

Article

Bacterial Communities in Informal Dump Sites: A Rich Source of Unique Diversity and Functional Potential for Bioremediation Applications

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Abstract: In this study, high-throughput metagenomic amplicon sequencing and physicochemical analyses were used to evaluate the structural composition and functional diversity of the soil bacterial communities at different illegal waste dump sites. Results showed that while the litter-free soil was dominated by the phylum *Proteobacteria*, dumpsite soils were enriched with phylum *Actinobacteria*, followed by *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Bacteroidetes*, and *Gemmatimonadetes*. Bacterial diversity differed significantly ($p > 0.05$) between the litter-free and contaminated sites, with each dumpsite having distinct genera that demonstrate the impact of waste type on the bacterial community composition. Genus *Nocardioiodes*, a versatile organic and inorganic pollutant-degrading bacteria in the class *Actinomycetia*, was dominant in the dump site soils, raising the possibility that this genus could serve as a potential biomarker for dump site soil pollution. PICRUSt functional profiling also showed the presence of genes involved in putative degradative pathways in the dump site soils. Furthermore, community-level physiological profile (CLPP) analyses revealed that the dump site soils are habitats to active bacterial communities with significant catabolic and carbon utilization capacity. Overall, this study provides a theoretical insight into the diversity and unique soil bacterial assemblages in illegal dump sites that could encode biotechnologically significant genes for biosynthesis and biodegradation.

Keywords: bacterial diversity; bioremediation; catabolic potential; heavy metals; informal/illegal dump site



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1. Introduction

Rapid global population growth and its associated anthropogenic pressure have been identified as a contributing factor to illegal dumping, which poses a serious threat to human health and natural ecosystems [1]. Toxic substances leaching from dump sites down the soil profile can cause significant degradation of the environment, threatening the taxonomic and functional diversity of soil microorganisms. From the standpoint of environmental protection and human ecology, it is critical to determine the impact of unregulated or illegal waste dumping on the soil physicochemical properties as well as on its microbial taxonomic and functional diversity [2,3]. According to Pardo et al. [4], prolonged soil pollution can affect its health directly by altering its physicochemical properties and indirectly by altering its microbial diversity and function, resulting in the alteration of its ecological function. Furthermore, pollution-induced changes in soil composition could

lead to a decrease in soil fertility [5,6]. Despite the loss of soil microbial diversity due to organic pollutants, pollution-tolerant microbes tend to flourish due to their metabolic plasticity, potentially resulting in the establishment of new microbial communities [7–9]. However, Bastida et al. [10] reported a lack of research on the microbial response in relation to inorganic and organic pollutants as well as the relationship between soil microbial communities and biotic/abiotic activities that influence their activities. Furthermore, soil microbes and their metabolic processes in a polluted environment can be better understood by investigating the interactions between soil microbes and soil components [11,12].

It has been suggested that the use of high-throughput sequencing, which reveals uncultivable microbes, can be used to compare the microbial ecology and taxonomic diversity of various soils with different types and levels of pollution [13]. This sequencing approach may be critical in identifying specific microbial biomarkers that can be used as pollution indicators as well as biotechnological applications in the development of bioremediation strategies. Jacquiod et al. [14] investigated the effects of pollutants on the diversity of microbes in relation to a multitude of other factors influencing the microbial response to various pollutants. For instance, *Proteobacteria* populations are abundant in the most hydrocarbon-polluted soil as well as coastal sediments and gold and uranium mines [15–19]. Dos Santos et al. [20] reported three oil-pollutant resistant genera: *Marinobacterium*, *Marinobacter*, and *Cycloclasticus*, and the genus *Halialia* as biomarkers in oil-polluted mangrove forest soils. Furthermore, Jeanbille et al. [21] also reported that both members of the class *Gammaproteobacteria* and *Deltaproteobacteria* dominated chronic polyaromatic hydrocarbon (PAH)-polluted coastal sediments. A recent study from Gupta et al. [22] also depicted the dominance of the phylum *Proteobacteria* and genes involved in biomolecule metabolism, aromatic compound degradation, stress tolerance, and xenobiotic biodegradation in municipal landfill soils. Furthermore, the author also proposed that microbial communities in landfill settings are far more complex than expected. Collectively, these studies provide evidence that pollution has a significant impact on the composition of soil microbial communities under different environments. However, the overarching pattern of change in microbial composition caused by pollution linked to anthropogenic activities such as the illegal dumping of wastes has not yet been fully explored.

A wide range of metabolites, from carbohydrates to proteins and lipids, are produced and consumed by microbes living in the complex environment. This results in a diverse microbial community, with cross feeding microorganisms able to take advantage of all available niches [23]. Some microbes have evolved the ability to use pollutants as carbon sources, whereas others have established toxic metal resistance mechanisms such as permeability barriers and enzymatic detoxification pathways [24]. A better understanding of the effects of pollutants on microbial communities and the utilization of various carbon sources by these microbes would allow us to assess the health and recovery of contaminated sites as well as identify potential bacterial members that can transform contaminants and could be used for bioremediation. The aim of this research was to determine the taxonomic and functional diversity of bacterial communities that have been impacted by pollution caused by a variety of anthropogenic activities at a number of different illegal dumping sites. Furthermore, the carbon substrate utilization ability of the soil bacterial communities in illegal dumping sites was also determined using Biolog Ecoplates™ based CLPP analysis to shed light on the bacterial metabolic activities in relation to various pollutants. This provides clues on the potential biomarkers to identify the types of pollutants present in the dump site environment and its application in bioremediation technologies.

2. Materials and Methods

2.1. Study Area and Sampling

The City of Johannesburg, South Africa, has over 180 informal settlements that house 16% of its population [25]. According to the City of Johannesburg, the term informal settlements refers to “an impoverished group of households who have illegally or without authority taken occupation of a parcel of land (with the land owned by the Council in the

majority of cases) and who have created a shanty town of impoverished illegal residential structures built mostly from scrap material without provision made for essential services and which may or may not have a layout that is more or less formal in nature." Due to their illegal and unplanned nature, most of the informal settlements are not serviced by municipal waste collection infrastructure. Therefore, these informal settlements are characterized by extensive littering and illegal dumping of wastes in the streets, public spaces, and vacant land [26]. In this study, soils from five illegal dump sites within the Princess informal settlement (S 26.135, E 27.850), located in the Roodepoort area, Gauteng Province, South Africa were analyzed.

The sampling sites included litter free soil (ID1), motor vehicle garage/repair shop dump (ID2), train station dump (ID3), sports ground dump (ID4), roadside dump 1 (ID5), and roadside dump 2 (ID6). The main wastes in roadside dump 2 included plastics, which were incinerated to reduce volume. Soil samples were collected from a depth of 5–15 cm using a dredge sampler (Kajak, KC-Denmark) based on a multi-point mixed sampling method. At every sampling site, 5–6 replicate samples were collected within an area of 2 × 2 m and then mixed into a composite sample. The collected soil samples were placed in clean polyethylene bags and transported immediately to the laboratory under cold storage for analysis.

2.2. Physicochemical Analysis

The soil samples were sifted through a 2 mm sieve to remove debris including stones, glass, plastic, wood, and rubber. The moisture content was calculated as a percentage weight difference between undried and oven-dried samples at 50 °C to constant weight. Soil electrical conductivity (EC) and pH values were measured using the Lovibond SD 70 conductivity meter (Lovibond Instruments Ltd, Dortmund, Germany) and the Adwa AD11 pH meter (Adwa Instruments, Szeged, Hungary), respectively. Total carbon (TC) and total nitrogen (TN) were determined using a LECO Trumac[®] Carbon, Nitrogen, and Protein Analyzer (Series 828, LECO, St. Joseph, MI, USA) fitted with a boat sampler (Series 828, LECO, MI, USA) by direct combustion of 0.2 g of the samples at 1350 °C. Following the method described by Debipersadh et al. [27], acid digestion of the samples was carried out and heavy metal concentrations measured using inductively coupled plasma-optical emission spectrometry (ICP-OES) (PerkinElmer Optima 5300 DV, Waltham, MA, USA).

2.3. DNA Extraction and Library Preparation

To dislodge bacterial cells from solid waste, 2 g of each soil sample was first mixed with 5 mL of phosphate buffered saline (PBS, pH 7.4), vortexed, and allowed to stand at room temperature for five minutes. An aliquot of 400 µL of the supernatant was used for DNA extraction using the Fecal/Soil Total DNA[™] Extraction Kit (Zymo Research Corporation, Irvine, CA, USA). The libraries of bacterial 16S rDNA V1-V3 hypervariable region gene fragments were amplified using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1429R (5'-TACGGYTACCTTGTACGACTT-3') according to the protocol described by Ogola et al. [28]. This was followed by a nested PCR using the 27F and 518R (5'-GTATTACCG CGGCTGCTGG-3') primer pairs having overhanging adapter sequences compatible with Illumina indexing and sequencing adapters. The purification of the amplified 16S rDNA gene fragments in each library was carried out using AMPure XP beads (Beckman Coulter, Agencourt Bioscience Corporation, Beverly, MA, USA) following the manufacturer's protocol. The cleaned products were subjected to a PCR (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s including initial denaturation of 95 °C for 3 min and final extension at 72 °C for 5 min) to add Illumina sequencing adapters and dual-index barcodes to each amplicon library using the full complement of Nextera XT indices (Illumina Inc., San Diego, CA, USA). The resultant barcoded PCR products were purified using AMPure XP beads, validated for fragment size (~630 bp) using a Bioanalyzer DNA 1000 chip (Agilent, Santa Clara, CA, USA), and quantified using the Qubit-HS assay (Life Technologies, Carlsbad, CA, USA), then pooled to a final DNA library before being denatured and sequenced

on an Illumina Miseq System (Illumina, San Diego, CA, USA) using paired 300-bp reads to generate high-quality, full-length reads of the V3 and V4 region. The dataset for this study was submitted to NCBI with the BioProject accession number PRJNA893411.

2.4. Bioinformatic Analysis

The fastq datasets were analyzed as previously described by Selvarajan et al. [29] using Mothur pipeline *v.1.40.0* [30]. Briefly, low quality sequence reads (nucleotides having <50 nts, >2% ambiguities and homopolymers) and mitochondrial and chloroplast sequences were excluded. The UCHIME algorithm [31] was used to remove chimeric sequences. The resultant quality-filtered sequences were then aligned against the SILVA database *version 132* [32] using the Naïve Bayesian classifier algorithm [33] at a confidence threshold of 80% to assign taxonomic identity of the bacteria. Finally, clustering of operational taxonomic units (OTUs) at 97% sequence identity was conducted using the furthest neighbor algorithm. To analyze the diversity of the bacterial OTUs, microbial community evenness indices (Shannon–Weaver and Simpson indices) and richness index (Chao 1) were calculated. The ggplot2 and heatmap.2 packages in R software (*version 3.6.1*) were used to generate stacked plots and heatmap, respectively, and to display the variations and distributions of bacterial communities found in dump site soils using dominant OTUs at the phylum, class, and genus levels.

Furthermore, to analyze the link between the microbial community distribution and dump site soil physicochemical data, canonical correspondence analysis (CCA) was conducted using the PAST software package (*version 3.2*) [34]. The phylogenetic investigation of communities by reconstruction of unobserved states (PICRUST) software package [35] was used to predict functional capabilities of the landfill bacterial communities. The bacterial OTUs were initially normalized and the prediction performed taking into account the influence of the 16S marker gene copy numbers in the species genomes and then obtaining KEGG Orthologs (KO) information and KO abundance corresponding to OTUs. To verify the accuracy of the predicted functional and metabolic pathways, the nearest sequenced taxon index (NSTI) value was used. A heat map of the predicted relative abundances of genes was generated using the heatmap.2 package in R software (*version 3.6.1*).

2.5. Assessment of Bacterial Catabolic Activities Using Biolog Ecoplates™

The Biolog Ecoplates™ were used to assess the catabolic diversity of the bacterial communities in the samples obtained. Three grams of the different soil samples were suspended in 27 mL of sterile 0.85% sodium chloride solution and vortexed for 5 min at maximum speed. After settling for 10 min, 180 µL of the supernatant was inoculated into each of the wells. All plates were sealed with parafilm on the sides and incubated at 25 °C in the dark. A VarioSkan Flash Multi Detection Microplate Reader (Thermo Fisher Scientific Inc, Waltham, MA, USA) was used to measure each well's optical density (OD at 590 nm, OD_{590nm}) at times 0, 24, 48, 72, 96, and 120 h. During the calculations, the OD were corrected by subtracting the blank well (inoculated, but without a carbon source) values from each plate well. The resultant data were used to generate growth curves that were adjusted according to the modified Gompertz equation [36], and GraphPad Prism™ software *version 8.2.1* (GraphPad Software Inc., San Diego, CA, USA) was used to determine the exponential phase as well as the corresponding incubation time and maximum absorbance. Statistical analysis of the OD values obtained at 48–120 h was carried out to determine the average well color development (AWCD), as previously described by Mendes et al. [37]. A heat map of the 31-carbon utilization patterns was generated using the heatmap.2 package in R (*version 3.6.1*).

3. Results

3.1. Physicochemical Variables

Table 1 summarizes the physicochemical and heavy metal concentrations for each dump site sample. Sample ID2 (8.09) and ID6 (8.03) had high pH values, indicating that the

dump site soils were alkaline. In contrast, other dump site locations were slightly acidic in nature (pH between 5.3 and 6.7), with ID4 being the most acidic. Furthermore, ID2 had the highest EC of 1196 $\mu\text{S}/\text{m}$, followed by the train station dump (ID3; 547 $\mu\text{S}/\text{m}$), while the litter-free zone (ID1) had the lowest EC value of 101.9 $\mu\text{S}/\text{m}$. Overall, the moisture content ranged between 13.7% (ID3) and 1.3% (ID2), while the TN and TC contents varied greatly among the dump sites. The two roadside dump samples (ID5; 9.47% and ID6; 8.9%) showed the highest values in carbon concentrations. Similarly, subtle variation in heavy metal concentrations was observed, with free-litter zone soil exhibiting higher levels of Fe, Mn, and P than the dump site samples. With a few exceptions, the majority of the heavy metal concentrations in both the free litter and dump site soils were within the recommended limits for soil by the World Health Organization (WHO). For example, ID1 and ID6 had cadmium (Cd) and lead (Pb) concentrations that were slightly higher than the recommended limits for soil. In the ID4 and ID5 samples, the chromium (Cr) and arsenic (As) contents were also higher than the permissible limits.

Table 1. Physicochemical parameters of the collected dumping site soil samples.

Parameters	ID1	ID2	ID3	ID4	ID5	ID6
Physicochemical						
pH	5.51	8.09	6.6	5.3	6.7	8.03
EC ($\mu\text{S}/\text{m}$)	101.9	1196	547	297	498	313
Moisture (%)	5.7	20.52	23.7	14.62	1.3	22.19
Total nitrogen (TN) (%)	0.13	0.176	0.295	0.16	0.345	0.125
Total carbon (TC) (%)	2.7	7.145	4.2	3.91	9.477	8.9
Heavy metals (HMs) (ppm)						
As	0.005	0.003	0.010	0.021	0.032	0.005
Cd	0.005	0.004	0.003	0.003	0.004	0.005
Pb	0.044	0.007	0.013	0.018	0.020	0.115
Mn	1.316	0.454	0.274	0.292	0.106	0.047
Co	0.010	0.011	0.012	0.029	0.010	0.010
Cu	0.145	0.064	0.102	0.099	0.062	0.074
Zn	0.067	0.110	0.089	0.077	0.098	0.175
Fe	12.863	6.545	3.185	4.094	4.712	5.221
Cr	0.072	0.053	0.101	0.100	0.094	0.073
Ni	0.002	0.025	0.038	0.036	0.035	0.030
Mg	0.982	2.300	4.500	2.682	3.911	0.446
Ba	4.116	3.911	4.401	4.386	4.220	1.305
P	15.178	1.092	0.101	4.245	0.171	0.391
S	0.013	0.012	0.016	0.013	0.015	0.013

3.2. Bacterial Diversity of Different Informal Dump Sites

Following the elimination of sequencing reads of poor quality, the number of quality sequencing reads in the collected samples ranged on average from 12,475 to 41,026 reads (Table 2). It was determined that Good's coverage of all of the samples was greater than 96%, which suggests that the sampling depth was sufficient for the vast majority of bacterial communities. After that, we determined the total number of OTUs that were found in each sample. According to the results of the rarefaction analysis, the level of diversity found in the collected soil samples was very close to being saturated. All six samples combined yielded a total of 22,125 OTUs; however, the number of OTUs obtained from each sample displayed a significant variation; in particular, samples ID1 and ID3 possessed a greater abundance of OTUs than the other samples. Bacterial richness and diversity were evaluated using the Shannon–Weaver and Simpson indices simultaneously, and the results showed that there were significant differences in richness and diversity between the various illegal dumping sites. The Shannon diversity magnitudes were as follows: ID3 (7.41) > ID1 (7.29) > ID4 (6.99) > ID6 (6.55) > ID5 (6.05) > ID2 (5.57). In addition, the Simpson indices for all samples showed infinite diversity in comparison to the other sampling locations, with IDs

1, 3, and 4 standing out as particularly diverse. According to these findings, there was a clear distinction between the bacterial diversity and richness of the various illegal dumping sites, with the litter-free zone (ID1) and the train station dumping site (ID3) exhibiting the highest levels of bacterial diversity and richness.

Table 2. Alpha diversity indices for bacterial communities in the different dump site soils.

Sample	Quality Reads	Observed OTUs	ACE	CHAO	Shannon	Simpson	Good's Coverage (%)
ID1	41,026	5658	6098	5808	7.29	0.00	98.04
ID2	21,886	2364	2700	2502	5.57	0.02	97.72
ID3	36,727	5058	5610	5308	7.41	0.00	97.56
ID4	37,906	4605	5048	4784	6.99	0.00	98.00
ID5	12,475	1595	1923	1767	6.05	0.01	96.74
ID6	22,370	2850	3364	3137	6.55	0.01	96.88

3.3. Bacterial Composition of Different Informal Dump Sites

Phylum-level analysis of the bacterial community composition revealed the presence of eight major bacterial phyla (Figure 1A). In all samples except ID1, sequences from the phylum *Actinobacteria* were the most dominant, followed by those from the phyla *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Bacteroidetes*, and *Gemmatimonadetes*. Bacterial sequences recovered from the litter-free zone (ID1) belonged to the phyla *Proteobacteria* (46.5%), *Actinobacteria* (41%), and *Chloroflexi* (4.0%). Other notable phyla include *Acidobacteria* (2.5%), *Firmicutes* (1.9%), and *Planctomycetes* (1.6%). In other samples, *Actinobacteria* was the most dominant phylum, with a relative abundance of between 42.7% in sample ID3 to 62.34% in sample ID5. The second most dominant phylum was *Proteobacteria*, whose relative abundance was 34.3%, 36.87%, 21.91%, and 24.13% in samples ID2, ID3, ID4, and ID5 respectively. Other major phyla were distributed as follows; *Firmicutes* (1.5% in sample ID6 to 18.09% in sample ID4), *Chloroflexi* (1.23% in sample ID2 to 6.53% in sample ID3), *Acidobacteria* (0.1% in sample ID5 to 2.95% in sample ID3), *Planctomycetes* (0.1% in sample ID5 to 3.49% in sample ID3), and *Gemmatimonadetes* (0.3% in sample ID2 to 1.16% in sample ID4). The distribution of the bacterial minor phyla detected in the different informal dump site soils is provided in Supplementary Table S1.

At the class level, 10 taxa were the most prevalent with discernible variations across the collected samples (Figure 1B). With the exception of ID1, unclassified *Actinobacteria* dominated all samples, with relative abundances ranging from 36.46% (ID3) to 61.55% (ID5), followed by *Alphaproteobacteria* (10.3% in ID2 and 29.65% in ID3). On the other hand, ID1 reflected the opposite trend, with *Alphaproteobacteria* (41%) being the most dominant class, followed by unclassified *Actinobacteria* (35.43%) members. A large percentage of *Gammaproteobacteria* sequences (20.68%) were recorded in ID2 compared to 1.03% reported in ID4. *Bacilli* was the fourth most abundant class, with a relative abundance ranging from 1.05% (ID6) to 17.95% (ID4), followed by *Acidimicrobiia* (0.35–5.64%), *Rubrobacteria* (0.07–3.84%), *Thermoleophilia* (0.18–3.75%), *Betaproteobacteria* (1.64–3.02%), and *Deltaproteobacteria* (0.21–2.49%). The distribution and relative abundance of minor bacterial classes in the six informal dump site soils is provided in Supplementary Table 2.

Analysis at the genus level revealed that 1268 distinct bacterial genera were present across all samples. Of these, 54 genera had 1% relative abundance in at least one sample (Figure 2), while the rest had less than 1% relative abundance and were thus classified as minor genera. Each sampling site had a distinct dominant genus. For instance, the ID1-litter free zone had *Microvirga* (11.59%), *Blastococcus* (8.72%), *Nocardioides* (4.18%), *Marmoricola* (2.53%), *Skermanella* (2.47%), and *Actinoplanes* (2.01%) as the dominant genera. The motor vehicle garage dump site (ID2) was dominated by *Pseudomonas* (17.11%), *Gordonia* (14.21%), *Williamsia* (13.16%), *Rhodococcus* (10.25%), *Nocardioides* (5.28%), and *Acinetobacter* (2.19%), whereas the train station dump site (ID3) had genus *Amaricoccus* (5.67%), *Nocardioides* (5.01%), *Nakamurella* (4.55%), *Skermanella* (3.79%), *Blastococcus* (3.12%), *Microbacterium*

(2.83%), *Paracoccus* (2.45%), and *Microtholunatus* (2.18%) as the enriched bacterial taxa. In contrast, ID4 (sports ground dump site soils) had distinct genera such as *Streptomyces* (11.42%), *Bacillus* (8.65%), *Nocardioides* (8.52%), *Geobacillus* (4.26%), *Rhodococcus* (3.52%), *Gaiella* (3.42%), *Microvirga* (2.73%), and *Anoxybacillus* (2.32%). Interestingly, samples ID5 and ID6, though from road side dump sites, exhibited different bacterial diversity. In ID5, genus *Dietzia* (10.82%) was most dominant, followed by *Microbacterium* (5.7%), *Pseudomonas* (3.55%), *Tessaracoccus* (3.47%), *Nocardioides* (3.26%), *Bacillus* (3.26%), *Rhodococcus* (3.07%), *Blastococcus* (3.05%), *Ornithinimicrobium* (2.97%), *Glutamicibacter* (2.59%), *Brachybacterium* (2.49%), *Sanguibacter* (2.46%), and *Cellulomonas* (2.33%). However, genus *Mycobacterium* (8.82%), *Blastococcus* (5.79%), *Microbacterium* (4.08%), *Gordonia* (3.59%), and *Nocardioides* (2.17%) dominated ID6 samples.

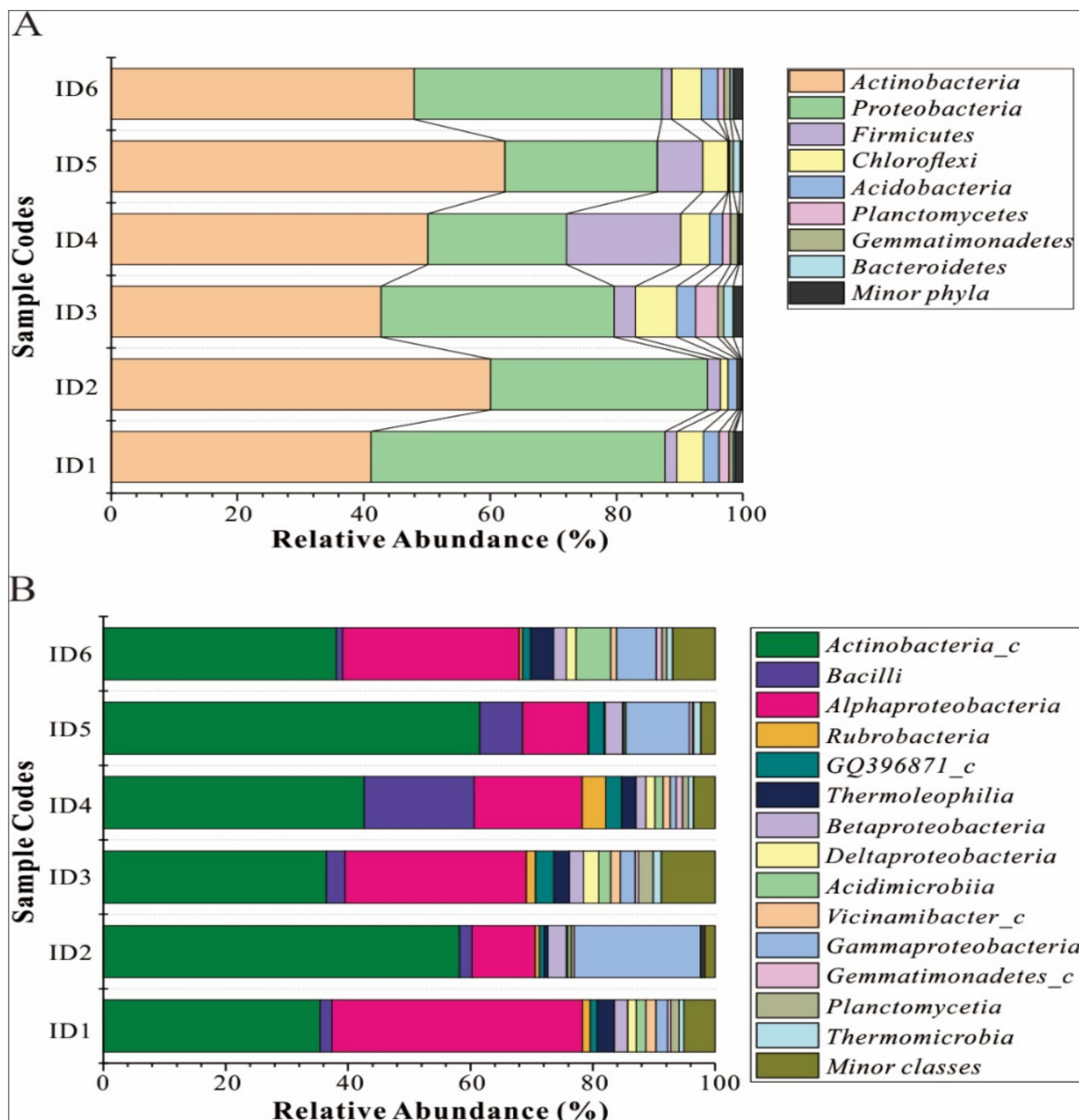


Figure 1. Relative abundance of the bacterial phyla (A) and classes (B) present in the various illegal dumping sites.

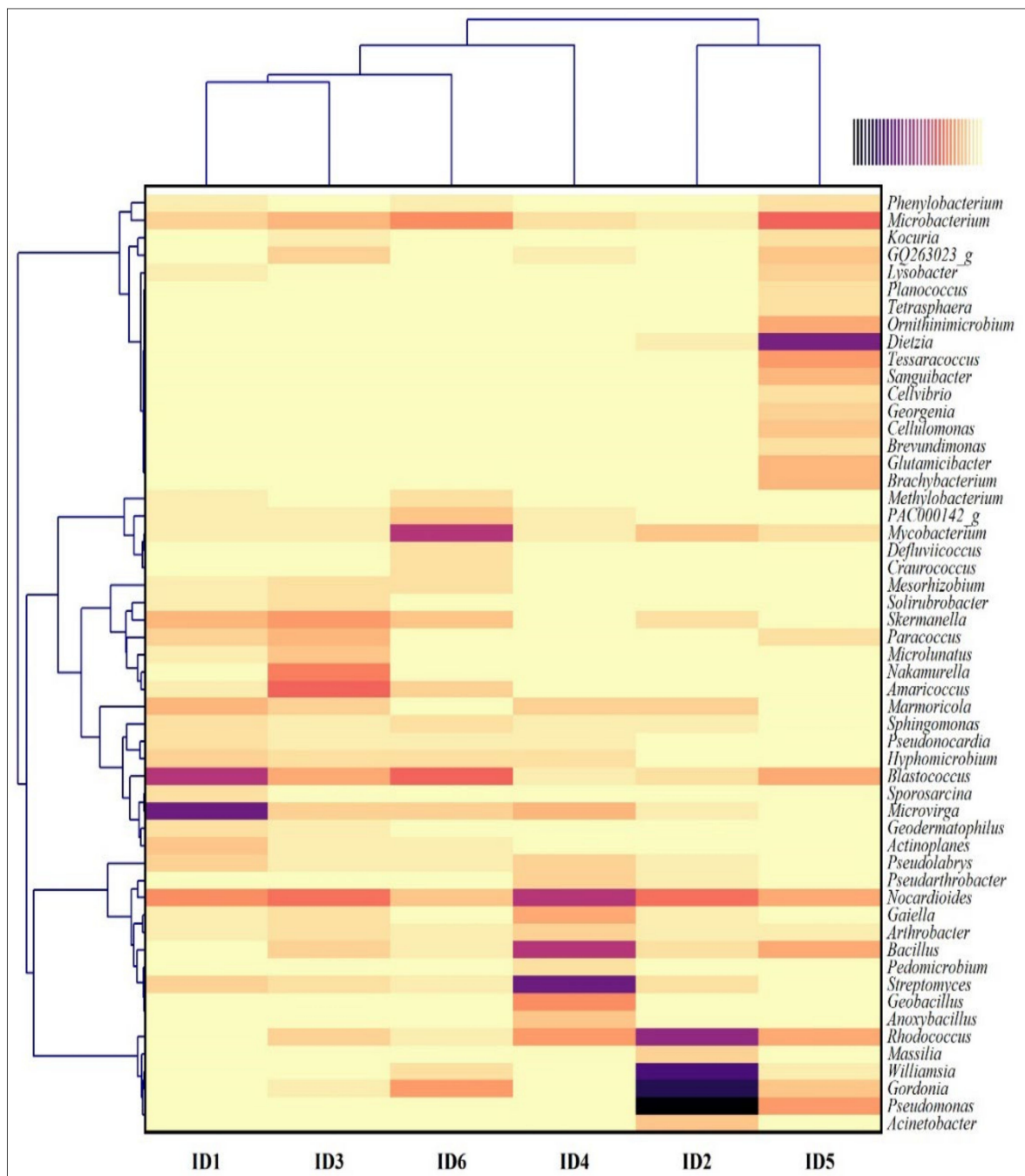


Figure 2. Heat map showing the distributive abundance of diverse bacterial genera in the illegal dumping sites.

3.4. CCA Analysis

The relationship between the bacterial communities and the environmental factors at informal dumping sites (ID2–ID6) along with ID1 (litter free zone) is depicted in Figure 3. The CCA triplot analysis showed that the abundance of *Betaproteobacteria* and *Gammaproteobacteria* (phylum *Proteobacteria*) was positively correlated with high TC and pH ($p < 0.05$). *Alphaproteobacteria*, another *Proteobacteria* class, were correlated with high Fe, Pb, and Cd concentrations. In contrast, the distribution of members of class *Actinobacteria* correlated highly with TN, while the distribution of members of the class *Thermomicrobia* and *Vicinamibacter* correlated to the high contents of Mn and Pb, respectively ($p < 0.05$) (Figure 3). Negative correlations between the distribution of *Betaproteobacteria* and *Gammaproteobacteria*

and Cu, Mn, Pb, Fe, and total phosphorus contents were also detected ($p < 0.05$). Similarly, *Actinobacteria* exhibited a negative correlation with S and Cr concentration, while *Acidimicrobiia* had a negative correlation with As concentration.

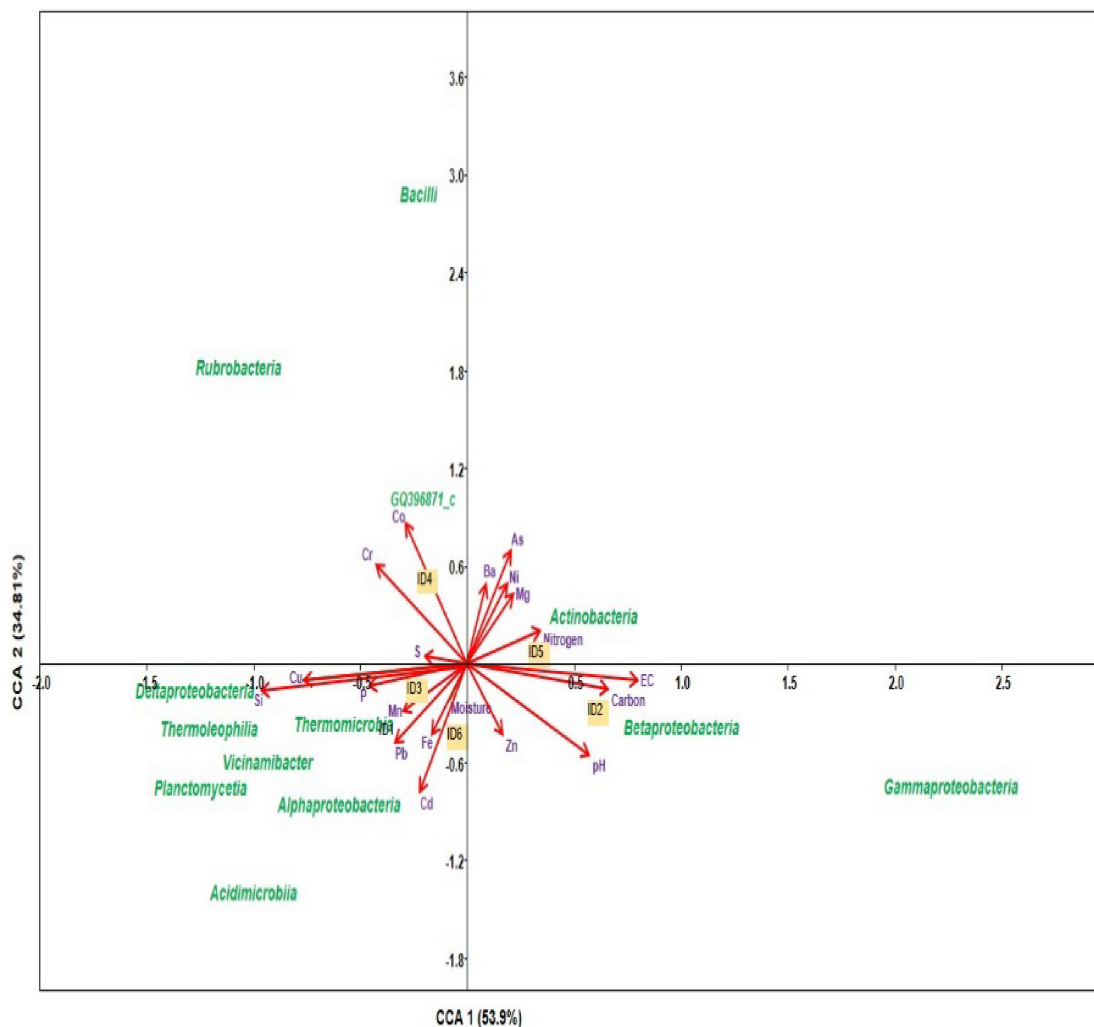


Figure 3. Canonical correspondence analysis (CCA) showing the relationship between microbial abundance and the measured environmental parameters.

3.5. PICRUSt Predictive Function Profiling

The study also performed PICRUSt profiling to complement the phylogenetic insights for the prediction of functional potentials associated with the dump site soil microbial community. Results revealed the existence of putative biosynthetic and degradative pathways across all six of the sample locations (Figure 4). The lipid biosynthesis genes, amino acid biosynthesis genes, and terpenoid biosynthesis genes were found in relatively higher abundance. Furthermore, genes responsible for xenobiotic aromatic compounds such as dioxin, xylene, toluene, aminobenzoate, nitrotoluene, ethyl benzene, bisphenol, cyclohexane, benzoate, flouorbenzoate, and polyaromatic degradation genes were enriched in the dump site samples (Figure 4). It should also be noted that other pathways of interest such as bacterial chemotaxis, bacterial motility, carbon fixation, electron transfer, germination, lipid metabolism, methane metabolism, plant–pathogen interactions, protein exports and kinases, secretion systems, signal transduction, sporulation, and transcription machineries were also abundant (data not shown in the figure).

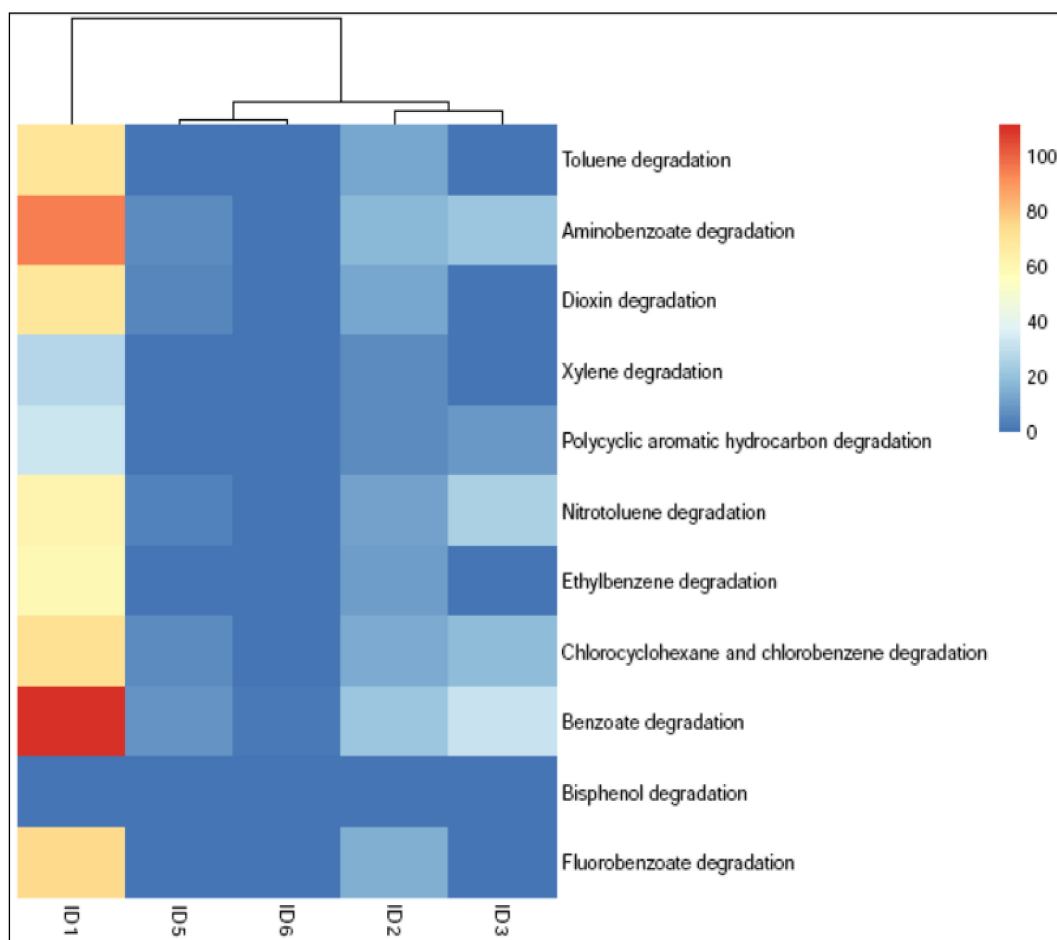


Figure 4. Predicted functional degradative genes in bacterial communities identified across informal dump site soil samples.

3.6. Catabolic Activity of Soil Bacterial Community in the Dumping Sites

The ability of bacterial consortiums in the soil of illegal dump sites to utilize a set of 31 carbon substrates was evaluated to gain insight into their in situ metabolic potential. Based on the CLPP metabolic fingerprints results, bacterial communities in the soil of informal dump sites demonstrated their ability to utilize a wide variety of organic substrates as carbon sources. Figure 5A depicts the progressive growth of bacterial communities over time as measured by the average amount of well color change at OD590 nm. Overall, the graph of the AWCD versus time exhibited a classical sigmoidal growth curve with the exponential phase of bacterial community growth observed between 48 and 120 h. However, growth was observed to be increasing over time for IDs 1, 2, 3, 4, and 6, but was limited for ID 5. Figure 5B shows the outcomes of profiling the carbon utilization of the bacterial consortia, which revealed that the utilization of different carbon sources varied between sampling locations. A total of 18 substrates out of 31 recorded a relative substrate utility greater than 3% for the car fix dump site samples (ID2) and train station dump soils (ID3), which was higher than that of ID1 (9) and ID4 (7) collected from the litter free zone and sports ground dumpsite soils, respectively. Soil sample ID6 collected from a roadside dump site showed 13 substrates utilized, whereas soil sample ID5 collected from a different roadside dump site showed no significant utilization on the given substrates. Notably some of the substrates were highly utilized, for instance, galacturonic acid, Gly_Glu, and Tween_80 (in ID1); methylpyruvate, beta_Methyl_D_Glucoside, D_Galactonic_acid_gama_lactone, and sodium_salicylate (in

ID2); L_Threonine, alpha_Cyclodextrin, Glycogen, and L_Phenylalanine (in ID3); L_Serine (in ID4); meso_Erythritol and D_Malic_acid (in ID6).

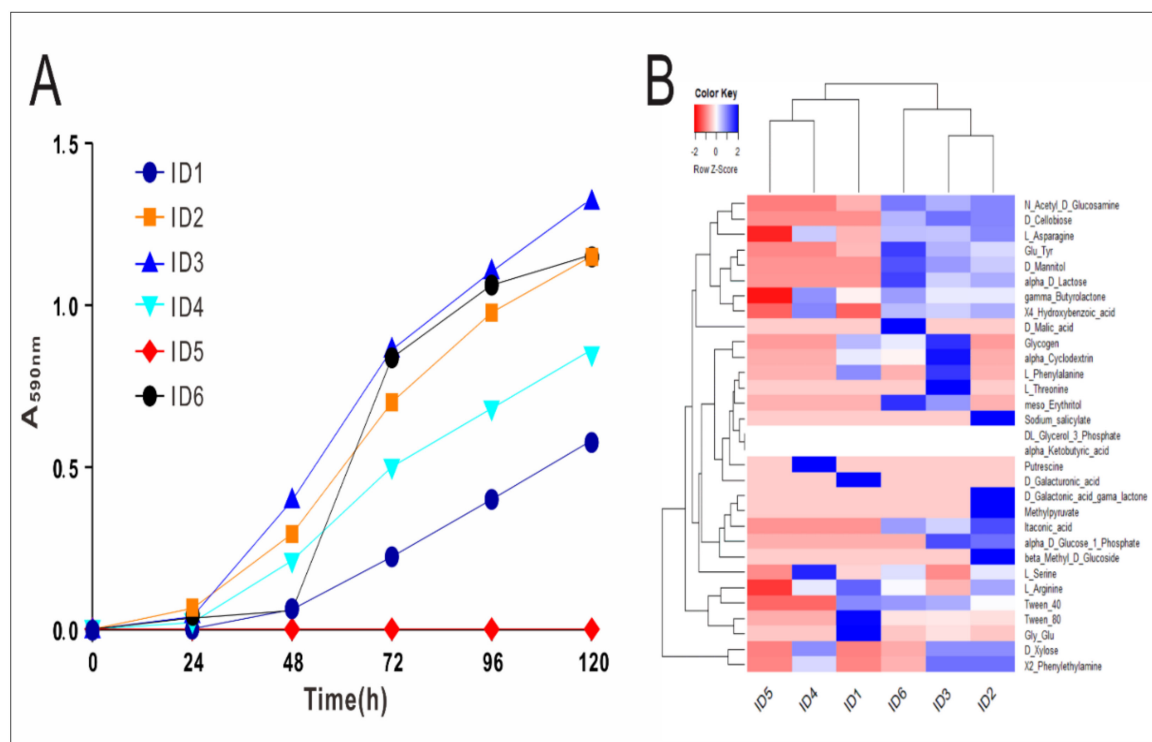


Figure 5. Community-level patterns of the different dump site soil bacterial community carbon metabolism. (A) Average well color development of the BIOLOG Ecoplate indicating the catabolic activity progression of the bacterial consortia in the different dumping sites over time. (B) Heat map showing the diverse bacterial catabolic activities against different substrates in the illegal dumping sites.

4. Discussion

Africa's rapid population growth, expanding economic activity, and ever-increasing urbanization have resulted in unprecedented waste material accumulation. Consequently, the number of potentially hazardous waste material sites has increased, despite the fact that the level of waste pollution caused by these sites has reached an emergency level across the African continent. However, to achieve the African Union's Agenda 2063: "The Africa We Want", Africa must pursue sustainable waste management approaches to ensure appropriate environmental preservation and to prevent further pollution. South Africa generates about 48 million tons of hazardous waste annually, and about 94% of that will eventually end up in dump sites [38]. According to the literature, dump sites represent an urban landscape with a distinct ecology and environmental conditions [39,40]. Dump site soils, for example, may form novel ecosystems with novel biodiversity composition, biogeochemistry, and ecological functions [41–43], which may be exploited for various biotechnological applications. However, the microbial and functional diversity of informal dumping sites is still poorly understood. As a result, we used a high-throughput sequencing approach to investigate the microbial community that is associated with informal dump waste soils from a variety of dumping sites in Johannesburg as well as its potential for functional applications.

Table 1 displays detailed data on the physicochemical properties of soil collected from various solid waste dump sites. It is important to note that the chemical profiles cannot be compared to those of long-term landfills because the samples studied were not taken from a landfill that has been operational for decades. It is our view, however, that the chemical and biological profiles of soils near these informal dumps are still significantly influenced. The

samples ID1 and ID4 in this study were slightly acidic in nature, whereas samples ID2 and ID6 were alkaline. Changes in pH might be caused by a variety of different types of waste being dumped. For example, ID2 dumps had more automobile-related products, which could raise the pH; however, soil pH also has a significant impact on the bioavailability of HMs in various media and their subsequent toxic effects on biota [44]. Furthermore, soil pH is one of the key factors influencing soil microbial abundance. Consistent with the findings by Wakelin et al. [45], our study found that the diversity of bacterial communities was profoundly affected by the soil pH. For example, samples ID2 and ID6 displayed high pH with low bacterial diversity compared to other samples. Microbial activity may be controlled by moisture in a variety of settings including salt water, food, wood, biofilms, and soils [46]. This study also demonstrates that sample ID5 had a very low moisture content (Table 1), implying that microbial activity during substrate utilization was low in comparison to the other samples (Figure 5A). CCA analysis of this study also further confirmed that the moisture content influences the bacterial communities of dump site soils (Figure 3), and it is well-known that soil moisture plays a key role in regulating the microbial population and its activity by influencing the redox potential, pH, oxygen, and CO₂ levels in soil [47]. In addition, the CCA results also corroborate the findings of a recent study showing that the majority of bacterial classes exhibit high resistance to a wide range of heavy metals [48].

Microorganisms including bacteria and archaea that have potential for extensive bioremediation activities are frequently found in landfills and/or dump sites [49,50]. Bacterial taxonomic distributions in this study revealed that the most dominant phyla across the samples were *Actinobacteria*, *Proteobacteria*, and *Firmicutes*. Numerous studies have found that the phyla *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* are prevalent in anaerobic ecosystems such as aquifer sediment [51], river sediment [52], and wastewater bioreactor [53] as well as landfills [54]. Furthermore, some studies have indicated the dominant presence of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* in various maturing landfills [50,55–57]; however, in the present study, we identified phylum *Actinobacteria* as the most dominant from the collected dump site soils, with the exception of ID1 (Figure 1A). This could indicate that most informal dump sites are not mature enough to have *Proteobacteria* or *Firmicutes* dominate over *Actinobacteria*. In addition, the breakdown of organic matter, which is a part of carbon turnover, is the primary activity of *Actinobacteria* [54,58]. Because dump sites receive waste from a wide range of sources, the amount of organic matter in the soil could be a factor in their presence in informal dumping soil. Not surprisingly, *Actinobacteria* are also important in the recycling of refractory biomaterials by decomposing complex polymer mixtures in dead plants and animals as well as fungal materials [59]. Next to *Actinomycetes*, the phylum *Proteobacteria* was the most abundant in all samples, suggesting that *Proteobacteria* may play a pivotal role in the degradation and assimilation of both organic and inorganic substances respectively, including heavy metal contaminants in dump site soil [49]. Dump site soils also harbored *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Bacteroidetes*, and *Gemmatimonadetes*, among other bacterial phyla. *Chloroflexi* bacterial members are assumed to play a major role in the environment by fermenting carbohydrates and degrading other complex polymeric organic compounds to low molecular weight substrates, which are used by other bacteria for growth [60]. *Bacteroidetes* are gaining attention for use in bioconversion industries along with *Actinobacteria*, *Firmicutes*, and *Proteobacteria* because they grow quickly on laboratory media and biodegrade biomass efficiently [61]. Phylum *Gemmatimonadetes* only has one described member, *Gemmatimonas aurantiacus*, a Gram-negative aerobic heterotroph isolated from sewage [62]. Previous studies have found the highest relative abundance of *Gemmatimonadetes* near neutral pH [63,64], which is consistent with our findings. Nonetheless, there is still much to understand about the diversity of this group.

The genus level analysis revealed that each sample had a distinct dominant genus. For example, the *Alphaproteobacteria* genus *Microvirga*, found predominately in soil sample ID1, has the ability to oxidize arsenite [65]. Similarly, the genus *Actinoplanes* is native to a wide range of habitats and is thought to play a role in the turnover of chitin, cellulose,

and lignin [66]. Other genus including *Skermanella* and *Marmoricola* from sample ID1 have demonstrated high resistance to heavy metals [67], but their biotechnological potential remains elusive. It was not surprising that *Pseudomonas* dominated the sample collected from the automobile dumps (ID2), which is consistent with other studies [68,69]. It is reasonable to hypothesize that the *Pseudomonas sp.* from these locations could be an essential component in the bioremediation of environmental settings that have been contaminated by oil and grease. *Rhodococcus* and *Nocardioideis* were the most common genera in the ID2 sample, followed by *Gordonia*. All of these members are phylogenetically close relatives, and their ability to degrade environmental pollutants has recently attracted greater interest from the biotechnology industry [70]. The soil sample from the train station dump site (ID3) was dominated by *Amaricoccus*, which had previously been detected in the activated sludge system [71] and was found to contribute to sulfamethoxazole biodegradation. Recent research has also shown that they are the primary degrader of Triclosan (5-Chloro-2-(2,4-dichlorophenoxy) phenol, TCS), an active component found in many common personal care products such as soaps, shampoos, and toothpastes [72]. Other dominant members of the respective samples, ID4, ID5, and ID6 were also reported in various landfill soils [50, 54,56,73,74], demonstrating its important ecological function. Intriguingly, *Nocardioideis* (Figure 2), a significant genus in *Actinomycetes*, was present in all samples used in this study. This genus was previously reported to be the dominant member of Dominican amber and Israeli amber preserved at about 120 million years ago [75]. This genus has the potential to withstand the exposure to toxic heavy metals and is involved in the breakdown of hexachlorobenzene (HCB) and pentachlorophenol (PCP) [76]. Findings from this study suggest the idea that this potentially contributes to biodegradation and thus could be an important bioinoculant for landfill pollutant sequestration.

Functional prediction analysis revealed the presence of putative degradative pathways in all dump site locations (Figure 4). To our knowledge, this is the first study to investigate functional genes from informal dump sites, whereas many studies have been reported in active or abandoned landfills that have matured for many years. However, our findings are quite consistent with those reported by others [50,74,77]. A possible explanation is due to the fact that long-term landfills host higher bacterial diversity than short-term informal dump sites, hence an enrichment of several functional genes cannot be expected as in the other landfill soils. In addition to function prediction, 31 different carbon substrates were used in this study to assess the active utilization and metabolic potential of the bacterial members residing in the dump site soils (Figure 5B). Sample ID5 showed no significant utilization on the given substrates because, as previously discussed, this sample had very low moisture content, which significantly affected the active microbial populations compared to other samples. Both the functional prediction analysis and the active utilization study found evidence supporting the idea that the dump site soils host active bacterial communities with significant degradative roles. A similar observation has previously been reported in heavy metal-contaminated soil, demonstrating that bacterial consortia have the ability to metabolize more complex substrates such as polymers [78]. Although the substrate utilization tends to vary between collected samples, the bacterial communities present in dump site soils clearly demonstrate that they have potential in the bioremediation of toxic contaminants and other biotechnological applications.

5. Conclusions

In conclusion, this study used next-generation sequencing technology to investigate bacterial communities in samples collected from a variety of informal dump sites impacted by a wide range of contaminant sources. Although the bacterial diversity between the contaminated sites did not differ significantly, each site had distinct genera, demonstrating its important ecological function. It has been shown that native microorganisms in polluted environments can efficiently and tenaciously detoxify their surroundings. Our study also showed that the dominant members of each dump site soil have the ability to break down complex polymers and other organic molecules, which was further confirmed by their

catabolic potential using different substrates as the sole carbon source. Notably, we observed that, despite numerous studies implying *Proteobacteria* to be the most metabolically versatile and thus abundant bacterial phyla in various landfills and other polluted environments, other bacterial phyla such as *Actinobacteria* also exhibit remarkable resilience to thrive in harsh environments. Finally, we reasonably concluded that microbial assemblages obtained from contaminated soils such as informal dump sites are a potential reservoir of bio-active important genes and could be used for in situ bioremediation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app122412862/s1>, Table S1: Distribution of Minor phyla obtained from informal dumpsites. Table S2: Distribution of Minor classes obtained from informal dumpsites.

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