

Review Article

# Microbial Diversity in Landfills through Metagenomics Profiling: A Review

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**Abstract**— Microbial communities are play a crucial role in the breakdown of organic waste, the production of biogas, and the reduction of environmental impact at landfills, which are complex ecosystems more than just places to dispose of waste. This study provides a comprehensive overview of the current knowledge of microbial diversity in landfills based on metagenomics studies. Metagenomics studies show that various microorganisms, including bacteria, archaea, and fungi, are found in landfills and interact to break down waste. Environmental factors such as pH, temperature, and waste composition impact these microbial communities. This review investigate how landfill age, waste composition, and operational methods impact microbial communities. Important microbial group functions in the waste breakdown, greenhouse gas emission, and soil leachate composition are also highlighted in this paper. The review examine how technologies, such as bioinformatics tools and high-throughput sequencing, enhance our knowledge of the landfill microbiome. Through metagenomics, researchers can identify the microbial genes responsible for important activities, including pollution bioremediation, production of methane, and organic matter breakdown. High-throughput sequencing methods such as 16S rRNA sequencing and shotgun metagenomics have improved the current understanding of landfill microbiomes by identifying new microbes with specialized metabolic roles. Identifying the plastic-degrading microbes and their enzymes is an emerging area of interest with potential for that may help develop biotechnological solutions to landfill plastic pollution. Metagenomics provides the opportunity to develop sustainable landfill management techniques and microbial-based waste treatment methods by identifying these microbial processes. This review emphasis the need of using the microbial processes for a better waste management and the significance of metagenomics in optimising landfill operations.

**Keywords**— Microbial diversity; Landfill, Metagenomics, Bioinformatics, Whole-Genome Shotgun (WGS)

## I. INTRODUCTION

A landfill is a site for the disposal of waste material by burying it in the ground [1]. Landfills are more than just sites to dispose of waste, as they function as regulated biological reactors where organic waste breaks down due to microbial activity. Microorganisms in landfills drive important

biochemical processes that help in the breakdown of waste [2]. Bacteria play a key role in separating complex organic matter into simpler compounds. At the same time, fungi and protozoa contribute to contaminant degradation [4]. This process reduces waste volume, minimizes environmental impact by lowering greenhouse gas emissions and toxic leachate, and generates

valuable byproducts such as biogas via anaerobic digestion [4]. Studying microbial communities in landfills through metagenomics provides valuable insights into their composition, function, and potential applications in waste management. Using the metagenomic analysis, researchers can identify bacterial taxa present in landfills and infer their ecological functions.

Metagenomics has revolutionized the study of microbial diversity by enabling the analysis of entire microbial communities without the need for cultivation [5]. Many microorganisms are unable to be grown using traditional lab culturing techniques. Metagenomics overcome this limitation by enabling researchers to analyse total genetic material directly from environmental sample. It provides a more comprehensive view of microorganisms in complex environments like landfills. This approach involves extracting DNA from environmental samples and sequencing it to determine microbial composition and functional potential [6]. By revealing the genes and pathways involved in key ecological processes, metagenomics enhances our understanding of landfill microbiomes and their role in waste degradation [7]. Metagenomics data provides insight into the functional roles of landfill associated microbes. For example, researchers can better understand the ecological roles of microbial communities in landfills by identifying the genes that break down organic waste or produce methane. High-throughput sequencing techniques overcome the limitations of traditional culture-dependent methods, providing deeper insights into microbial diversity and metabolic functions [8].

This review aims to provide a comprehensive overview of microbial diversity in landfills using metagenomic approaches. It explores current knowledge on landfill microbial communities, their ecological roles, and the factors influencing their composition. Additionally, it highlights the potential of metagenomics in advancing sustainable waste management practices. Finally, it discusses future research directions to improve landfill management through microbial insights.

## II. IMPACT OF ENVIRONMENTAL FACTORS ON MICROBIAL COMMUNITY STRUCTURE

Metagenomic research on landfill microbiomes has implications for the environmental preservation and management of waste. Harnessing microbial diversity can improve biogas yields, facilitate pollutant degradation, and mitigate greenhouse gas emissions from landfill systems. Integrating metagenomic insights with biotechnological strategies can potentially advance sustainable landfill management practice.

Various environmental factors such as pH, temperature, moisture content, heavy metal concentration, oxygen availability, and waste composition can influence the structure of the microbial population in the landfill [9-11]. These factors affect the microbial diversity, abundance, and metabolic processes that can break down waste, produce methane, and degrade pollutants. Consequently, improving bioremediation procedures and optimizing landfill management requires comprehending how these environmental factors impact microbial communities [11].

Temperature differences significantly affect the metabolic activity and microbial diversity in landfills. Note that the different landfill layers are dominated by mesophilic (20-45°C)

and thermophilic (>45°C) microbial populations, with thermophilic bacteria and archaea being essential to the faster decomposition of the organic material at higher temperatures [12, 13]. Microbial communities are further differentiated by oxygen availability, where the anaerobic bacteria, such as *Clostridium*, and methanogenic archaea, such as *Methanosarcina* and *Methanobacterium*, flourish in a deeper, oxygen-depleted layer, while aerobic bacteria, such as *Pseudomonas* and *Bacillus*, dominate the top layers [14, 15]. Moreover, pH changes in the waste sites impact enzymatic activity and microbial growth. Neutral to slightly alkaline conditions with pH 6.5 to 8.5 can enhance microbial variety and metabolic efficiency. In contrast, acidic conditions with a low pH may inhibit bacteria activity [16]. Since microbial activity is dependent on the availability of water, the moisture content is another important consideration. The increase in moisture speeds up the breakdown of organic waste, thus promoting the formation of leachate and the microbial-mediated biogeochemical cycling process [17]. However, excessive moisture in landfills, often greater than 70%, can promote anaerobic conditions, causing microbial communities shift towards methanogenic and fermentative pathways. According to studies, when moisture in waste is more than 70%, methanogens rapidly appear and methane production increases as a result. Production of methane jumps at 80% moisture and increase even more with moisture level above 90% [18]

## III. RICHNESS AND DIVERSITY OF MICROBIAL COMMUNITY IN LANDFILLS

The biogeochemical cycling of essential elements and the breakdown of organic waste are greatly assisted by various microbial communities found in landfills. Metagenomic investigation reveal high microbial diversity in landfills with substantial richness and evenness among fungi, archaea, and bacteria each contributing to various phases of waste degradation [19]. Bacterial populations are the most dominant and important in decomposing organic materials. At the phylum level, landfills usually contain a variety of microbial communities dominated by *Proteobacteria*, *Firmicutes*, *Bacteroidota*, and less well-known phyla like *Patescibacteria* [20]. Microbial diversity discovered in the landfills varies depending on the condition of the environment. *Pseudomonas* and *Bacillus* are the major aerobic bacteria in aerobic zones, which promote the breakdown of organic matter [21, 22].

On the other hand, the facultative and anaerobic bacteria that promote fermentation and methanogenesis, such as *Clostridium* and *Methanosarcina*, are more common in the anaerobic zones [21, 23]. Methanogenic archaea converts acetate and hydrogen into methane, especially methanogens from the phylum *Euryarchaeota*, which helps in the last phase of anaerobic decomposition [24]. *Aspergillus*, *Penicillium*, and *Trichoderma* species are fungal communities that help break down resistant organic compounds [25].

Various microorganisms are found in the landfills, including specialized and unique taxa adapted to harsh and changing environmental conditions. These microorganisms are essential for the detoxification of pollutants, the breakdown of organic matter, and biogas production. A novel microbial species with unique metabolic capabilities that support the functioning of the landfill ecosystem has been identified by metagenomic research [26-28].

Table 1 presents the microbial distribution across the various landfill depth zones, highlighting these microorganisms' environmental factors, dominant taxa, and degrading activities. The landfills have five depth zones with microbial populations and environmental characteristics. The composition of the microbial community varied by zone, with some taxa uniquely abundant or restricted to specific areas. In contrast, the composition of other zones was more varied or unclear [29]. As soil depth increases, environmental conditions change, resulting in primarily anaerobic conditions on the deeper layer, whereas the surface stays aerobic. The covered soil, which is

exposed to atmospheric oxygen, supports the methane-oxidizing microbes like *Methalohalobius*, which play an important role in minimizing landfill methane levels [29]. Microbial populations help in the first stage of decomposition in the top waste layer, which has both aerobic and anaerobic conditions. The deeper layer, especially the middle and deep waste zones, becomes completely anaerobic, thus allowing methanogens like *Syntrophomonas* and *Fastidiosipila* to promote methanogenesis along with the complex polymer breakdown [29]. Meanwhile, leachate supports different

Table 1. Spatial And Environmental Distribution Of Microbial Communities In Landfills

Depth Zone	Dominant Taxa	Environmental Conditions	Degradation activity	Source
Cover soil (0-30 cm)	<i>Methylohalobius</i>	Potentially aerobic, exposed to atmosphere	Methane oxidation	Wang et al. 2017 [29]
Upper waste layer (30-90 cm)	Varied	Transition zone, mixed aerobic/anaerobic	Diverse, including an initial waste breakdown	Wang et al. 2017 [29]
Middle waste layer (90-150 cm)	<i>Syntrophomonas</i> , <i>Fastidiosipila</i>	Predominantly anaerobic	Methanogenesis, complex polymer degradation	Wang et al. 2017 [29]
Deep waste layer (>150 cm)	-	Anaerobic, potentially high pressure	Long-term anaerobic degradation	Wang et al. 2017 [29]
Leachate	<i>Epsilonproteobacteria</i> , <i>Gammaproteobacteria</i> , <i>Clostridia</i>	Liquid phase, variable chemistry	Dissolved organic matter degradation	Stamps et al. 2016 [30]
Plastic waste layer	<i>Ideonella sakaiensis</i>	Aerobic, enriched with PET degradation products	PET biodegradation and valorization	Han et al., 2022 [31]
Trade waste layer	Mixed microbial consortia	Variable, depending on waste composition	Biodegradation of mixed plastics	Elliott et al., 2024 [32]
Leachate treatment zone	<i>Pseudomonas spp</i>	Anaerobic or bioaugmented conditions	Bioremediation of organic pollutants	Mendoza-Burguete et al., 2023 [33]
Organic waste layer	Fungal/bacterial cellulases	Predominantly anaerobic	Cellulose hydrolysis and lignin decomposition	Chander et al., 2023 [34]
Landfill surface	<i>Bradyrhizobiaceae</i> , <i>Pseudarthrobacter</i>	Aerobic, exposed to the atmosphere	Plastic degradation, xenobiotic metabolisms	Yeon et al., 2022 [35]

microbial species, such as *Clostridia*, *Gammaproteobacteria*, and *Epsilonproteobacteria*, that assist in the breakdown of dissolved organic waste [30].

Beyond the general depth zones, various waste layers have different microbial communities adapted to the chemical and physical properties of their substrates. In PET-rich zones, *Ideonella sakaiensis*, which produce the enzymes PETase and MHETase, helps in the enzymatic depolymerisation of polyethylene terephthalate into monomeric components such as terephthalic acid and ethylene glycol, hence enhancing plastic biodegradation [31]. Mixed microbial consortia dominate in the trade waste layer, which can be identified by its diverse waste composition and varying environmental conditions. These include taxa that can degrade a wide range of synthetic polymers and industrial wastes, as well as those that participate in the biodegradation of mixed plastics [32]. *Pseudomonas spp*. Dominate the leachate treatment zone, where bioremediation techniques are frequently used. They are well-known for their ability to break down organic pollutants in anaerobic or

bioaugmented conditions [33]. Aerobic conditions on the landfill surface promote the development of microbial taxa like *Bradyrhizobiaceae* and *Pseudarthrobacter*, which play important roles in the degradation of plastics and xenobiotic metabolism [35]. These microbial activities reveal the vertical and functional differentiation of the microbial communities in the landfill, which is influenced by environmental factors like waste composition, pressure, and oxygen availability.

Although most bacteria belong to the *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* genera, some unique genera have been observed to have particular waste-degrading abilities. For example, *Anaerolinea* and *Clostridium* are involved in the fermentation of complex organic compounds, generating intermediates for methanogenic archaea [36]. Furthermore, landfill soils often contain *Acidobacteria* and *Gemmatimonadetes*, indicating that these microorganisms have adapted to anaerobic and nutrient-limited environments [37]. These specialized microbial populations contribute to our knowledge of the landfill ecosystems. They

may have been used in waste-to-energy conversion, bioremediation, and sustainable waste management. Novel microbial species and metabolic pathways are being discovered through metagenomic research, which may enhance landfill operations and environmental sustainability.

The type of waste dumped there influences microbial diversity in the landfill. Hydrolytic and fermentative bacteria like *Firmicutes* and *Bacteroidetes*, which can break down proteins, lipids, and carbohydrates, are supported by organic-rich waste [38]. Furthermore, waste containing much plastic might have specific microorganisms that can break down plastic, such as *Pseudomonas* and *Ideonella*, which create enzymes like PETase to break down synthetic polymers [39]. Compounds containing nitrogen, phosphorus, and sulphur also influence the composition of the microbial communities, which impact the processes involved in the cycling of nitrogen and sulphur [40, 41]. Heavy metals and other hazardous substances are frequently found in landfills. They may cause selective pressure on the microbial communities. Specific bacteria, such as *Acidobacteria* and *Actinobacteria*, can contribute to bioremediation by detoxifying pollutants. They also exhibit a strong resistance to metals. However, too much heavy metal buildup can be harmful, thus decreasing enzyme efficiency and microbial diversity [42, 43].

#### IV. COMPARATIVE ANALYSIS OF BACTERIAL DIVERSITY IN FOREST AND LANDFILL SOILS

Environmental elements such as nutrient availability, pH, organic matter composition, and moisture content significantly impact the microbial diversity in the soil ecosystems. Both forest and landfill soils harbour diverse bacterial communities, however, their taxonomic composition and ecological functions differ substantially due to contrasting environmental conditions. The diversity of bacteria in various environments has been more fully understood due to the metagenomic research [44].

The decomposition of organic matter, nutrient cycling, and the interactions between microbes and plants are greatly aided by the stable microbial population found in forest soil. *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Bacteroidetes* are usually the most dominant bacterial phyla in forest soils [45]. These bacteria help to maintain vital biogeochemical cycles by breaking down organic materials and driving key processes like nitrogen and carbon cycling. For example, *Proteobacteria* are typically involved in nitrogen fixation and denitrification, whereas *Actinobacteria* are important in the breakdown of complex chemical compounds and humic substances. On the other hand, landfill soil is a highly dynamic and harsh environment. Anaerobic conditions and waste content greatly influence the microbial community in landfill soils, favouring bacterial taxa involved in pollutant breakdown, fermentation, and methane production [3].

The composition of microbial communities in forest soils and landfills varies depending on environmental factors such as leachate composition, oxygen availability, and organic matter content. Table 2 compares the abundant microbial phyla across various locations categorized by the sample type.

Table 2. Most Abundant Phyla In Different Landfill Environments

Type of sample	Location	Most abundant phyla	Source
Leachate	Municipal landfills in the United States	<i>Bacteroidetes</i>	Stamps et al., 2016 [30]
Soil	Tropical Botanical Garden in China	<i>Annelida</i> (earthworm)	Singh et al., 2019 [45]
Leachate	Salt River Landfill in Scottsdale	<i>Euryarchaeota</i>	Reynolds et al., 2022 [46]
Soil	Forest-steppe zone in Siberia	<i>Proteobacteria</i>	Naumova et al., 2021[47]
Soil	Cool-Temperate Montane Forests in Japan	<i>Proteobacteria</i>	Shigyo et al., 2019 [48]
Soil	Tropical Forest soil in Brazil	<i>Proteobacteria</i>	Tamburini et al., 2020 [49]
Soil	Temperate Urban Forest in China	<i>Actinobacteriota</i>	Fu et al., 2022 [50]
Soil	Landfill in China	<i>Proteobacteria</i>	Wan et al., 2021 [51]
Soil	Forest in Chile	<i>Proteobacteria</i>	Quinteros-Urquieta et al., 2024 [52]
Leachate and Soil	Municipal landfill in Canada	<i>Patescibacteria</i>	Sauk & Hug, 2022 [53]

*Bacteroidetes*, *Euryarchaeota*, *Proteobacteria*, and *Patescibacteria* are the most abundant in landfill environments, especially in soil and leachate samples. These phyla are important for the breakdown of the organic matter, methane production, and anaerobic degradation. The ability of the *Bacteroidetes* to decompose complex organic materials is well known, and it helps in the initial stages of waste degradation. *Euryarchaeota*, which are mostly made up of methanogenic archaea, help produce methane in anaerobic landfill conditions, an essential step in biogas production. The recently discovered phylum *Patescibacteria* promotes the breakdown of organic waste in landfill habitats [30, 47, 52, 54]. These microbial populations stabilize landfills by turning organic waste into simpler compounds, detoxifying waste in landfill leachate and managed the release of greenhouse gases through methane oxidation and carbon dioxide fixation. Across all climate zones, including the tropical, temperate, and montane forests, *Proteobacteria* and *Actinobacteriota* are the most abundant phyla in forest soils. For example, *Proteobacteria* were most common in forest soils from Siberia [47], Japan [48], Brazil [49], Chile [52], and demonstrating their ecological resilience and flexibility to changing climatic conditions. The breakdown of resistant organic substances such as cellulose and lignin can make *Actinobacteriota* especially important for nitrogen cycling in natural and landfill-affected soil. These phyla have a role in important soil processes such as symbiosis, organic matter breakdown, and nitrogen fixation. Interactions between microbial communities and soil macrofauna, such as *Annelida* (earthworm), enhance nutrient cycling and soil structure, thereby improving microbial habitat quality [46, 49, 51, 55]. These interactions between the microbes show how the native soil communities and landfill microbiomes improve environmental sustainability by enhancing soil health and promoting waste breakdown. The contrasting bacterial

communities in natural and landfill environment reflect functional specialization, where native soils support nutrient cycling and biodiversity, whereas landfill microbiomes are adapted for pollutant degradation and organic waste conversion, all of which contribute to overall ecosystem resilience.

## V. APPLICATION OF METAGENOMICS IN LANDFILL MICROBIOMES STUDIES

Metagenomics studies genetic materials obtained directly from environmental samples without the need to isolate and culture single microbes. This technique has significantly advanced the study of waste microbiomes, showing the important functions in bioremediation, waste decomposition, and cycling of nutrients, with potential implications for managing the environment and sustainability [56]. Furthermore, it has uncovered previously uncultured microbes, enhancing our understanding of how bacteria live and its ecological importance. Researchers can study microbial populations without cultivating them due to metagenomics, which has significantly changed how landfill microbiomes are studied. The study is limited to species cultivated artificially because traditional microbiology techniques rely on lab-based microbe culture [57]. For example, researchers can better understand microbial diversity's ecological roles in landfills by identifying the genes that break down organic waste or produce methane. A deeper understanding of microbial diversity and metabolic processes becomes possible through high-throughput sequencing techniques, which get around the drawbacks of conventional culture-depend approaches [8].

Metagenomics enables researchers to identify genes that are involved in important processes like pollutant bioremediation, organic waste degradation, and production of methane gas by directly extracting and sequencing DNA from landfill samples [3, 58]. For example, gene-encoding cellulases, ligninases, and proteases have been linked to the decomposition of organic matter. In contrast, methyl-coenzyme M reductase (*mcrA*) genes show the presence of methanogenic archaea that are involved in the production of methane [59]. This functional information helps us understand how microbial population contributes to waste stabilization and biogas production.

Researchers can investigate new biotechnological applications, such as improving biogas yields, improving landfill microorganism management, and developing microbial-based waste treatment strategies, by identifying the rare genes and the metabolic pathways [60, 61]. Additionally, metagenomics can help track microbial changes as they respond to environmental changes, waste decomposition, and landfill aging, providing valuable information for sustainable landfill operations [61]. Metagenomic research has also revealed the presence of novel and specialized microbes that play important roles in landfill ecosystems.

Microbial communities in landfills can be thoroughly characterized using high-throughput sequencing methods such as 16S rRNA amplicon sequencing shotgun metagenomics and Whole-Genome Shotgun (WGS) sequencing [62, 63]. Researchers may reconstruct microbial genomes, analyze strain-level diversity within the landfill microbiomes, and analyze the functional gene potential using the WGS sequencing metagenomics. This provides a more thorough method by sequencing the genetic material in a sample [64, 65].

The WGS metagenomics provides a more comprehensive understanding of the waste microbiomes by detecting wide range of microorganisms, including viruses, fungi, and archaea, that are typically missed by targeted sequencing techniques like the 16S rRNA sequencing [64, 65]. WGS metagenomics also facilitates the discovery of novel genes associated with heavy metal resistance, plastic degradation, and hydrocarbon bioremediation, each of which supports environmentally friendly waste management [65-67]. Furthermore, WGS sequencing metagenomics enables a comparative genomic analysis, which helps the researchers monitor microbial evolution and functional adaptation over time in response to environmental changes and landfill aging [65, 67]. These techniques provide a more profound and accurate knowledge of microbial diversity and metabolic capability, thus avoiding the disadvantage of the culture-dependent methods. Metagenomics continues to offer an essential insight into optimizing microbial-driven processes for better waste degradation, less environmental impact, and increased resource recovery as landfill management changes toward more sustainable waste treatment options [62, 68].

## VI. METAGENOMIC APPROACHES FOR IDENTIFYING PLASTIC-DEGRADING MICROORGANISMS

Plastics have long breakdown times and considerable landfill accumulation, making them a significant environmental contaminant. One promising strategy for mitigating plastic pollution involves harnessing microorganisms that can break down plastics. However, due to the disadvantages of traditional culturing methods, many of these microbes remain unidentified or poorly characterized [69, 70]. The discovery of the plastic-degrading microbial population and the functional genes that can contribute to the breakdown of the synthetic polymer has been made possible by the development of metagenomics [71]. Without the requirement for culture, metagenomic sequencing enables researchers to examine the complete genetic composition of the microbial population in the waste sites. Researchers have discovered important microbial taxa involved in the breakdown of plastics by analyzing the waste microbiomes. These include fungi like *Aspergillus* and *Penicillium*, while bacteria including genera *Pseudomonas*, *Bacillus*, *Ideonella*, and *Rhodococcus* [72-74]. These bacteria produce enzymes such as PETases, cutinases, and laccases, which are essential for the breakdown of plastics like polyethylene terephthalate (PET), Polyethylene (PE), and Polystyrene (PS) into the smaller, biodegradable compounds [75].

On the other hand, Shotgun and functional metagenomics techniques allowed for the direct identification of novel plastic-degrading genes from waste microbiomes. In order to find the gene that is related to the production of the hydrolytic enzymes, these methods involve extracting DNA from waste samples, sequencing the entire genetic material and analyzing the results [28, 76]. Furthermore, these genes can also be linked to specific metabolic pathways responsible for plastic degradation aided by bioinformatics tools such as protein structure modelling and KEGG pathway analysis [77].

Using Krona plots, which enable the hierarchical and dynamic visualization of taxonomic composition, metagenomic data can be visualized to gain a better

understanding of the microbial diversity structure and their potential for plastic degrading capabilities [78, 79]. Krona plot allow researchers to examine the microbial diversity at various levels, from phylum to species, by providing a circular, multi-layered visualization of the taxonomic data. By showing the relative abundance of these microorganisms in the waste microbiomes and identifying the possible candidate taxa for additional research, this visualization helps identify plastic-degrading microorganisms [78, 80]. Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) are multivariate statistical techniques used to interpret complex microbiological datasets. CCA assists in determining how landfill conditions, such as pH, temperature, and pollution levels, affect the plastic-degrading microorganisms since it helps to find the relationship between microbial diversity and environmental factors [81]. On the other hand, PCA helps researchers find patterns and clustering among microbial populations by breaking down big datasets into principle components [81, 82]. These tools assist in identifying the important microbial species that play a role in degrading plastic and offer information on the environmental factors that can encourage their activity.

Microbial diversity within and between waste sites can be quantified using alpha and beta diversity indices. The complexity of the microbial population involved in the plastic degrading can be analyzed with the use of alpha diversity indices, such as Simpson, Shannon, Berger-Parker, and Fisher,

which quantify the species richness and evenness within samples [83, 84]. Beta diversity indices, such as UniFrac and Bray-Curtis dissimilarity, compare the microbial composition of various landfill sites and show how environmental factors, waste composition, and landfill age can affect the microbial populations [47, 84]. By improving knowledge of microbial interactions and community dynamics, these diversity metrics can help identify highly effective plastic-degrading microorganisms in various landfill environments.

Table 3 summarizes the findings from various studies on the microbial diversity related to plastic degradation. Although MacLean et al. (2021) [85] measured alpha and beta diversity, they did not find any particular enrichment of the plastic-degrading microbes in the plastic debris compared to the surrounding soil. The genes associated with plastic biodegradation were found in the landfill microbiomes by Kumar et al. (2021) [86]. However, no particular diversity measurements were specified. Using alpha diversity indices to examine the microbial population, Pawano et al. (2024) [87] discovered particular strains related to Polypropylene (PP) breakdown in the enrichment cultures. In contrast to traditional plastic, Pinnel and Tunner (2019) [88] discovered different biofilm community compositions and beta diversity by measuring the biodegradation capacity. Lastly, although diversity metrics were not explicitly discussed, Wróbel et al. (2024) [89] determined several strains in the enrichment cultures that could degrade plastic.

Table 3. Overview Of Microbial Diversity And Plastic Degradation Across Different Studies

Source	Diversity Measure	Community type	Environmental factor	Biodegradation Association
MacLean et al., 2021 [81]	Alpha diversity (Chao1, Pielou, Shannon-Weaver, Simpson); Beta diversity (Bray-Curtis)	Plastic debris vs surrounding soil	Soil plastic content	No specific enrichment of plastic-degrading bacteria
Kumar et al., 2021 [82]	No mention found	Landfill microbiome	Sample type (soil, leachate, compost)	Genes associated with plastic biodegradation detected
Pawano et al., 2024 [83]	Alpha diversity (Shannon and Simpson reciprocal indices)	Enrichment cultures	Plastic type and pretreatment	Specific strains associated with PP degradation
Pinnel & Tunner, 2019 [84]	Beta diversity (weighted UniFrac)	Biofilms on different substrates	Plastic-type (conventional vs bioplastic)	Distinct communities of bioplastics associated with degradation
Wróbel et al., 2024 [85]	-	Enrichment cultures	Environmental source and plastic type	Multiple strains identified with plastic degradation potential

Moreover, metagenomic data can improve or engineer microbial strains for bioremediation. Researchers aim to create bioaugmentation techniques to accelerate the biodegradation of plastic waste in landfills and other polluted places by identifying and improving plastic-degrading enzymes. Scientists can speed up and enhance waste decomposition by improving the effectiveness of the microbial consortia involved in plastic degradation by utilizing metagenomic insight. Furthermore, metagenomic research makes finding new enzymes with unique degrading characteristics easier, which can be further enhanced using synthetic biology and genetic engineering methods. Metagenomics provides opportunities for creative solutions to plastic pollution. The ongoing development of sequencing technologies and metabolic pathway analysis encourages more environmentally friendly waste management techniques..

## VII. DISCUSSION

This review highlights the importance of metagenomics in improving our knowledge about these complex ecosystems while offering a thorough summary of the microbial diversity in landfills. Landfills are more than places to dispose of waste. They are controlled biological reactors where organic waste breaks down due to microbial activity [2]. This review evaluates the current knowledge regarding the microbial population in landfills, analyzing how operational methods, waste composition, and landfill age affect the structure of microbial communities [61]. It highlights the revolutionary impact of bioinformatics tools and high-throughput sequencing methods while examining the major microbial groups involved in waste decomposition, greenhouse gas emissions, and soil leachate composition. These developments have allowed scientists to discover landfill microorganisms' metabolic and

genetic potential, providing information about environmentally friendly waste management techniques.

Bacteria, fungi, and archaea have unique functions in the breakdown of waste, and the microbial communities found in landfills are incredibly varied and dynamic. Although methanogenic archaea that are from the *Euryarchaeota* phylum promote methane production, bacterial phyla, including *Proteobacteria*, *Bacteroidota*, and *Firmicutes*, are dominant and essential for the decomposition of the organic matter [20, 90]. *Aspergillus* and *Penicillium* are two examples of fungi that help break down resistant organic substances [25]. The structure and function of the microbial population are greatly influenced by environmental factors, including waste content, oxygen availability, pH, moisture, and temperature [44]. For instance, anaerobic bacteria and methanogens predominate in the oxygen-depleted layers, but thermophilic microbes flourish in the higher-temperature zones, speeding up the decomposition [12-14]. Comprehending these factors is crucial for enhancing the bioremediation methods and landfill management.

Moreover, landfills are habitats for unique microbial taxa that have adapted to harsh environments and are essential for the breakdown of organic matter, the detoxification of pollutants, and the production of biogas [26-28]. The microbial variety is greatly influenced by the type of waste, such as waste rich in the organic matter that supports the hydrolytic and fermentative microbes. In contrast, waste rich in plastic may contain microbes that break down plastic, such as *Pseudomonas* and *Ideonella* [38, 39]. As a result of their selective pressure, heavy metals favour resistant taxa that can help in bioremediation, like *Acidobacteria* and *Actinobacteria* [42, 43]. Identifying the genes and pathways associated with the essential processes, such as the breakdown of pollutants, methane production, and organic waste decomposition, has been made possible using metagenomics methods. For example, the degradation of organic matter is associated with the genes encoding enzymes such as cellulases, ligninases, and proteases. In contrast, the presence of the methanogenic archaea is indicated by *mcrA* genes [59]

In addition to fungi like *Aspergillus* and *Penicillium*, researchers have discovered microbial taxa, including *Pseudomonas*, *Ideonella*, *Phodococcus*, and *Bacillus*, that may produce enzymes such as laccases, cutinases, and PETases—which catalyse plastics degradation [72-75]. Novel genes and metabolic pathways that degrade plastics can be found using the shotgun and functional metagenomics approaches. Bioinformatics methods that assist in visualizing and comprehending complicated datasets, such as Krona plots, PCA, and CCA, show how environmental factors can affect microbes that break down plastics [78, 81, 82]. Consequently, alpha and beta diversity indexes are further assessed by measuring microbial diversity within and between the waste sites and identifying effective plastic-degrading microorganisms in various conditions [83, 84].

The review highlights the potential of metagenomics to promote sustainable waste management. Researchers may develop bioaugmentation methods to accelerate the decomposition of plastic waste and increase biogas production by identifying the landfill microbiomes' genetic and metabolic capacities. New approaches to plastic pollution and more effective waste management are made possible by

developments in metabolic pathway analysis and sequencing technologies. Overall, this analysis highlights the importance of landfill microbial ecosystems and the revolutionary potential of metagenomics in managing environmental issues and advancing sustainability.

## VIII. CHALLENGES AND LIMITATIONS

Metgenomics has significantly advanced our understanding of landfill microbial communities. Nevertheless, several challenges and limitations still prevent it from achieving its full potential. The complex ecology of landfill ecosystems, in which various environmental factors, including pH, temperature, and waste content, produce highly dynamic and diverse microbial populations, is one of the primary challenges [3]. Due to this complexity, obtaining a complete and representative sample for the metagenomic analysis is challenging. Another important limitation is the difficulty of accurately identifying functional genes within metagenomic datasets. Nonetheless, the reference database for identifying genes from the metagenomic sequence is still lacking, especially for uncultured or new microbial species [91]. This gap limits our ability to link specific genetic sequences with metabolic pathways, obscuring a full understanding of microbial contributions to waste degradation and bioremediation.

High-throughput sequencing technologies produce vast amounts of data that must be processed and interpreted using advanced bioinformatics tools and processing power. However, many researchers face difficulties, especially in developing countries, due to the lack of standardized pipelines and bioinformatics knowledge [92]. Contamination, sequencing errors, and the presence of highly homologous genetic sequences further complicate data analysis and may lead to misannotation or redundancy [93]. Moreover, metagenomic research is usually limited to DNA-based analysis, which details the microbial communities' possible metabolic roles but not the actual activity [94]. Metagenomics must be merged with other omics methods, such as metatranscriptomics and metaproteomics, for a more thorough understanding of the microbial activity and interaction in the landfill ecosystems. Finally, a significant limitation to the widespread use of metagenomics in landfill research, especially for long-term monitoring studies, is the expense of the high-throughput sequencing and related bioinformatics analysis [3]. This financial barrier can limit access to modern technology, especially for research institutes with limited funding or those in developing countries, resulting in disparities in data availability and limiting microbiome research. Addressing these challenges, will require broad interdisciplinary collaboration, standardized analytical protocols and the development of comprehensive curated reference database.

## IX. CONCLUSIONS

Metagenomics methods have significantly advanced our understanding of microbial diversity in waste ecosystems by enabling culture-independent, in-depth analysis of microbial communities. Metagenomic research has also clarified the roles of microorganisms in key processes such as waste degradation, methane production, and pollution bioremediation. Therefore,



identifying the novel bacterial species and the metabolic pathways has significantly contributed to the biotechnological applications and microbial-based waste management techniques.

However, several challenges, such as costly expenses, computational complexity, and a lack of reference databases, still limit the full potential of metagenomics in waste studies. Expanding microbiological reference libraries, integrating multi-omics methods, and enhancing bioinformatics tools are essential steps to overcome these limitations. Hence, the main goals of future studies should be to identify the microorganisms that break down plastic, investigate the ecological functions of an uncultured microbial species, and develop synthetic biology techniques. Metagenomics has the potential to contribute to global environmental sustainability and accelerate the transition to more ecologically friendly waste management practices by harnessing microbial diversity.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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