: response to oxidative stress (asterisk), reduction and resistance to heavy metals (square, triangle, circle), electron transport system (cross), production of EPS, biosurfactants and siderophores (diamond).

their ecological role in contaminated environments [52]. A decrease was seen in the lavender treatments. The genus *Microvirga* had increased abundance in the BIOAUG condition and decreased abundance in PLANT+BIOAUG, suggesting competition or adverse adaptation to the rhizosphere [37]. *Bacillus* had increased substantially in all treatments, peaking in PLANT+BIOAUG after 90 days, showing the genus's capacity to inhabit disturbed, enriched environments and perform key functions in bioremediation [1,62]. *Steroidobacter* and *Sphingomonas* mainly gave rise to the BIOAUG condition, indicating strong artificial selection for taxa that degrade organic contaminants and manage heavy metals [39].

The distinct taxonomic profile in PLANT+BIOAUG treatment confirms that the interplay between the plant and inoculated bacteria is the principal ecological force in shaping community composition [29], favouring the enrichment of genera with documented roles in heavy metal tolerance, redox transformations [63], siderophore and bio-surfactant production [64], and EPS-mediated protection [65]. BIOAUG is less effective than expected, which is consistent with previous results [66]. In nature, native microbial populations tend to outcompete inoculated strains, exhibiting reduced persistence [34]. This finding is further validated by our LEfSe analysis. The BIOAUG treatment demonstrated a substantial 66% reduction in significant features following LDA filtration, underscoring the considerable heterogeneity and instability of the inoculum's effect when administered independently. In contrast, the PLANT condition retained the majority of its discriminant features (294 biomarkers, LDA > 5.0), confirming that the root system is the primary and most consistent ecological driver in restructuring the soil microbiome. This competitive exclusion may also provide a rationale for the discrepancy between microbial abundance

and activity observed in the complementary microbiological dataset.

Functional predictions derived from the metagenomic data further substantiate the superiority of the combined treatment. The PLANT+BIOAUG system exhibited the highest degree of functional redundancy in metabolic pathways directly implicated in heavy metal mitigation. This functional enrichment is indicative of a community that is metabolically versatile and highly resilient [67]. It can withstand toxic pressure while maintaining biogeochemical processes that are essential for immobilising and transforming metals [20]. Functional redundancy is likely to play a stabilising role, thereby enabling the system to maintain its functional integrity despite taxonomic fluctuations. It is evident that the pollutant removal, taxonomic reorganisation and functional enrichment observed in PLANT+BIOAUG are indicative of the synergy between *L. angustifolia* and the selected microbial consortium being central to the remediation performance. Plants alone promote microbial abundance and activity, improve soil quality, and modulate contaminant availability, whereas the consortium alone struggles to persist and contribute meaningfully [12]. However, when combined, plant roots create an ecological niche that supports both native and inoculated microbes [68]. This facilitates metabolic complementarity and enhances the overall capacity of the system to tolerate and sequester heavy metals [24]. Such collaborative dynamics are being increasingly recognised as the basis of effective plant-assisted bioremediation systems and represents a sustainable approach for the recovery of contaminated soils.

The presence of certain taxa and functions related to efflux supports the occurrence of enzymatic transformations and biosorption phenomena that reduce the bioavailability of toxic metals [69]. The

plant-microbe system acts as an integrated biocatalytic unit, where *L. angustifolia* directly controls metal dynamics through its own rhizosphere processes, and indirectly by modulating the microbial community composition and metabolism in the root zone. This allows the plant-microbe system to collectively enhance the phytoremediation of metal(loid) contaminants [70].

The combined treatment PLANT+BIOAUG showed high capabilities in recovering Sn and Pb contaminated soils evidencing how these bioremediation techniques (PABR and Bioaugmentation) are in line with the goals of Nature-Based Solutions and the circular economy framework promoted by the European Commission [18]. By reinforcing functional redundancy and stability, this system also supports the long-term recovery of soil ecological services [32].

5. Conclusions

This study evidenced that the combination of *L. angustifolia* and the bacterial consortium represents a promising nature-based solution (NbS) for the remediation of historically heavy metals contaminated soils. The combined treatment (PLANT+BIOAUG) achieved the most significant and apparent reduction of heavy metals, particularly Pb (reduced by 44.75%) and Sn (reduced by 66.87%). It is important to note that plant tissue analysis revealed bioaccumulation factors (BAFs) consistently lower than 1 (average BAF for Pb 0.02 in PLANT treatment and 0.01 in PLANT+BIOAUG), confirming that *L. angustifolia* acts primarily through phytostabilization rather than phytoextraction, effectively limiting metal translocation to its aerial parts. Metagenomic analyses have further clarified the mechanisms underlying this synergy. While microbial α -diversity remained stable, significant changes in β -diversity highlighted a structural reorganization of the microbial community. This dynamic indicates a robust community turnover: rather than depleting overall microbial richness, the treatment drove the substitution of sensitive taxa with specialized degrader populations. Specifically, LEfSe analysis confirmed the synergistic enrichment of functional taxa, such as *Comamonadaceae* (LDA > 4.0), in the combined treatment.

These findings emphasise the ecological and technological value of plant-assisted bioaugmentation (PABR) as a scalable, low-impact strategy for environmental restoration and soil quality improvement. *L. angustifolia* retains heavy metals in the root-soil compartment, ensuring its aerial biomass remains protected from harmful accumulation. Therefore, the implementation of this in situ bioremediation on degraded reclamation soils should be combined with controlled biomass management, particularly essential oil extraction, while also establishing clear practical guidelines for in situ bioremediation within a circular economy. To further validate and expand these findings, future research will aim to overcome the inherent limitations of this study. Although this laboratory-scale NbS shows strong potential, its applicability on a real scale needs to be tested in relation to fluctuating environmental variables and the economic feasibility of large-scale inoculum production. Additionally, while 16S rDNA-based predictions provide valuable insights into the metabolic potential of the microbial community, they are inherently limited by their reliance on reference databases and do not represent confirmed active gene expression. Future efforts will extend the monitoring period, assess the persistence of inoculated strains under natural conditions, and apply metatranscriptomic or metabolomic analyses to validate functional predictions.

CCRediT authorship contribution statement

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Visualization. **Antonio Bucci:** Writing – review & editing, Visualization, Conceptualization. **Gino Naclerio:** Writing – review & editing, Visualization. **Valeria Ancona:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Domenico De Paola:** Writing – review & editing, Visualization, Conceptualization. **Vito Felice Uricchio:** Supervision. **Cristina Cavone:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Fulvio Celico:** Writing – review & editing, Visualization.

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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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nbt.2026.05.013.

Data availability

Data will be made available on request.

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