

Global biogeographic patterns and assembly processes of landfill leachate microbiomes

Xinqiang Chen^{a,b}, Yunqi Huang^{b,c}, Xiangrong Zhu^{b,d}, Cuifen Gan^b, Wenwen An^b,
Yongqin Liu^{a,*}, Shaofeng Zhou^{b,*}, Meiyang Xu^{b,*}

^a Center for Pan-Third Pole Environment, Lanzhou University, Lanzhou, 730000 China

^b Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, State Key Laboratory of Applied Microbiology Southern China, Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, 510070, China

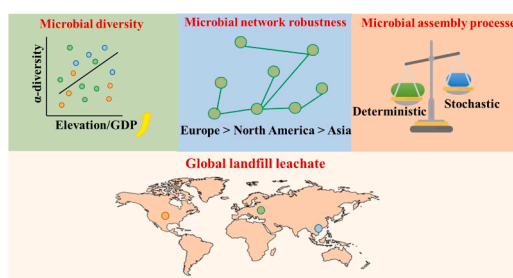
^c Guangzhou Key Laboratory Environmental Catalysis and Pollution Control, Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, China

^d College of Life Sciences, South China Agricultural University, Guangzhou, 510642 China

HIGHLIGHTS

- Regional differences exist in landfill leachate microbial community composition.
- Deterministic factors primarily shaped landfill leachate microbial communities.
- Regional microorganisms have the potential to degrade emerging pollutants.

GRAPHICAL ABSTRACT



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ABSTRACT

Approximately 95 % of municipal solid waste is disposed of in landfill globally, generating leachate that is known as a complex mixture of biodegradable and persistent toxic compounds. Microbes are main forces for tackling the toxic leachate but the patterns of microbial assembly in such treatments are largely unknown, limiting the proper optimization of leachate treatment efficiency. This study, for the first time, presents a global-scale analysis involving 151 landfill leachate treatment samples for uncovering mechanisms of microbial assembly from an ecological perspective. The information of microbiome from 97 treatments in Asia, 41 treatments in Europe, and 13 treatments in North America were collected. The results revealed pronounced biogeographic divergence, with Asian samples (particularly those from India) exhibiting lower microbial diversity and richness compared to Europe and North America counterparts. Geographical-climatic and socio-economic factors significantly influenced microbial composition, with elevation and per capita GDP being primary drivers. Further, the community assembly was predominantly governed by deterministic processes. Co-occurrence network analyses demonstrated distinct microbial interaction patterns across continents, with Asian networks being more vulnerable to collapse under external disturbances. This study provides critical insights into the global microbial ecology of landfill leachate treatment, offering a foundation for developing targeted bioremediation strategies.

* Corresponding authors.

E-mail addresses: yqliu@itpcas.ac.cn (Y. Liu), zsfking@hotmail.com (S. Zhou), xumy@gdim.cn (M. Xu).

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1. Introduction

Landfill has long served as the primary method for managing municipal solid waste (MSW). Over 95 % effectively managed MSW was disposed of via landfilling, exerting immense pressure on surrounding land and the following leachate treatment systems (National Bureau of Statistics Data, 2024 <https://www.stats.gov.cn>; United Nations Environment Programme, 2024; Emenike et al., 2024). Landfill leachate is a high-strength waste liquid composed mainly of organic and inorganic compounds including heavy metals, and trace-level emerging pollutants (Jayawardhana et al., 2016; Gong et al., 2024). Over time, increasing toxic contaminants from MSWs are mobilized into the leachate, causing excessive levels of emerging contaminants being widely detected (Jia et al., 2024; Laiju et al., 2023). These include per- and polyfluoroalkyl substances (PFAS), pharmaceuticals and personal care products (PPCPs), endocrine-disrupting compounds (EDCs), and related emerging contaminants (Qian et al., 2024). Owing to their persistence, bio-accumulative nature, and toxicity, these pollutants pose significant threats to the environment, ecosystems, and human health (Hu et al., 2024).

Microorganisms play a pivotal role in landfill leachate treatment by breaking down complicated toxic pollutants into smaller and less harmful compounds, thereby facilitating the pollutant removal (Miao et al., 2019). Their structural composition, functional redundancy, and ecological interactions enable the community to adapt to the fluctuating and chemically complex nature of landfill leachate. However, the microbial communities in the leachate treatment plants are “black boxes” characterized by high complexity and diversity of interspecific and intraspecific connections, transcending simple definitions of competition or collaboration. In addition, the physicochemical properties of leachate are far from uniform and are profoundly influenced by the compositions of the landfill waste, the landfill age, and the engineering practices. In particular, variations in regional climate conditions (e.g., temperature and precipitation) and lifestyle factors (e.g., dietary habits and consumption patterns) lead to differences in municipal solid waste composition, resulting in significant heterogeneity of landfill leachate across different geographic regions (Mor and Ravindra, 2023; Wu et al., 2017). This heterogeneity not only affects the concentrations and compositions of pollutants, but also directly influences the structure and function of microbial communities within landfill leachate treatments. In this regard, a mechanistic understanding on assembly of microbial communities, that is a key to deciphering the black box, remains limited.

In recent years, culture-independent techniques, such as high-throughput sequencing, have complemented the traditional culture-dependent methods (e.g., isolation and cultivation) for exploring and deeply understanding microbial communities in landfill leachate facilities (Abiriga et al., 2021; Qian et al., 2024; Yang et al., 2022). Despite these methodological advances, some studies focused on microbial community composition of leachate treatment from different regions have shown significant inconsistencies within the microbiota. For instance, one study identified *Thiomonas* as the dominant genus in landfill leachate in Shaanxi, China (Liu et al., 2022), whereas another research collectively demonstrated landfill leachate from eleven Chinese cities indicated that *Deffluviitoga*, *Sphaerochaeta*, *Arcobacter*, *Syntrophomonas*, and *Petrimonas* were the predominant taxa (Zhao et al., 2021). These discrepancies might stem from variations in landfill design, waste composition, treatment strategies, and geographic factors. However, most existing studies are restricted to individual leachate treatment plants or localized areas, leaving a comprehensive investigation from a global level remain unexplored (Lepcha et al., 2025; Zhang et al., 2024a).

This study presents a global meta-analysis of microbial communities in landfill leachate treatments, investigating their composition, biogeographic patterns, and assembly mechanisms. We synthesized published datasets and analyzed raw 16S rRNA gene sequences from public databases to characterize: (i) worldwide microbial diversity

patterns, (ii) co-occurrence network structures, and (iii) deterministic vs. stochastic assembly processes. Furthermore, through comparative genomics and functional prediction, we identified regionally distinctive taxa with putative capacities for degrading emerging contaminants. These findings provide a framework for optimizing leachate treatment strategies and advancing global pollutant mitigation efforts.

2. Materials and methods

2.1. Collection of microbial sequencing data and anthropogenic parameters

Bacterial 16S rRNA gene sequences were obtained from the NCBI Sequence Read Archive (SRA) using the SRA Toolkit (data accessed by September 2024). Raw sequencing reads were identified through database queries using the search terms “landfill leachate” or “landfill leachate microbial community”. Only paired-end Illumina datasets derived from landfill leachate or leachate-associated treatment systems were included. Datasets with incomplete metadata, unclear sample origin were excluded. A total of 270 microbial community datasets from samples of landfill leachate treatments in Asia (China, India, and Japan), North America (Canada), and Europe (Italy, Norway and Poland) were collected from publicly available sources. Detailed information on each sample is provided in the *Supplementary Material*.

The anthropogenic parameters like elevation, human footprint, and per capita GDP etc. surrounding locations of landfill treatments were mainly collected and calculated from publicly released dataset. The detailed anthropogenic parameters and climate information are included in Table S1.

2.2. Bioinformatic analysis

The raw sequencing reads were merged (fastq_mergepairs, VSEARCH), followed by primer trimming and quality filtering. The quality-filtered reads were dereplicated and low abundance sequences (minimum size: 13) were removed. An operational taxonomic unit (OTUs) was generated after clustering OTUs at a 97 % similarity threshold using USEARCH. Chimeric sequences were identified and removed using VSEARCH with the SILVA 138 database, and the resulting data were used for feature table construction and taxonomic assignment. The reads with no taxonomic match were discarded. Non-bacterial OTUs, as well as those assigned to mitochondria and chloroplast were removed in R platform (version 4.2). A re-sampling depth of 5.496×10^3 reads per sample was applied to ensure the data availability for bacterial analysis (R package *vegan*). Least squares linear regression was performed in SPSS to evaluate the relationships between anthropogenic parameters and α -diversity indices. Distance-based redundancy analysis (db-RDA) was conducted in R platform (version 4.2) using forward selection and VIF filtering to identify key anthropogenic drivers shaping microbial community composition. The community's functional profiles were predicted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST), and PICRUST2 was used to predict functions (Chen et al., 2024a). The detailed procedures for sequence quality control, OTU clustering, and functional prediction are provided in *Supplementary Material*.

To evaluate compositional differences in microbial communities between countries and continents, principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarities was performed (Chen et al., 2024b). The significance comparisons were tested using a permutational multivariate analysis of variance (PERMANOVA) with the *adonis* function in the R package *vegan* (Chen et al., 2023). The significance of alpha diversity across countries and continents was determined using Kruskal-Wallis tests in R platform (version 4.2).

2.3. Co-occurrence network and robustness analysis

To reduce dataset complexity, low-abundance OTUs (<0.01 %) and those with low occurrence frequency (present in <10 % of samples at the continental level and <25 % at the national level) were removed prior to network construction. The *P*-values of the *t*-test were corrected using the “FDR” method (Zhang and Ren, 2023). Spearman correlation (*P*-value < 0.01 and $|r| > 0.7$) were used to generate sub-networks using the R *igraph* package) and exported as a GML format network file for visualization in Gephi software (Zhang et al., 2024b). Topological features of each network were calculated using Gephi, including node count, positive/negative edges, average degree, average path length (APL), network diameter, network density, clustering coefficient (CC), and modularity. The network robustness and vulnerability were estimated using *igraph* and WGCNA package in R for predicting the structural stability of microbial networks.

2.4. Identification of key microorganisms in landfill leachate

The linear discriminant analysis effect size (LefSe) was used to identify significantly different bacterial taxa as potential biomarkers across different continents or countries (Li et al., 2025). In order to achieve a larger linear discriminant analysis (LDA) value, the standardized scaling factor was set to 1×10^6 . In addition, based on the network analysis in Section 2.3, the nodes in the network were categorized into four groups based on their within-module connectivity (*Z*_i) and among-module connectivity (*P*_i). Specifically, the nodes with *Z*_i ≥ 2.5 and *P*_i < 0.62 were classified as module hubs, while those with *Z*_i < 2.5 and *P*_i ≥ 0.62 were defined as connectors and the nodes with *Z*_i ≥ 2.5 and *P*_i ≥ 0.62 were recognized as the network hubs. Lastly, the nodes with *Z*_i < 2.5 and *P*_i < 0.62 were categorized the peripheral nodes.

Among these, the module hubs, connectors, and network hubs are widely recognized as keystone taxa in the structuring of networks (Kajihara and Hynson, 2024). These biomarkers and keystone taxa are regarded as key microorganisms in landfill leachate.

2.5. Quantification of the community assembly process

A null model approach was used to disentangle community assembly mechanisms for quantification of variations in phylogenetic and taxonomic diversity (Gao et al., 2024). The β-nearest taxon index (βNTI) was calculated for indication of phylogenetic β-diversity while the “Bray–Curtis”-based Raup–Crick (RC_{bray}) was employed for quantification of taxonomic β-diversity. A $|\beta\text{NTI}| > 2$ indicates a deterministic process with $\beta\text{NTI} > 2$ representing heterogeneous selection and $\beta\text{NTI} < -2$ representing homogeneous selection. In contrast, $|\beta\text{NTI}| < 2$ indicates stochastic processes, further differentiated by RC_{bray} values: < −0.95 for homogenizing dispersal, > 0.95 for dispersal limitation, and intermediate values indicating undominated processes.

3. Results and discussion

3.1. Diversity of bacterial communities from various leachate biotreatments

After quality control filtering and resampling, 151 high-quality samples out of 270 global samples were retained, with geographic distribution as follows: 97 samples from Asia (China: 75, India: 19, Japan: 3), 41 samples from Europe (Poland: 15, Italy: 26), and 13 samples from North America (Canada) (Fig. 1, Supplementary Information). The alpha-diversity results indicated that landfill leachate treatments in Asia exhibited the lowest microbial diversity and richness compared to those

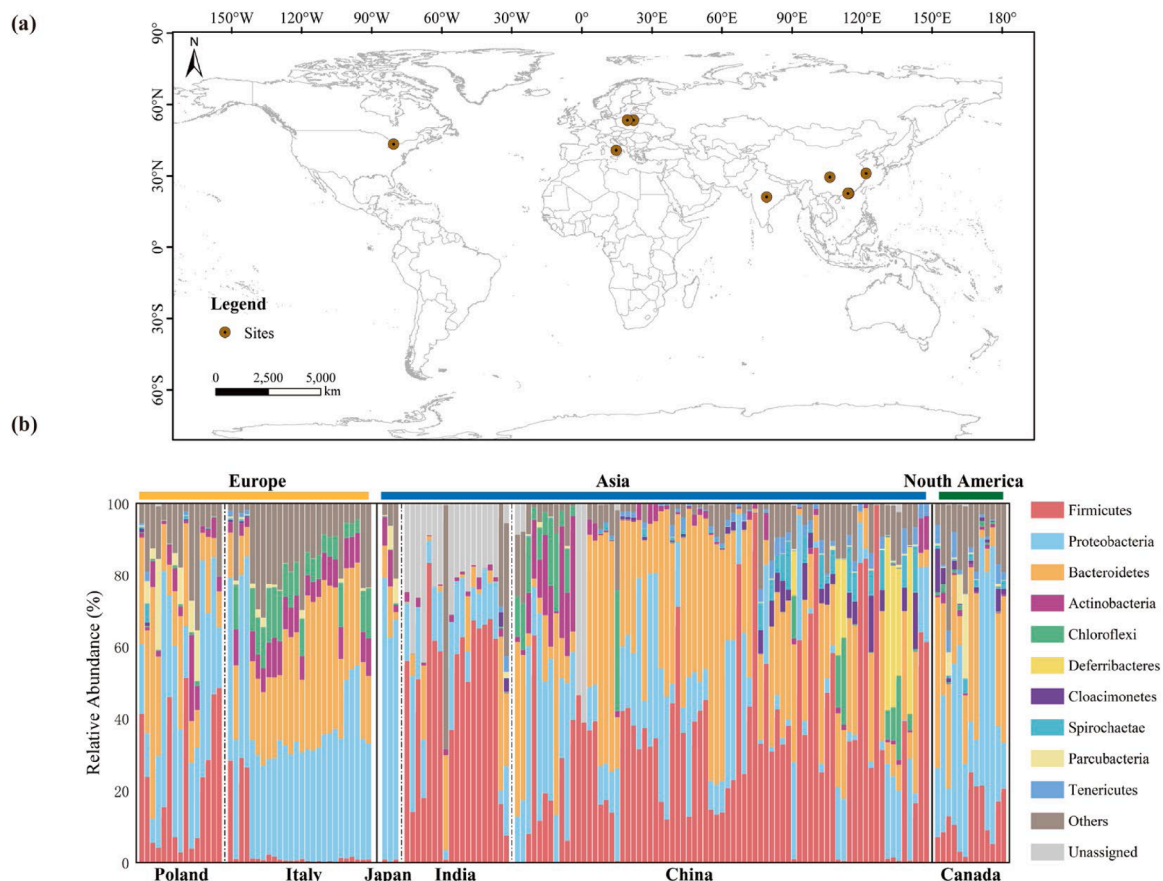


Fig. 1. Sample distribution of microbial communities in landfill leachate. (a) collection location, (b) horizontal stack of phylum level.

in Europe and North America, with India showing significantly lower diversity than China and Japan ($P < 0.05$, Kruskal-Wallis test, Fig. S1). Across all samples, a total of 59 bacterial phyla were identified. Though most leachate samples were dominated by members of phyla *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*, significant differences in microbial composition across continents were observed (Fig. 2 and Table S2). Compared with the continental level, the composition of microbial communities shows greater differences at the national level (Kruskal-Wallis test, Fig. 2c, Table S3).

Among the dominant phyla, *Firmicutes* and *Bacteroidetes* are recognized as the key hydrolytic bacteria, playing critical roles in initial stages of organic matter degradation through hydrolyzing macromolecules such as proteins, polysaccharides, lipids, and nucleic acids. These macromolecules are then fermented into small molecules, including amino acids, sugars, and volatile fatty acids, accompanied by the production of metabolic end-products such as hydrogen, CO_2 , ethanol, acetate, and other organic acids (Kim et al., 2019; Kvesić et al., 2022; Mayer et al., 2016; Shi et al., 2021). In Asia, the relative abundance of *Firmicutes* was significantly higher than those in the other two continents, while *Bacteroidetes* were significantly more abundant in Europe than in Asia ($P < 0.05$, Kruskal-Wallis test). This variation may be closely related to differences in organic pollution levels in landfill leachate across regions. In particular, landfill leachate in China is characterized by high chemical oxygen demand (COD) levels, ranging from 0.5 to 75.9 g L^{-1} (Qian et al., 2024). Members of *Firmicutes* were particularly efficient in cellulose degradation (Jin et al., 2023; Wei et al., 2021), and some of its genera could withstand extreme environments (Jun et al., 2025; Ziels et al., 2018). In addition, *Bacteroidetes* possess diverse extracellular enzymes and polysaccharide utilization loci that enable them to degrade complex polysaccharides and proteins, contributing to nutrient cycling and

breakdown of organic matters under anaerobic conditions (Hao et al., 2021; Hu et al., 2016).

The Venn diagram analysis revealed 46 phyla were shared among microbiomes from global landfill leachate treatments (Fig. 2), while only few unique phyla were identified in specific regions. The Asia and North America shared four phyla (*Hyd24-12*, *Miscellaneous_Crenarchaeotic*, *Aenigmarchaeota*, and *Acetothermia*), whereas only one unique phylum was found between Asia and Europe (*Candidate_division_WS6*). Notably, *Dictyoglomi* and *Nanohaloarchaeota* were found exclusively in China and India, respectively, whereas Canada displayed a higher phylum-level uniqueness with five distinct phyla: *Parvarchaeota*, *SM1K20*, *SBYG-2791*, *PAUC34f*, and *LCP-89* (Fig. 2b). The variation in types and concentrations of pollutants in landfill leachates across countries might explain the region-specific distribution of certain non-dominant phyla, such as *Dictyoglomus*, *Nanohaloarchaeota*, and *Parvarchaeota*. *Dictyoglomus* generally produces a variety of hydrolytic enzymes, including amylase, mannanase, and xylanase, which degrade polysaccharides (Hamm et al., 2019; Nishida et al., 2011). These enzymes enable *Dictyoglomus* to efficiently break down cellulosic substances commonly detected in landfill leachate, facilitating metabolic interactions among microbial communities and improving the overall removing efficiency of organic matters (Nishida et al., 2011). *Nanohaloarchaeota* and *Parvarchaeota* are typical archaeal taxa adapting to hypersaline environments. Their presence in landfill leachate treatment systems may reflect elevated salinity levels and specific local physicochemical conditions, leading to the selective enrichment of halotolerant species (Nishida et al., 2011). In particular, landfill leachates from Asian regions often contain high concentrations of recalcitrant organic compounds, as well as relatively high salinity, which may contribute to the observed differences in these non-dominant

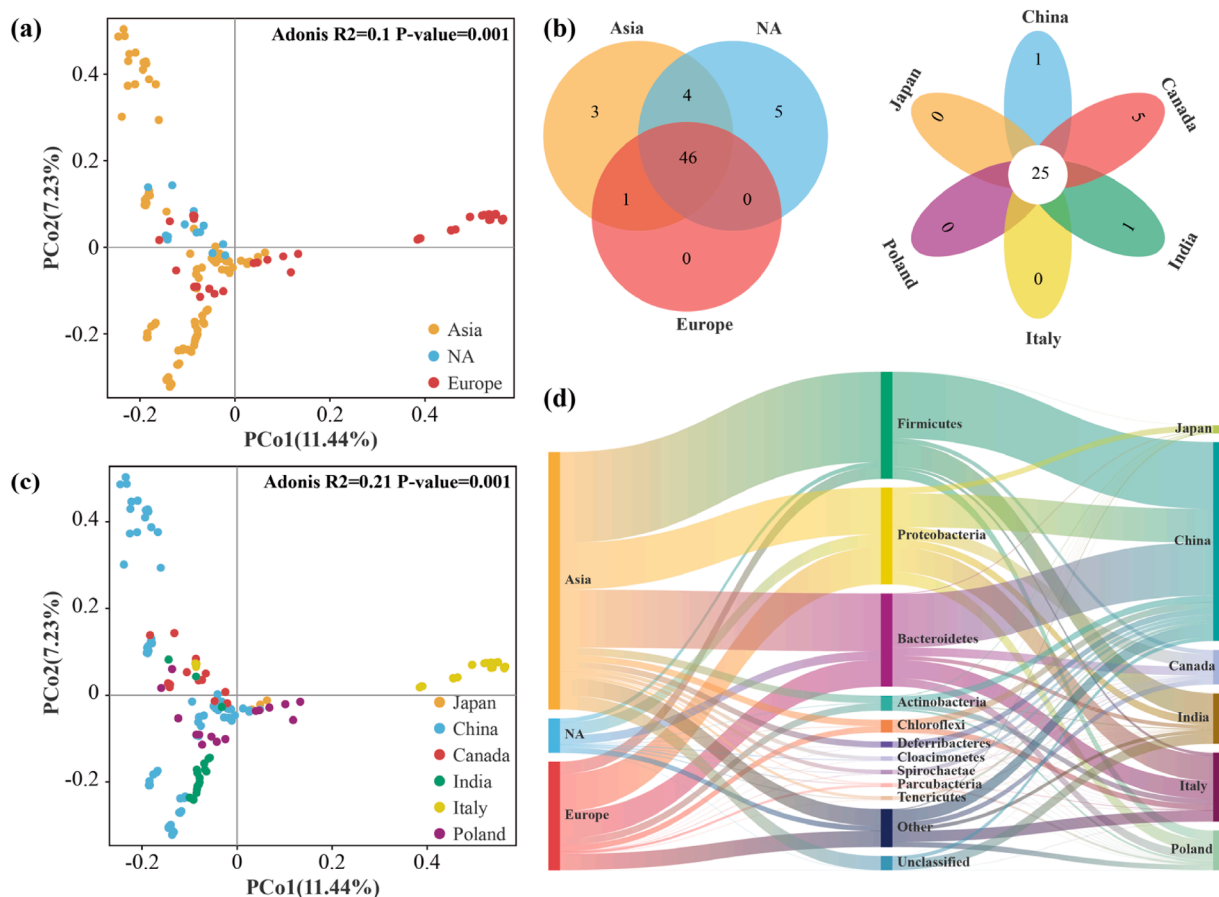


Fig. 2. Differences in microbial community composition of landfill leachate across continents and countries. (a) and (c) PCoA based on Bray-Curtis distances; (b) Venn diagram of phylum-level taxa across continents or countries; (d) Phylum-Level taxa composition across different continents or countries.

microbial phyla (Qian et al., 2024).

3.2. Effects of geographical-climatic and socio-economic factors on the diversity of leachate biotreatments

Regional variations in climate (e.g., precipitation) and lifestyle factors (e.g., dietary habits and consumption patterns) shape the composition of municipal solid waste, thereby contributing to pronounced heterogeneity in landfill leachate across different geographic regions, which in turn alters the diversity of microbial communities. (Mor and Ravindra, 2023; Wu et al., 2017). Accordingly, the correlations between these factors and microbial community diversity were analyzed. The bulk density (oven dry) of surface soil at a depth of 0–5 cm (BDOD 0–5 cm), was identified as one of the most critical factors of leachate microbial α diversity and community composition ($P < 0.05$) (Fig. S2–3). Higher BDOD 0–5 cm values, indicating a higher level of soil compaction and decreasing porosity that significantly resulted in poor aeration and permeability, was unfavored regarding the release of organic matter from MSW. Ultimately, microbial diversity was reduced during

biological leachate treatment (Wang et al., 2025a). On the contrary, total precipitation was positively associated with microbial diversity and richness (Shannon and Chao1 indices). This might be due to the enhanced leaching effect on landfilled waste under higher rainfall (Linh et al., 2020), which created moist conditions that accelerated the hydrolysis and fermentation of organic matter (Cossu et al., 2016), thereby increasing microbial diversity in leachate treatment processes.

Among the socio-economic variables, only per capita GDP significantly increased the Shannon and Chao1 indices (Fig. S2–3). Economic development often leads to a higher proportion of kitchen and food waste in municipal solid waste streams (Gu et al., 2017). Such changes in waste composition provide a broader range of substrates and niches for microbial colonization, thereby enhancing community diversity. By contrast, Population Count, Population Density, and Road Density were significantly negatively correlated with microbial α diversity (Fig. 2). Higher values of these factors typically indicate stronger anthropogenic disturbances, such as land-use change, industrial and domestic wastewater discharge, increased waste generation, and traffic-related pollution (Valderrama et al., 2024). These disturbances may increase the

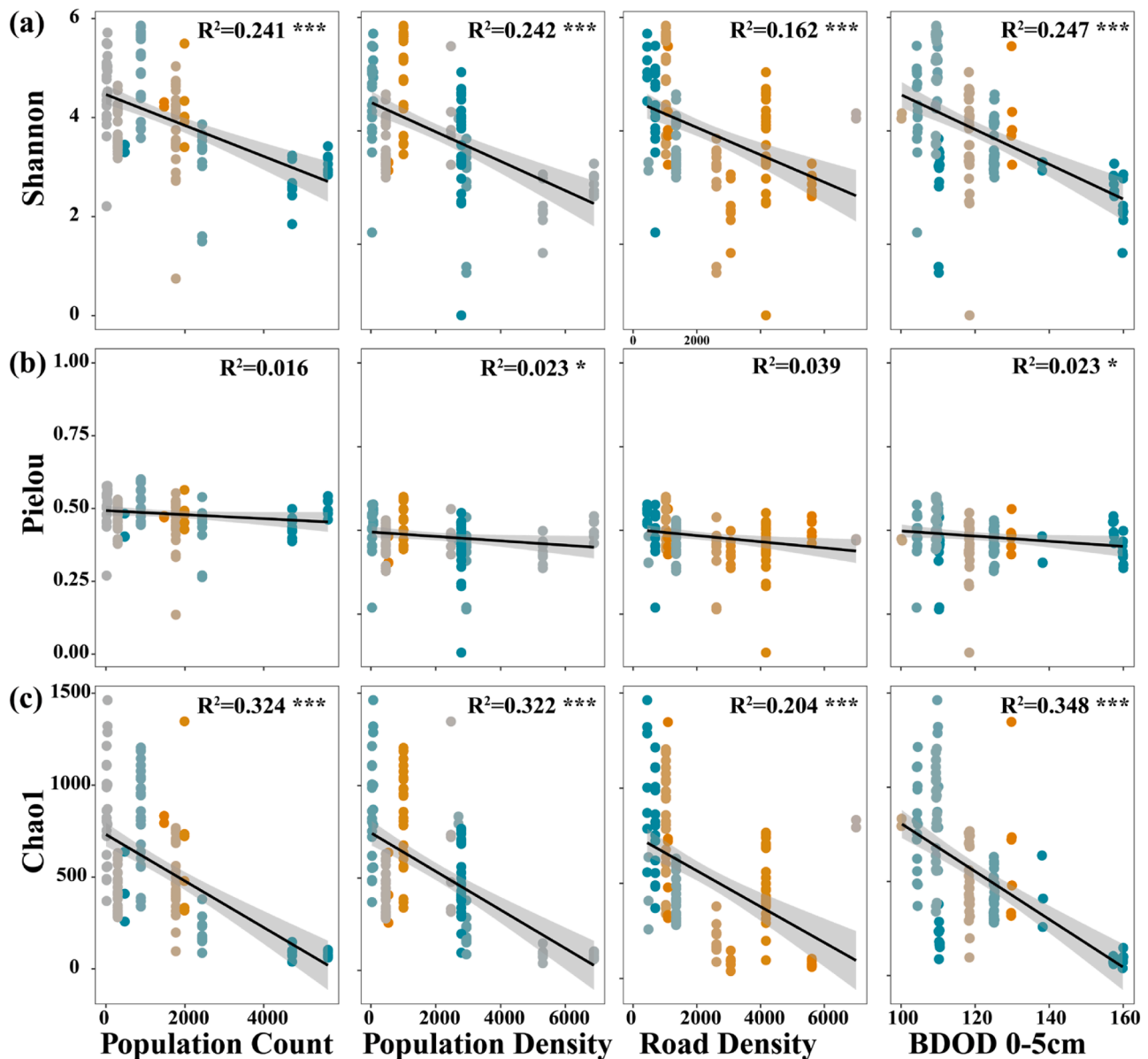


Fig. 3. Effects of geographical-climatic and socio-economic factors on the α -diversity of leachate microbial communities. Adjusted R^2 and P-values are shown in the figure. Note: BDOD 0–5 cm represents the bulk density (oven dry) of surface soil at a depth of 0–5 cm.

concentrations of emerging contaminants in leachate, including EDCs, PPCPs, heavy metals, and microplastics (Qian et al., 2024), which impose selective pressures on microbial communities and exert complex, mixed effects on α diversity (Gamboa et al., 2025).

Overall, these results demonstrate that the composition of landfill leachate microbial communities is governed by an interplay of geographic-climatic and socio-economic factors, with significant synergistic effects between these drivers determining community composition.

3.3. Patterns of co-occurrence complexity

Beyond the taxonomic structures, potential biological interactions identified from co-occurrence networks could complement new insights into complex communities (Zhang and Ren, 2023). The co-occurrence network for Asia consisted of 531 nodes and 8014 edges, whereas the networks for Europe and North America comprised 858 nodes with 36,000 edges and 851 nodes with 20,545 edges, respectively (Table S4). The bacterial communities in European and North American treatment plants exhibited higher α -diversity, indicated more complex networks compared to those observed in Asian (Fig. 3, S1). Theoretically, complex interactions are often associated with greater robustness and higher stability of the entire community, making it more resilient to environmental disturbances such as hydrologic shock of unfavored contaminants and fluctuation of substrates bioavailability (Liu et al., 2025; Steele et al., 2011). Similarly, a larger network diameter and higher modularity coefficient generally reflect increased resistance to disturbance, as modularity buffers the impacts of taxa loss by localizing disruption effects within individual modules (Table S4) (Hernandez et al., 2021). However interestingly, though communities in Asian treatment plants exhibited the highest network diameter (Asia: 22,

Europe: 10 and North America: 18) and modularity coefficient (Asia: 0.561, Europe: 0.36 and North America: 0.35) among the three continents, its network was the most fragile likely due to its significantly lower number of keystone taxa (Asia: 2, Europe: 7 and North America: 33) (Fig.S4). It was also concluded from network architecture in European and North American landfill treatment plants as they harbored more key nodes, contributing to enhanced robustness by mitigating the cascading effects of node loss. Additionally, the results of co-occurrence network suggest that the microbial communities predominantly exhibit cooperative, rather than antagonistic interactions. While cooperative interactions can enhance functional complementarity, they may also generate positive feedback loops, rendering the community more vulnerable to collapse if key taxa decline (Hernandez et al., 2021).

At the national level, notable differences in network composition were observed. (Fig. 4). For examples, the network of Italian treatments show the interactions (62.70 %) between nodes are primarily dominated by *Planctomycetes* and *Proteobacteria*, which were reported widespread in the environment and mainly involved in global carbon and nitrogen cycles (Jeske et al., 2015; Spain et al., 2009; Wiegand et al., 2018). Additionally, the association of *Proteobacteria* with the degradation of organic contaminants, including pharmaceuticals, has been well documented in constructed wetlands (Li et al., 2024). In these systems, *Planctomycetes* have been associated with the biodegradation of emerging organic contaminants (e.g., sulfamethoxazole) and even proposed as potential bioindicator for pollutant removal (Peng et al., 2025). These two phyla may exhibit higher community adaptability, which enhances the complexity of co-occurrence networks and contributes to greater microbial community stability (Fig. 4).

ZiPi analysis was performed to identify the keystone taxa (i.e., nodes) in the co-occurrence networks by classifying them into network hubs,

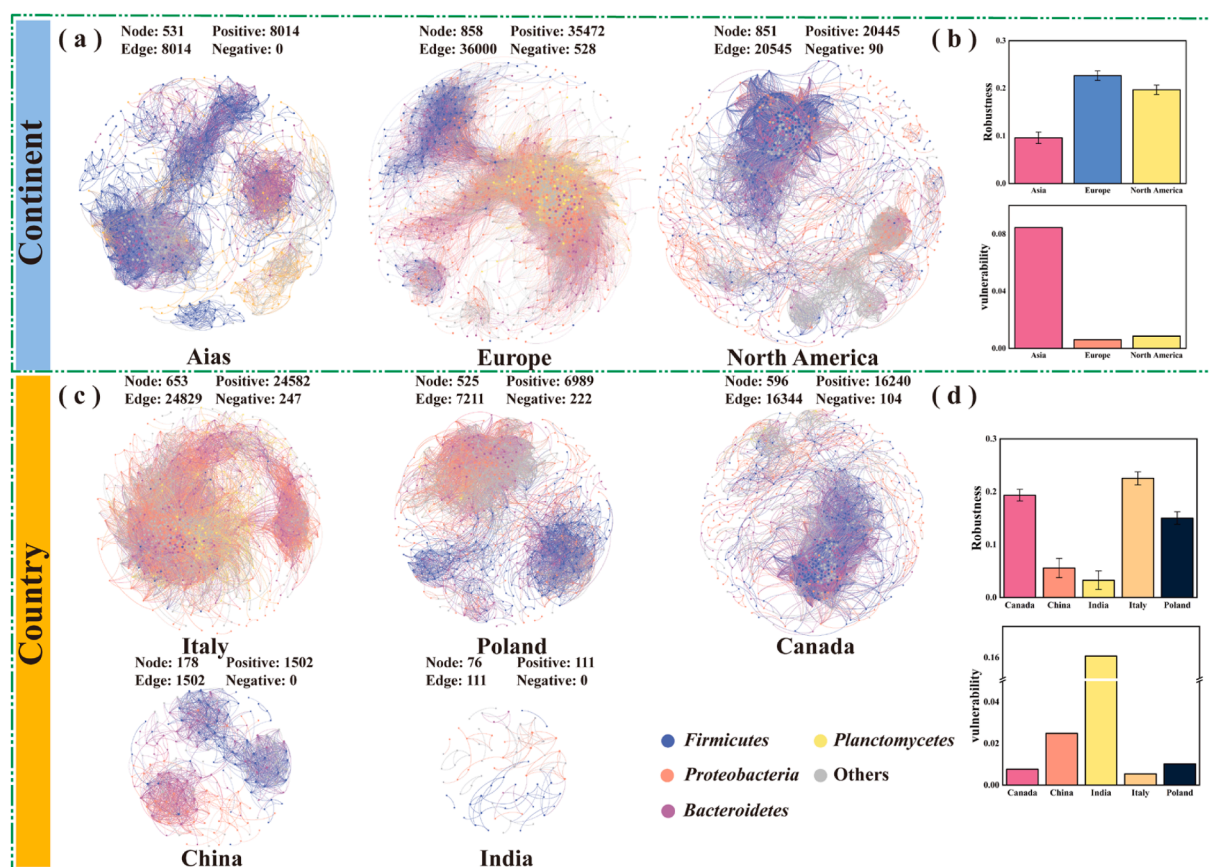


Fig. 4. (a) and (c) Co-occurrence network of Continent and Country. The sizes of the nodes (OTUs) are proportional to the degree. The taxonomy of each node is shown in different colors in the phylum level. (b) and (d) Network robustness and vulnerability. Robustness measured as the proportion of taxa remained with 50 % of the taxa randomly removed from the network. Each error bar corresponds to the standard deviation of 100 repetitions of the simulation.

module hubs, and connectors (Fig. S4) (Zhou et al., 2024). It turned out that the network of microbial community in North American leachate treatments exhibited the highest number of key nodes (with 3 connectors and 30- module hubs) compared to those in Asian (2 module hubs) and Europe (2 connectors, 3 module hubs, and 2 network hubs) leachate treatments. These key nodes were predominantly affiliated with *Bacteroidetes* (47.62 %) and *Firmicutes* (26.20 %), along with representatives from eight other phyla. At the national level, the network derived from Italian leachate treatments contained the highest number of key nodes (23 connectors, 8 module hubs, and 6 network hubs). The taxonomic composition of these nodes indicated a significant diversity in microbial communities spanning 12 phyla. Notably, among the six network hub nodes identified in Italian samples, five were affiliated with *Saccharibacteria*, which are widely distributed across various natural environments and have been recognized as active participants in hydrocarbon-degrading microbial consortia (Dong et al., 2024; Figueroa-Gonzalez et al., 2020). Their presence in contaminated habitats suggests a key ecological role in degrading complex organic pollutants and maintaining microbial community stability under stress conditions.

3.4. Deterministic processes dominate microbial community assembly

Microbial community assembly is generally influenced by deterministic and stochastic processes (Zhou and Ning, 2017). Understanding the assembly of microbial community in landfill leachate, specifically the balance between deterministic or stochastic processes, is crucial for predicting ecological stability and treatment performance. Deterministic processes, driven by environmental filtering, shape microbial composition and can lead to adaptive evolution, which is reflected in the structure of interaction networks. Consequently, the deterministic assembly pattern provides valuable insights into the long-term performance and stability of the microbial communities underpinning

leachate treatment. Linking these assembly processes to microbial adaptation strategies not only clarifies the ecological mechanisms at play but also informs strategies for enhancing treatment efficiency and controlling environmental risk (Wu et al., 2025). To further elucidate their relative contributions in shaping landfill leachate microbiomes, β NTI and RCbray were used to assess the microbial assembly mechanisms. The results indicated that both deterministic and stochastic processes significantly influenced microbial assembly in landfill leachate treatments (Fig. 5). Neutral model analysis reveals that deterministic processes dominated bacterial community assembly in most countries (except China), indicating that microbial β -diversity is shaped by both abiotic and biotic factors (Wang et al., 2013). Stochastic processes contribute to the construction of a more comprehensive organic matter metabolic network and enhance the functional potential of microbial communities involved in landfill leachate treatment (Wu et al., 2025). The deterministic processes affecting community assembly were mainly composed of homogeneous selection, which played a crucial role in the assembly of prokaryotic communities and might promote the similarity of communities (Wang et al., 2022; Zhou and Ning, 2017).

3.5. Regional divergence of potential functions

In recent years, growing quantities and diversity of emerging contaminants have been detected in landfill leachate (Jayawardhana et al., 2016; Qian et al., 2024). Enhancing microbial tolerance and degradation capabilities for these contaminants has thus become critical in leachate biotreatment systems. This study conducted functional predictions of key microorganisms (Table S5–6) to further elucidate their potential roles in the degradation of emerging contaminants. KEGG annotation was employed to characterize pollutant-degradation genes among differentially abundant microbes (Fig. S5). Several species within *Bacteroidetes* contained a significant number of genes associated with the

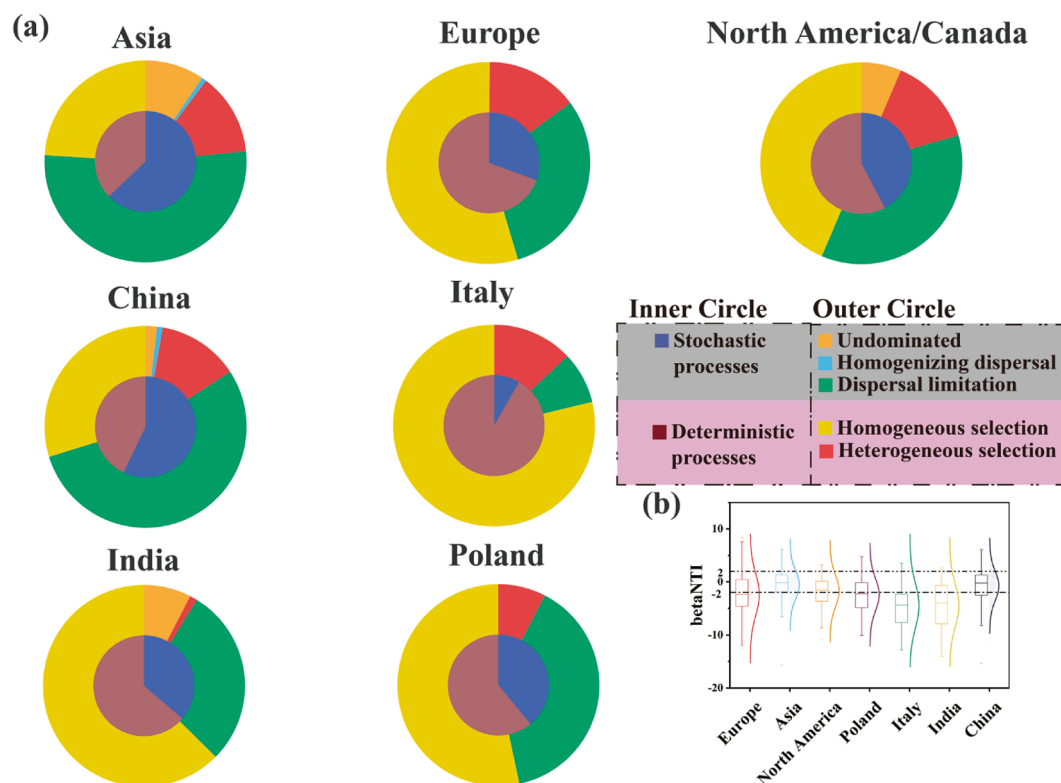


Fig. 5. Neutral models and null models assessed random/deterministic assembly processes of the community. (a) Assembly of bacterial communities based on the null-model-based framework. The inner circle represents the contribution of stochastic and deterministic processes to community assembly. The outer circle represents the percentage of detailed ecological processes statistically assigned to stochastic or deterministic processes. (b) Beta-nearest taxonomic index of communities.

degradation of plastics (Lv et al., 2024; L. Wang et al., 2025), PFAS (Lu et al., 2024), and ibuprofen (Rutere et al., 2020) with their abundance being significantly higher in Asian leachate treatments, particularly the ones in China and Japan ($P < 0.05$). Notably, *Pseudomonas*, a *Proteobacteria* genus, exhibited significantly greater abundance in leachate treatment systems from North America, Poland, and Italy relative to other geographical regions. This genus is known for its capacity to degrade various emerging contaminants, such as endocrine disruptors (Liao et al., 2010), bisphenol A (Vijayalakshmi et al., 2018), plastics (Wilkes and Aristilde, 2017), and PFAS (Bygd et al., 2021). Collectively, these microbial taxa demonstrate dual significance: as functional candidates for contaminant bioremediation and as ecological indicators of pollutant dynamics in diverse environments.

4. Conclusion

This study, for the first time, provides comprehensive characterization of landfill leachate microbiomes on a global scale, analyzing 151 samples collected from 10 cities across 6 countries spanning three continents. Altitude and per capita GDP are recognized as the key factors determining the structure of the community. Moreover, the deterministic processes were found to be the dominant microbial community assembly in landfill leachate. At the co-occurrence network level, *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota* exhibited significant continental differences in their relative abundances, which in turn contributed to distinct variations in network structures across continents. By establishing the global framework for landfill leachate microbiomes, this work provides a critical basis for future research to identify the environmental drivers of community assembly and experimentally validate the ecological functions of keystone taxa, thereby paving the way for tailorable biotechnologies for regional leachate treatments and development of contaminant removal strategies.

CRedit authorship contribution statement

Xinqiang Chen: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Yunqi Huang:** Writing – original draft. **Xiangrong Zhu:** Writing – review & editing, Supervision, Conceptualization. **Cuifen Gan:** Validation, Software, Formal analysis. **Wenwen An:** Visualization, Software, Formal analysis. **Yongqin Liu:** Writing – review & editing. **Shaofeng Zhou:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Meiying Xu:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2025.124922](https://doi.org/10.1016/j.watres.2025.124922).

Data availability

All data supporting the findings of this study, including sequence accession numbers and associated metadata, are provided in the Supplementary Information.

References

- Abiriga, D., Jenkins, A., Alfsnes, K., Vestgarden, L.S., Klempe, H., 2021. Characterisation of the bacterial microbiota of a landfill-contaminated confined aquifer undergoing intrinsic remediation. *Sci. Total. Environ.* 785, 147349.
- Bygd, M.D., Aukema, K.G., Richman, J.E., Wackett, L.P., 2021. Unexpected mechanism of biodegradation and defluorination of 2,2-difluoro-1,3-benzodioxole by *Pseudomonas putida* F1. *mBio* 12 (6), e0300121.
- Chen, X., Jiang, Q., Cui, Y., Deng, H., Mao, H., Wang, Z., Huang, L., Shen, P., 2024a. Lipid-extraction of *Tribonema minus* avoids foaming facilitating anaerobic co-digestion with molasses vinasse. *J. Clean. Prod.* 448, 141660.
- Chen, X., Mao, H., Cui, Y., Deng, H., Zhao, T., Liu, J., Huang, L., Shen, P., 2024b. An algal regulation-based molasses vinasse anaerobic digestion strategy for enhancing organic matter removal and methane production. *Renew. Energ.* 234, 121257.
- Chen, X., Mao, H., Cui, Y., Jiang, Y., Liu, J., Zha, X., Huang, L., Shen, P., 2023. Multi-angle evaluation of the anaerobic digestion of Molasses vinasse using two different feeding patterns. *Water Cycle* 4, 170–178.
- Cossu, R., Morello, L., Raga, R., Cerminara, G., 2016. Biogas production enhancement using semi-aerobic pre-aeration in a hybrid bioreactor landfill. *Waste Manag.* 55, 83–92.
- Dong, P.T., Tian, J., Kobayashi-Kirschvink, K.J., Cen, L., McLean, J.S., Bor, B., Shi, W., He, X., 2024. Epibiotic saccharibacteria induce intracellular lipid droplet production in their host bacteria. *ISME J.* 18 (1).
- Emenike, C., Omo-Okoro, P., Pariatamby, A., Barasath, J., Hamid, F.S., 2024. Remediation of leachate-metal-contaminated soil using selected bacterial consortia. *Soil. Syst.* 8 (1), 33.
- Figuerola-Gonzalez, P.A., Bornemann, T., Adam, P.S., Plewka, J., Revesz, F., von Hagen, C.A., Tancsics, A., Probst, A.J., 2020. Saccharibacteria as organic carbon sinks in hydrocarbon-fueled communities. *Front. Microbiol.* 11, 587782.
- Gamboa, J., Le, G.H., Wong, S., Alteza, E.A.I., Zachos, K.A., Teopiz, K.M., McIntyre, R.S., 2025. Impact of antidepressants on the composition of the gut microbiome: a systematic review and meta-analysis of in vivo studies. *J. Affect. Disord.* 369, 819–833.
- Gao, S., Li, S., Cao, S., Zhong, H., He, Z., 2024. Disclosing the key role of Fe/As/Cu in community co-occurrence and microbial recruitment in metallurgical ruins. *J. Hazard. Mater.* 480, 135889.
- Gong, H., Hu, J., Rui, X., Wang, Y., Zhu, N., 2024. Drivers of change behind the spatial distribution and fate of typical trace organic pollutants in fresh waste leachate across China. *Water. Res.* 263, 122170.
- Gu, B., Jiang, S., Wang, H., Wang, Z., Jia, R., Yang, J., He, S., Cheng, R., 2017. Characterization, quantification and management of China's municipal solid waste in spatiotemporal distributions: a review. *Waste Manag.* 61, 67–77.
- Hamm, J.N., Erdmann, S., Eloe-Fadros, E.A., Angeloni, A., Zhong, L., Brownlee, C., Williams, T.J., Barton, K., Carswell, S., Smith, M.A., Brazendale, S., Hancock, A.M., Allen, M.A., Raftery, M.J., Cavicchioli, R., 2019. Unexpected host dependency of Antarctic Nanohaloarchaeota. *P. Natl. Acad. Sci. U.S.A.* 116 (29), 14661–14670.
- Hao, L., Liu, Y., Chen, N., Hao, X., Zhang, B., Feng, C., 2021. Microbial removal of vanadium (V) from groundwater by sawdust used as a sole carbon source. *Sci. Total. Environ.* 751, 142161.
- Hernandez, D.J., David, A.S., Menges, E.S., Searcy, C.A., Afkhami, M.E., 2021. Environmental stress destabilizes microbial networks. *ISME J.* 15 (6), 1722–1734.
- Hu, P., Qian, Y., Radian, A., Xu, M., Guo, C., Gu, J., 2024. A global metagenomics-based analysis of BPA degradation and its coupling with nitrogen, sulfur, and methane metabolism in landfill leachates. *J. Hazard. Mater.* 477, 135395.
- Hu, Y., Hao, X., Wang, J., Cao, Y., 2016. Enhancing anaerobic digestion of lignocellulosic materials in excess sludge by bioaugmentation and pre-treatment. *Waste Manag.* 49, 55–63.
- Jayawardhana, Y., Kumarathilaka, P., Herath, I., Vithanage, M., 2016. Prasad, M.N.V. and Shih, K. (eds), pp. 117–148, Academic Press.
- Jeske, O., Schuler, M., Schumann, P., Schneider, A., Boedeker, C., Jogler, M., Bollschweiler, D., Rohde, M., Mayer, C., Engelhardt, H., Spring, S., Jogler, C., 2015. Plantomycetes do possess a peptidoglycan cell wall. *Nat. Commun.* 6, 7116.
- Jia, W., Zhang, M., Gao, F., Bai, H., He, L., He, L., Liu, T., Han, Y., Ying, G., 2024. Antibiotic resistome in landfill leachate and impact on groundwater. *Sci. Total. Environ.* 927, 171991.
- Jin, P., Bian, S., Yu, W., Guo, S., Lai, C., Wu, L., Zhao, H., Xiao, K., Liang, S., Yuan, S., Huang, L., Wang, S., Duan, H., Gan, F., Chen, W., Yang, J., 2023. Insights into leachate reduction in landfill with different ventilation rates: balance of Water, waste physicochemical properties, and microbial community. *Waste Manag.* 156, 118–129.
- Jun, Z., Fan, Z., Xiaofeng, L., Han, L., Hao, C., Li, L., Xiaowei, X., 2025. Insight of biological transformation process in the anaerobic codigestion of kitchen waste with landfill site closure leachate. *Sci. Rep.-U.K.* 15 (1), 22930.
- Kajihara, K.T., Hynson, N.A., 2024. Networks as tools for defining emergent properties of microbiomes and their stability. *Microbiome* 12, 184.
- Kim, M., Abdulazeez, M., Haroun, B.M., Nakhla, G., Keleman, M., 2019. Microbial communities in co-digestion of food wastes and wastewater biosolids. *Bioresour. Technol.* 289, 121580.

- Kvesić, M., Kalinić, H., Dželalija, M., Šamanić, I., Andričević, R., Maravić, A., 2022. Microbiome and antibiotic resistance profiling in submarine effluent-receiving coastal waters in Croatia. *Environ. Pollut.* 292, 118282.
- Laiju, A.R., Gandhimathi, R., Nidheesh, P.V., 2023. Removal of pharmaceutical and personal care products in landfill leachate treatment process. *Curr. Opin. Environ. Sci. Health* 31, 100434.
- Lepcha, A., Kumar, R., Dindhorja, K., Bhargava, B., Pati, A.M., Kumar, R., 2025. Metagenomic insights into the functional potential of non-sanitary landfill microbiomes in the Indian Himalayan region, highlighting key plastic degrading genes. *J. Hazard. Mater.* 484, 136642.
- Li, M., Zhou, W., Sun, M., Shi, W., Lun, J., Zhou, B., Hou, L., Gao, Z., 2024. Decoupling soil community structure, functional composition, and nitrogen metabolic activity driven by salinity in coastal wetlands. *Soil Biol. Biochem.* 198, 109547.
- Li, W., Wang, K., Wang, P., Yang, P., Xu, S., Tong, J., Zhang, Y., Yang, Y., Han, L., Ye, M., Shen, S., Lei, B., Liu, B., 2025. Impact of glyphosate on soil bacterial communities and degradation mechanisms in large-leaf tea plantations. *J. Hazard. Mater.* 483, 136626.
- Liao, C., Chen, L., Chen, B., Lin, S., 2010. Bioremediation of endocrine disruptor di-n-butyl phthalate ester by *Deinococcus radiodurans* and *Pseudomonas stutzeri*. *Chemosphere* 78 (3), 342–346.
- Linh, H.N., Tamura, H., Komiya, T., Saffarzadeh, A., Shimaoka, T., 2020. Simulating the impact of heavy rain on leaching behavior of municipal solid waste incineration bottom ash (MSWI BA) in semi-aerobic landfill. *Waste Manag.* 113, 280–293.
- Liu, L., Shi, L., Li, P., Ma, X., Hou, X., Jiang, S., Lv, J., Xu, H., Cheng, Y., Han, B., 2022. Seasonal dynamics survey and association analysis of microbiota communities, antibiotic resistance genes distribution, and biotoxicities characterization in landfill-leachate. *Ecotox. Environ. Safe.* 245, 114103.
- Liu, X., Wang, M., Liu, B., Chen, X., An, L., Nie, Y., Wu, X., 2025. Keystone taxa mediate the trade-off between microbial community stability and performance in activated sludges. *Nat. Water*.
- Lu, L., Yan, X., Kang, G., Qi, D., Tang, N., Zhu, R., Lv, C., Bu, Y., Zhang, H., Zhang, S., 2024. Unraveling drivers of per- and polyfluoroalkyl substances (PFASs) occurrence and removal in leachate: insights from disposal methods, geo-climate, and biodegradation. *Sci. Total. Environ.* 954, 176444.
- Lv, S., Li, Y., Zhao, S., Shao, Z., 2024. Biodegradation of typical plastics: from microbial diversity to metabolic mechanisms. *Int. J. Mol. Sci.* 25 (1).
- Mayer, R.E., Bofill-Mas, S., Egle, L., Reischer, G.H., Schade, M., Fernandez-Cassi, X., Fuchs, W., Mach, R.L., Lindner, G., Kirschner, A., Gaisbauer, M., Piringer, H., Blaschke, A.P., Girones, R., Zessner, M., Sommer, R., Farnleitner, A.H., 2016. Occurrence of human-associated bacteroidetes genetic source tracking markers in raw and treated wastewater of municipal and domestic origin and comparison to standard and alternative indicators of faecal pollution. *Water. Res.* 90, 265–276.
- Miao, L., Yang, G., Tao, T., Peng, Y., 2019. Recent advances in nitrogen removal from landfill leachate using biological treatments - A review. *J. Environ. Manage* 235, 178–185.
- Mor, S., Ravindra, K., 2023. Municipal solid waste landfills in lower- and middle-income countries: environmental impacts, challenges and sustainable management practices. *Process Saf. Environ.* 174, 510–530.
- National Bureau of Statistics Data, 2024. <https://www.stats.gov.cn>. China Statistical Yearbook 2024.
- Nishida, H., Beppu, T., Ueda, K., 2011. Whole-genome comparison clarifies close phylogenetic relationships between the phyla dictyoglomi and thermotogae. *Genomics* 98 (5), 370–375.
- Peng, F., Feng, X., Li, S., Yu, X., Chen, J., Liu, S., Ying, G., Liu, Y., 2025. Removal of emerging organic contaminants in a subsurface wastewater infiltration system: a preliminary study of microbial mechanism. *Water. Res.* 284, 123960.
- Qian, Y., Hu, P., Lang-Yona, N., Xu, M., Guo, C., Gu, J., 2024. Global landfill leachate characteristics: occurrences and abundances of environmental contaminants and the microbiome. *J. Hazard. Mater.* 461, 132446.
- Rutere, C., Knoop, K., Posselt, M., Ho, A., Horn, M.A., 2020. Ibuprofen degradation and associated bacterial communities in hyporheic zone sediments. *Microorganisms* 8 (8).
- Shi, Z., Campanaro, S., Usman, M., Treu, L., Basile, A., Angelidaki, I., Zhang, S., Luo, G., 2021. Genome-centric metatranscriptomics analysis reveals the role of hydrochar in Anaerobic digestion of waste activated sludge. *Environ. Sci. Technol.* 55 (12), 8351–8361.
- Spain, A.M., Krumholz, L.R., Elshahed, M.S., 2009. Abundance, composition, diversity and novelty of soil proteobacteria. *ISME J.* 3 (8), 992–1000.
- Steele, J.A., Countway, P.D., Xia, L., Vigil, P.D., Beman, J.M., Kim, D.Y., Chow, C.E., Sachdeva, R., Jones, A.C., Schwalbach, M.S., Rose, J.M., Hewson, I., Patel, A., Sun, F., Caron, D.A., Fuhrman, J.A., 2011. Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *ISME J.* 5 (9), 1414–1425.
- United Nations Environment Programme, 2024. *global waste management outlook 2024*.
- Valderrama, C., Diaz, L., Ceron, A., 2024. Trends of the ecological footprint and urban development: a systematic and bibliometric review. *Ecol. Front.* 44 (5), 865–873.
- Vijayalakshmi, V., Senthilkumar, P., Mophin-Kani, K., Sivamani, S., Sivarajasekar, N., Vasantharaj, S., 2018. Bio-degradation of bisphenol A by *Pseudomonas aeruginosa* PAb1 isolated from effluent of thermal paper industry: kinetic modeling and process optimization. *J. Radiat. Res. Appl. Sci.* 11 (1), 56–65.
- Wang, D., Wang, B., Han, X., Yang, X., Zhu, Y., Shi, Z., Indree, T., 2025a. Mapping of Hulun Buir steppe topsoil bulk density based on different sets of predictors. *Catena* 256, 109076.
- Wang, J., Shen, J., Wu, Y., Tu, C., Soininen, J., Stegen, J.C., He, J., Liu, X., Zhang, L., Zhang, E., 2013. Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. *ISME J.* 7 (7), 1310–1321.
- Wang, L., Wang, X., Wu, H., Fan, S., Lu, Z., 2025b. Integration of metagenomic analysis and metabolic modeling reveals microbial interactions in activated sludge systems in response to nanoplastics and plasticizers. *Water. Res.* 271, 122863.
- Wang, Z., Feng, K., Lu, G., Yu, H., Wang, S., Wei, Z., Dang, N., Wang, Y., Deng, Y., 2022. Homogeneous selection and dispersal limitation dominate the effect of soil strata under warming condition. *Front. Microbiol.* 13, 801083.
- Wei, Y., Li, Z., Ran, W., Yuan, H., Li, X., 2021. Performance and microbial community dynamics in anaerobic co-digestion of chicken manure and corn stover with different modification methods and trace element supplementation strategy. *Bioresource Technol.* 325, 124713.
- Wiegand, S., Jogler, M., Jogler, C., 2018. On the maverick planctomycetes. *FEMS Microbiol. Rev.* 42 (6), 739–760.
- Wilkes, R.A., Aristilde, L., 2017. Degradation and metabolism of synthetic plastics and associated products by *Pseudomonas* sp.: capabilities and challenges. *J. Appl. Microbiol.* 123 (3), 582–593.
- Wu, D., Huang, X., Sun, J., Graham, D.W., Xie, B., 2017. Antibiotic resistance genes and associated microbial community conditions in aging landfill systems. *Environ. Sci. Technol.* 51 (21), 12859–12867.
- Wu, J., Wang, X., Fu, Y., Yu, Z., Meng, F., 2025. Recruiting high-efficiency denitrifying consortia using *Pseudomonas aeruginosa*. *Water. Res.* 277, 123303.
- Yang, J., Xiang, J., Xie, Y., Yu, K., Li, J., Wang, H., Li, P., Gin, K.Y., He, Y., 2022. Removal behavior and key drivers of antibiotic resistance genes in two full-scale leachate treatment plants. *Water. Res.* 226, 119239.
- Zhang, C., Ren, Z., 2023. The role of subsurface ice in sustaining bacteria in continental and maritime glaciers. *Sci. Total. Environ.* 896, 165324.
- Zhang, L., Li, J., Li, Y., Min, X., Kong, D., Han, Y., Jiang, C., Xiao, H., Liang, B., Zhang, Z., 2024a. Tracing residual patterns and microbial communities of pharmaceuticals and personal care products from 17 urban landfills leachate in China. *J. Hazard. Mater.* 477, 135295.
- Zhang, Z., Liu, Y., Liu, K., Chen, Y., Guo, X., Ji, M., Zhao, W., 2024b. Supraglacial and subglacial ecosystems contribute differently towards proglacial ecosystem communities in Kuoyonggangri Glacier, Tibetan Plateau. *Commun. Earth. Environ.* 5 (1).
- Zhao, R., Liu, J., Feng, J., Li, X., Li, B., 2021. Microbial community composition and metabolic functions in landfill leachate from different landfills of China. *Sci. Total. Environ.* 767, 144861.
- Zhou, J., Ning, D., 2017. Stochastic Community Assembly: does it matter in microbial ecology? *Microbiol. Mol. Biol. Rev.* 81 (4).
- Zhou, L., Wu, Y., Zhou, Y., Zhang, Y., Xu, H., Jang, K., Doling, J., Spencer, R.G.M., Jeppesen, E., 2024. Terrestrial dissolved organic matter inputs drive the temporal dynamics of riverine bacterial ecological networks and assembly processes. *Water. Res.* 249, 120955.
- Ziels, R.M., Sousa, D.Z., Stensel, H.D., Beck, D.A.C., 2018. DNA-SIP based genome-centric metagenomics identifies key long-chain fatty acid-degrading populations in anaerobic digesters with different feeding frequencies. *ISME J.* 12 (1), 112–123.