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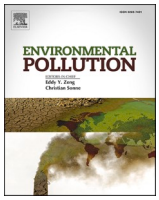

Inverse relationship between microplastic abundance and microplastic biodegradation potential in soils from both natural and anthropogenic sites in Lithuania

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ABSTRACT

Biodegradation is a sustainable strategy for removing microplastics (MP), owing to its environmental compatibility and cost-effectiveness. This study investigated MP distribution, characterized microbial communities, and assessed the presence of plastic-degradation-associated genes across three locations in Lithuania: an operating Kazokiškės landfill, a closed Kariotiškės landfill, and a natural grassland. Polyamide was the most abundant plastic across all locations, followed by polyethylene, with the highest MP concentrations detected in the operating landfill. Sequencing of 16S rRNA and ITS genes revealed site-specific microbial communities with significantly different β-diversity. Operating landfill soils were enriched in plastic-associated genera, including *Methylocaldum*, *Rhizobium*, *Brevundimonas*, *Aspergillus*, and *Malassezia*. Based on the microbiome analysis, both studied landfills had distinct communities yet shared some plastic-degradation-associated genera. The key novel finding is contrary to conventional assumptions: the active landfill had the most MP and the highest number of plastic-associated microbial genera, yet the narrowest range of plastic-degradation-associated genes was limited to alkane hydroxylases and styrene monooxygenases, possibly due to suppression by constant input of fresh, labile organic waste. The grassland and closed landfill, despite lower MP abundance, hosted a broader diversity of studied genes: cutinases in both and urethanases exclusively in the grassland, suggesting broader plastic polymer utilization potential in non-operating landfill soils. These findings suggest that ordinary and closed-landfill soils are an underappreciated reservoir of plastic-degradation potential, and that the closed-landfill topsoil microbiome may be influenced by buried waste beneath it.

1. Introduction

Increasing amounts of microplastics (MP) are a rapidly growing concern across social and scientific areas due to their accumulation in the environment (Lalrinfela et al., 2024). Plastics are low–cost to produce, lightweight, physically and chemically stable, and are hardly biodegradable (Lv et al., 2024). MP are plastic particles smaller than 5 mm in diameter (Lv et al., 2024) and occur in various shapes, including beads, fragments, fibers, and films (Fox et al., 2024). MP can be found in diverse locations, ranging from waste management facilities to the top of Mount Everest (Napper et al., 2020), Arctic sites, the Alps (Bergmann et al., 2019), as well as in food products, including honey (Basaran et al., 2024), the surface of fruits (Nassar et al., 2025), and seafood (Süssmann et al., 2026). MP pollution was first documented in marine environments in the 1970s (Andrady, 2011), but the discovery that most MP in the oceans originate from land, where MP are found at higher concentrations, drew attention to studies of soil pollution by MP. Different types of plastics have different environmental impacts: some are more recyclable or biodegradable than others (Zhang et al., 2022), can differently absorb and transport contaminants such as hydrophobic organic pollutants, heavy metals, and antibiotics (Menéndez-Pedriza and Jaumot, 2020), slowly release plasticizers and other additives (Menger et al., 2024), and can alter microbial communities by creating a new niche, the “plastisphere,” for microorganisms to grow. These particles, along with microorganisms, may be transported through air, water, or by fauna, altering different microbial communities in which

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they deposit (Senathirajah and Pattiaratchi, 2025).

Although recycling has gradually increased, most plastic waste ends up in the environment (Wojnowska-Baryła et al., 2022). Municipal landfills are among the most widely used methods of waste management worldwide, including Europe, where they remain indispensable. For example, in Europe, about 23% of all plastic waste is landfilled, 35% recycled, and 42% incinerated (Zu Castell-Rüdenhausen and Marttila, 2025), while part of the plastic waste ends up in the environment. Hence, various MP are found in large amounts in landfills. Landfills represent one of the most dynamic terrestrial ecosystems, where microbial communities are highly diverse, with the potential to metabolize complex substrates (Sekhohola-Dlamini and Tekere, 2020) due to the spectrum of extracellular and intracellular enzymes (Luo et al., 2025). Microorganisms such as fungi and bacteria may attach to the surface of the petro–polymer, propagate on it, creating a plastisphere, and utilize plastic as a carbon source (Agostini et al., 2021). Therefore, it is worthwhile to screen for microorganisms and/or enzymes capable of degrading various MP in both open and closed landfills since biological degradation of MP is considered an environmentally friendly and cost–effective strategy (Zeng et al., 2025).

Hydrolysis and oxidation are the two main types of reactions that occur during plastic biodegradation (Heris, 2024). Among hydrolases, esterases (e.g., cutinases, whose active pockets are large enough to accommodate high molecular weight substrates (Maurya et al., 2020), carboxylesterases, lipases), proteases, and ureases exhibit catalytic activity on plastic substrates such as polyethylene terephthalate (PET) and polyurethane (PU), as they contain hydrolyzable ester and urethane bonds (Maestri et al., 2023; Suresh et al., 2025). Several examples are: *Fusarium solani* cutinase, due to its ability to modify the surface of PET (Okal et al., 2023); cutinase 1 and cutinase 2 from *Thermobifida fusca*, capable of hydrolyzing ester bonds of PET (Müller et al., 2005); polyester hydrolase Leipzig 7 (PHL7), which exhibits high activity on amorphous PET (Sonnendecker et al., 2022); Leaf Branch Compost Cutinase (LCC), capable of PET cleavage (Sulaiman et al., 2012); *Ideonella sakaiensis* specific PETase and MHETase that are involved in PET utilization; *Pseudomonas chlororaphis* polyurethanase (lipase) PueA, due to the ability to utilize PU (Stern and Howard, 2000); *Lysinibacillus fusiformis* urethanase (glutamyl–tRNA– amidotransferase), with its ability to target the urethane, amide, and urea bonds (Raczyńska et al., 2024); as well as *Rhodococcus erythropolis* MP_{50} amidase, due to the activity against urethane bonds (Raczyńska et al., 2024; Kang et al., 2021).

Oxidoreductases represent another key class of enzymes involved in plastic biodegradation. Among them are monooxygenases, peroxidases, and laccases (Suresh et al., 2025). Alkane 1-monooxygenases (alkane hydroxylases, AHs) potentially attack highly recalcitrant polyethylene (PE) and polystyrene (PS) (Lv et al., 2024). Further examples of oxidoreductases that can potentially target MP for biodegradation include: laccase–like multicopper oxidases (LMCOs) from *Rhodococcus opacus* R7 (Zampolli et al., 2023), which have the ability to degrade PE; styrene monooxygenases (SMOs), which attack and oxygenate the vinyl side chain of the PS intermediate-product, styrene (Oelschlägel et al., 2018) as a step in the PS degradation pathway (Lv et al., 2024). Therefore, we screened samples for these enzymes.

Microbial community composition and functional capabilities vary considerably among environments with different MP profiles. While biodegradation capabilities are still being explored worldwide, it is expected that the microbiome has greater MP-degrading capacity in high MP environments. In Lithuania, the implementation of EU landfill regulations resulted in the closure of landfills. This provides an opportunity to compare reclaimed landfill sites, active landfills, and natural grasslands that have never been subjected to waste disposal activities, which may provide insights into microbial adaptation and biodegradation potential. In the current study, amplification of 16S rRNA and ITS genes enabled characterization of bacterial and fungal communities, including uncultivated microorganisms, whereas detection of functional genes may reveal potential for biodegrading MP. This is the first study to combine microplastic characterization, soil microbiome composition, and screening for plastic-degradation-associated genes, such as hydrolases and oxidoreductases, across three locations in Lithuania: an active Kazokiškės landfill, a closed Kariotiškės landfill, and a grassland with no prior farming, mulching, or exposure to waste.

2. Methods

2.1. Sample collection

Samples analyzed in this study were collected from both natural and anthropogenic sites in 2021. 7 samples were collected from Kazokiškės landfill, which has been operating since 2007 (KZ, 54.807033° N, 24.821911° E). KZ1.T sample was taken from the active site (waste materials intermixed with soil cover); other Kazokiškės soil samples were taken from inactive parts of the operating landfill: KZ2.T (operational during 2007–2009); KZ3.T, KZ5.T, and KZ5.B (operational during 2009–2014); KZ4.T and KZ4.B (operational during 2014–2021). Another 8 samples were collected from Kariotiškės landfill (KR, 54.715513° N, 24.959925° E). This landfill operated from 1987 to 2008 and was covered with a revegetation soil layer after closure. Maps of landfills and collection points are presented in Supplementary Materials, Fig. S1. To compare artificial landfill soils with natural soils, 8 samples were collected from the grassland in Molėtai district, Lithuania (C, 55.378529° N, 25.360082° E). This area has been grassland for >24 years, has not been affected by agricultural activities, has not been exposed to waste, and is 17 km from the nearest town. Additionally, C4.T and C4.B soil samples were collected from the forest, ~1 km from the grassland. A detailed description of the soil samples and their physicochemical properties is presented in Supplementary Materials, File S1.

Letters T or B indicate that the sample was collected from the top (T) or at a depth of 10 cm (B) at the sampling point. During transportation, samples were kept in a sampling bag with an ice pack in a polyurethane foam cool box and stored at −20 °C or −70 °C for further analysis.

2.2. Extraction and identification of MP

To identify the distribution of microplastics, a group was formed by pooling equal amounts of samples from the same location. Each location was represented by one group. MP extraction from complex matrices involves multiple steps, including density separation, filtration, digestion of organic material, and removal of inorganic compounds (Larrea et al., 2025). The method proposed by Ragoobur et al. (2021) was slightly adapted, with minor modifications based on the level of sample contamination (Ragoobur et al., 2021). Previous studies have reported recovery rates of 79–98% for similar microplastic extraction protocols, suggesting that the extraction method applied in the present study is suitable and provides reliable results (Ruffell et al., 2024; Lares et al., 2019; Hurley et al., 2018). However, in this study, the matrix-specific recovery tests were not performed. As recovery efficiency can vary depending on polymer type, particle size, morphology, and soil matrix, the microplastic concentrations reported here are presented as apparent abundance rather than absolute values. The full microplastic extraction procedure, as well as quality assurance and control, are provided in Supplementary Materials, File S2, Chapters 1.1.1 and 1.1.2, respectively.

The chemical composition of the <1 mm MP fraction was analyzed using a micro-FTIR LUMOS II (Bruker, Germany) equipped with a focal-plane-array (FPA) detector. Due to the size detection limit of the micro-FTIR analysis (>5 μm), only microplastics larger than this threshold were identified in this study. Infrared spectra were recorded in transmission mode over the wavenumber range of 4000–680 cm^{-1} at a resolution of 4 cm^{-1}, collecting four scans per sample. Background measurements were taken before analyzing each sample. The obtained data were processed using OPUS (version 9.1.4) (Bruker Optik)

software, employing the MP–ID function for automated identification and characterization of MP, including their chemical composition, size, and shape. Polymer identification was accepted only for matches above 70% in accordance with recommendations from previous studies (Rathore et al., 2023). For justification, see Supplementary Materials, File S2, Chapter 1.1.3.

2.3. Metagenomic DNA extraction and microbiome analysis

Microbiome population analysis was performed by extracting metagenomic DNA from an individual sample. The ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Cat. No. D4300) was used to isolate metagenomic DNA from the samples in accordance with the manufacturer's instructions, with a few alterations: increased amount of starting material (up to 1000 mg) and incubation with DNase/RNase-free water was prolonged for 10 min. The concentration and quality of the isolated genomic DNA were evaluated with a NanoDrop™ One Microvolume UV–Vis (Thermo Scientific, USA) or NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA). Isolated DNA samples were stored at −20 °C for further analysis.

The composition of the bacterial, archaeal, and fungal communities was determined by sequencing the amplicons of the bacterial and archaeal V3–V4 16S rRNA and fungal ITS1 genes. Sequencing was performed by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) on Illumina paired–end (250 bp) platform. Primers and amplicon lengths are presented in the Supplementary Materials, File S2, Chapter 1.2.

The letter F before the name of the sample indicates that analysis was performed for ITS ASV. Samples that passed sequencing quality requirements and produced successful sequencing data were included in microbiome analysis.

The 10 cm depth did not influence microbial community composition, as indicated by alpha- and beta-diversity analyses; therefore, samples were analyzed by location.

2.4. 16S rRNA and ITS data preparation

Raw reads from different sequencing batches were processed separately using QIIME2 (version 2025.10) (Bolyen et al., 2019) with default settings, unless stated otherwise. Barcodes and adapters from 5' ends were trimmed using cutadapt (Martin, 2011), and untrimmed sequences were discarded. Read quality was assessed before and after trimming using FastQC (Babraham Bioinformatics - FastQC) and MultiQC (Ewels et al., 2016). Amplicon sequence variants (ASV) were constructed using the DADA2 workflow (Callahan et al., 2016). Parameters used in the DADA2 workflow are described in the Supplementary Materials, File S2, Chapter 1.3.

Taxonomic classification was performed using the q2-feature-classifier plugin (Bokulich et al., 2018) with a Naive Bayes classifier at a confidence threshold of 70%. For 16S rRNA V3-V4 sequences, the classifier was trained on the SILVA v138.2 SSU Ref NR 99 database (Quast et al., 2012). For ITS sequences, the UNITE database (version 10.0, released 19 February 2025) was used (Abarenkov et al., 2024). For ITS data, only ASV classified as kingdom Fungi (73.4% of all ITS reads) were retained for downstream analysis. The 16S rRNA dataset comprised 18 samples, totaling 1,012,325 sequences (an average of 56,240 reads per sample). The ITS dataset comprised 17 samples, totaling 706,538 sequences (an average of 41,561 reads per sample). ASV counts for different-level classification are presented in Supplementary Materials, Table S1.

2.5. Amplification of genes associated with MP degradation

PCR analysis of metagenomic DNA was performed using 3–100 ng of DNA per 50 µl reaction. 2 µM of primers (Invitrogen, Lithuania, and Eurofins, Germany) targeting genes or gene fragments encoding enzymes potentially involved in plastic biodegradation were used. Reactions were performed with DreamTaq PCR Master Mixes (Thermo Scientific, USA). Conditions for each PCR reaction were chosen based on data from the literature or developed in this study (for the primers designed in this study). For the negative control, an equal volume of water was added instead of metagenomic DNA. PCR reactions were performed in a T100 Thermal Cycler (Bio-Rad Laboratories, USA) and in a PTC Tempo Thermal Cycler (Bio-Rad Laboratories, USA). Three PCR repeats per individual sample were performed to reduce the risk of false-negative results. Genes, PCR conditions, primers, and amplicon lengths are listed in Supplementary Materials, Table S2. As no positive controls were performed, the absence of the gene is considered "not detected".

Tools used for primer design included Primer–BLAST (NCBI) and OligoArchitectTM (Sigma-Aldrich). The criteria involved increased annealing temperature due to metagenomic DNA, absence or minor presence of secondary structures and primer–dimers, and extended length (26–30 bp). Designed primers are indicated in Supplementary Materials, Table S2.

2.6. Amplicon sequencing

Isolated amplicons from electrophoresis gels (representative gel electrophoresis images are presented in Supplementary Materials, File S3; extraction of amplicons is described in Supplementary Materials, File S2, Chapter 1.4) were diluted according to the requirements of the SeqVision sequencing facility (Lithuania) and distributed into pools, each containing 1–3 amplicons differing in length by at least 200 bp. The pooled samples were barcoded using Native Barcoding Kit 96 V14 (SQK–NBD114.96) and sequenced on a MinION™ device (R10.4.1 Flow Cell) at the SeqVision sequencing facility. The resulting reads were demultiplexed by barcode, yielding datasets for each pool.

2.7. Bioinformatic analysis of functional gene amplicons

Primary bioinformatic analysis was performed by SeqVision (Lithuania). Obtained reads were basecalled using Dorado (version 0.9.0) with the dna_r10.4.1_e8.2_400bps_sup@v5.0.0 model. Read length histogram (200 bins) was drawn using Matplotlib, and reads were split by length using Seqkit (version 2.6.0) to represent the expected read lengths. Each read file was then analyzed using amplicon_sorter, which combines sequences into a consensus based on their nucleotide similarity (at least 96% in this study) and length (differing by no more than 8% in this study). This method is not well-suited for resolving strain-level, or in some cases species-level, identity among closely related sequences, as single-nucleotide substitutions critical for strain discrimination may be averaged out during consensus generation. However, sequences with $\geq$ 5% divergence in the target gene are reliably resolved into distinct consensus groups (Vierstraete and Braeckman, 2022). Consensus sequences were analyzed with the BLASTx (Basic Local Alignment Search Tool X) tool available on the Galaxy Web service, which performs protein homology searches. The search parameters were set to default, and the number of hits displayed was reduced to 5–10.

2.8. Microbiome statistical analysis

Microbiome statistical analyses were performed using R (version 4.4.3) (R Core Team, 2025) with the mia microbiome analysis framework (Borman et al., 2026) and TreeSummarizedExperiment (Huang et al., 2021). Rarefaction was used as the method that consistently removes correlation between sequencing depth and alpha and beta diversity analyses (Schloss, 2024) and performed by repeated subsampling (100 iterations) to the sequencing depth of the least abundant sample, and diversity values were averaged across iterations. Sequencing depth for 16S rRNA ASVs was set to 49,732 reads, for ITS ASVs – 23,539 reads. Statistical significance was set at $\alpha = 0.05$.

Alpha diversity was assessed at the ASV level using the Shannon diversity index (Shannon, 1948), observed feature richness, and the Berger-Parker dominance index (Berger and Parker, 1970). Beta diversity was assessed using Jaccard (presence-absence) and Bray-Curtis (Bray and Curtis, 1957) (abundance-weighted) dissimilarity matrices, computed on rarefied data with 100 repeated iterations. Ordination was performed using Principal Coordinates Analysis (PCoA), with variance explained by each axis derived from eigenvalue decomposition. Statistical analyses of taxonomic composition, alpha, and beta diversity are further described in Supplementary Materials, File S2, Chapter 1.5.

For further identification of differences in specific taxa between groups, analysis using MaAsLin 3 was performed (Nickols et al., 2026). All genera were analyzed separately for 16S rRNA and ITS. Statistical analyses of differential abundance and correlation analysis between genes and environmental variables are further described in Supplementary Materials, File S2, Chapter 1.5.

3. Results and discussion

3.1. MP identification and distribution in the studied samples

The composition of detected plastics varies by sample location (Fig. 1a–d), with the highest apparent concentration of particles less than 1 mm in diameter in KZ samples (495 particles/g) and the lowest in KR (170 particles/g). The MP levels in the tested landfills are higher than those in the Lapės landfill in Lithuania (Sholokhova et al., 2023) and in other landfills (Leitão et al., 2023; Yatim et al., 2025; Lin et al., 2023). Grassland soil samples contained a higher abundance of MP than other soil samples worldwide (Leitão et al., 2023; Crossman et al., 2020; Feng et al., 2021; Liu et al., 2022; Feng et al., 2023; Van Den Berg et al., 2020; Scheurer and Bigalke, 2018; Tunali et al., 2022; Adhikari et al., 2024; Choi et al., 2021). The comparative table is presented in Supplementary Materials, Table S3. The most abundant plastic was polyamide (PA), which was detected in all tested sample mixtures. PE was also detected in all groups, with the highest apparent concentration in KZ, lower in KR, and least in C. Polypropylene (PP), similarly to PE, was more abundant in KZ but less in KR (1.2 particles/g, 0.71%) and in C (0.8 particles/g, 0.35%) groups. Ethylene–Vinyl Acetate (EVAc) plastic was found in higher relative abundance in KZ and KR, but less in relative abundance in C samples (1.8 particles/g, 0.78%). PET was absent in KR samples, and accounted for 0.09% (0.2 particles/g) and 0.08% (0.4 particles/g) in C and KZ, respectively. Poly(methyl methacrylate) (PMMA) was detected in all tested samples: 1.6 particles/g, 0.32% in KZ; 0.2 particles/g, 0.12% in KR; and 0.2 particles/g, 0.09% in C samples. PS and polyvinyl chloride (PVC) were detected only in the KZ samples (0.8 particles/g, 0.16%, and 0.8 particles/g, 0.16%, respectively). The remaining apparent abundance of larger plastic particles was 135 mg for KZ. The absence of specific plastics does not indicate the complete absence in the studied locations. To provide additional evidence for the identification of microplastic polymer types, FTIR spectra of selected particles from the analyzed samples, along with their characteristic absorption bands, are presented in Supplementary Materials, Fig. S2. The provided spectra confirm the presence of identified polymer types and support the reliability of the MP classification reported in this study.

The size distribution analysis (Fig. 1b–e) showed that the most abundant plastic size was 20–50 μm (42–58%) across all locations, and KZ had the highest variety of size distribution. Analysis of MP shapes (Fig. 1c–f) showed that fragments accounted for the largest proportion

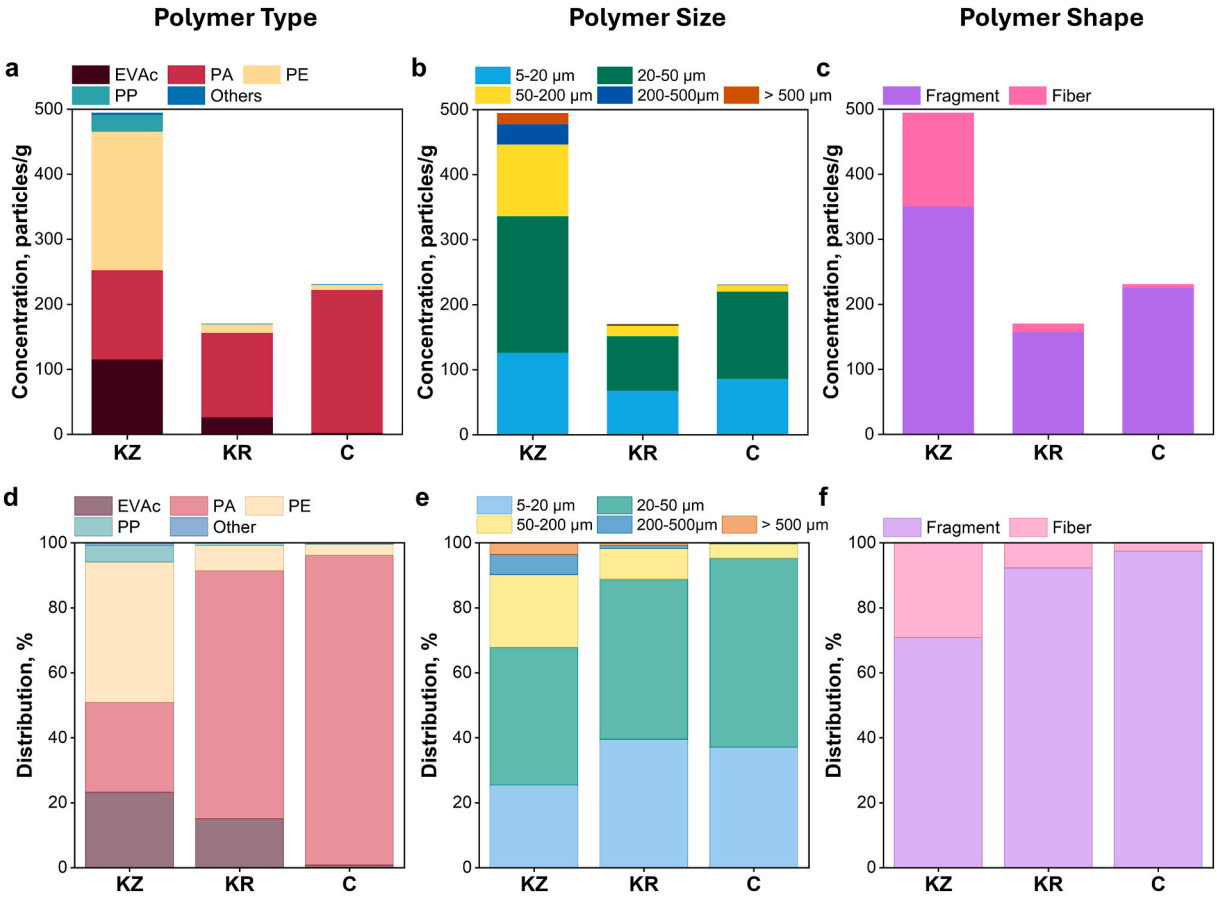

Fig. 1. MP type (a,d), size (b,e), and form (c,f) distribution in the studied groups: operating Kazokiškės landfill (KZ), closed Kariotiškės landfill (KR), and grassland (C) based on concentration (top row) and distribution (bottom row). EVAc – ethylene-vinyl acetate copolymer; PA – polyamide; PE – polyethylene; PP – polypropylene.

of particles. Exceptionally, fibers accounted for >25% in KZ samples.

3.2. Taxonomic analysis

3.2.1. Amplicon sequence variant (ASV) analysis

The DADA2 analysis aimed to prepare ASV and link paired–end reads. Final per-sample read counts after DADA2 plus downstream filtering (group subset, Kingdom Fungi for ITS, zero-count ASV pruning) vs the percentage of the pre-DADA2 input reads they represent are presented in Supplementary Materials, Fig. S3. 16S rRNA and ITS sequences accounted for 49,732 to 68,517 and 23,539 to 67,594 of the final sequence count and 46–60% and 22–61% of ASV after DADA2 reads, respectively. In general, the percentage of ASV reads was considered good quality for further analysis for both cases.

3.2.2. Alpha and beta diversity analysis

The microbiome alpha–diversity analysis (Shannon index and Observed features) based on 16S rRNA and ITS ASV indicated that KR, KZ, and C samples had similar diversity (Fig. 2a,b,d,e). Statistical testing using the Kruskal-Wallis test showed no significant differences in alpha diversity between groups in all tested cases (results provided in Supplementary Materials, Table S4). The Berger–Parker (DBP) index (Fig. 2c–f) indicates that the tested samples were not dominated by a single species.

The PERMANOVA pairwise analysis of beta diversity for 16S rRNA ASV indicated significant differences in community composition among the three study locations. Jaccard distance (Fig. 2g) analysis of bacterial 16S rRNA sequences indicated that all groups formed separate but close clusters (R^2 values between 0.116 and 0.148, F values between 1.19 and 1.79, p–values < 0.05). Bray–Curtis distances (Fig. 2h) showed stronger separation between the C and the other two groups ($R^2 = 0.28$, $F = 4.67$, p–value < 0.05 and $R^2 = 0.28$, $F = 3.48$, p–value < 0.05 for KR and KZ, respectively). The KR and KZ groups formed closer but moderately separate clusters ($R^2 = 0.147$, $F = 1.55$, $p < 0.05$), suggesting greater similarity between the two groups. The PERMANOVA pairwise analysis of Jaccard and Bray–Curtis distances for ITS ASV (Fig. 2i and j) indicated an even distribution but significant differences among all studied locations in every case (R^2 values between 0.097 and 0.157, F values

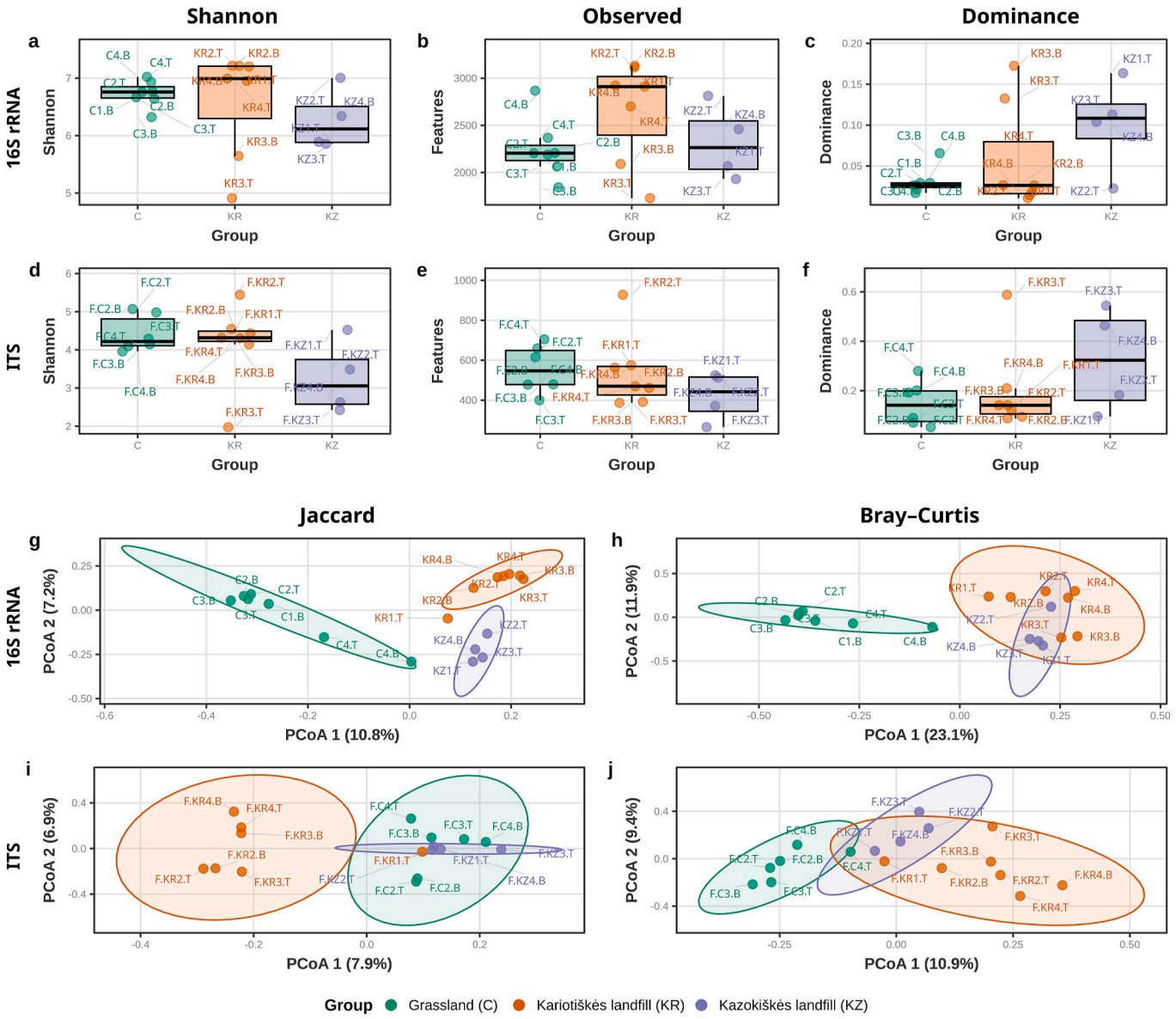

Fig. 2. Alpha diversity analysis of grassland (C), closed Kariotiškės landfill (KR), and operating Kazokiškės landfill (KZ) based on 16S rRNA and ITS ASV: Shannon index analysis for 16S rRNA (a) and ITS (d), Observed features for 16S rRNA (b) and ITS (e), and DBP for 16S rRNA (c) and ITS (f); Beta diversity analysis based on 16S rRNA and ITS ASV: Jaccard analysis for 16S rRNA (g) and ITS (i), and Bray–Curtis analysis for 16S rRNA (h) and ITS (j).

between 1.06 and 1.79, $p < 0.05$). A detailed result of the PERMANOVA pairwise analysis is presented in Supplementary Materials, Table S4. It should be noted that PERMANOVA tests (except for the 16S rRNA Jaccard) may be confounded by unequal group dispersions, as indicated by the betadisper analysis and shown in Supplementary Materials, Table S4.

3.2.3. Taxonomic composition at the phylum level

The results of phyla-level bacterial 16S rRNA and fungal ITS sequences of analyzed groups are presented in Fig. 3. In total, 997,503 bacterial ASV reads were found, with a mean abundance with 95% CI of 53,334 ± 4090 for C, 56,510 ± 5062 for KR, and 57,148 ± 4391 for KZ. Actinomycetota was the most abundant bacterial phylum in C and KR, and the 2nd most abundant in KZ samples. Pseudomonadota was the 2nd-largest phylum in C and KR, and the largest in KZ.

A total of 706,538 ASV fungal reads were found with a mean abundance with 95% CI of 44,560 ± 8346 for C, 38,898 ± 13,741 for KR, and 41,723 ± 20,848 for KZ. Ascomycota was the most abundant phylum across the samples, followed by Basidiomycota. In total, 19 fungal phyla were detected in the samples.

The presence of plastic particles is one of the factors that influences the structure of soil microbial communities. Among the dominant phyla detected in soil bulk, Pseudomonadota, Bacillota, Actinomycetota, Bacteroidota, and Cyanobacteriota (detected in all locations) were also found in biofilms on various plastics (Zhai et al., 2023; MacLean et al., 2024), according to previous studies. On the contrary, even though Chloroflexota and Acidobacteriota are present in the plastispheres, they are associated with a decrease in relative abundance on plastic biofilm compared to the surrounding environments (Wang et al., 2025). Accordingly, there was a slight decrease in relative abundance at the plastic-richer location (KZ), as indicated by median values. The two largest fungal phyla, i.e., Ascomycota and Basidiomycota, are recognized as the most potent plastic-degradation-associated taxa and are reported to occur on plastic particles (Khatua et al., 2024; Ekanayaka et al., 2022).

3.2.4. Taxonomic composition at the genus level

The composition of bacterial genera in C samples (Fig. 4a) showed that *Gaiella* was the most relatively abundant genus. The distribution among the C group samples showed similar patterns of the top 10 genera. By contrast, the distribution of genera in KR samples (Fig. 4c) differed. *Methylocystis* is considered the most abundant genus due to the KR3 samples. There, it represented 3.3% and 23% for KR3.B and KR3.T, respectively. In KR3.T, this genus accounted for the highest relative abundance among the Beijerinckiaceae family (27.5%) members. An uneven distribution of genera in relative abundance could be seen in the KZ group as well (Fig. 4e). *Terrimicrobium* was the most abundant genus in the KZ group due to KZ3.T, where it represented 14.1%. It constituted the majority of the family Terrimicrobiaceae, which was the most abundant in the KZ3.T (14.2%). *Methylocaldum* was another dominant genus in the KZ3.T (9.19%) sample, accounting for most of Methylococcaceae family (9.23%).

Taxonomic analysis revealed diverse relative abundances of fungal genera across all samples and groups. *Mortierella* was the most abundant genus in the C group (Fig. 4b), due to high relative abundance in C4.T and C4.B (10.25–31.84%). Only *Exophiala* showed a distribution similar to that across C samples. Greater genus-level variation in relative abundance was observed in the KR and KZ groups (Fig. 4d and f). *Scedosporium* accounted for 75.8% in the KR3.T and was therefore identified as the most abundant genus in the KR group. It correlates with the high relative abundance of Microascaceae family (75.9%) in the KR3.T. Among the KZ group, each sample had its own most relatively abundant genus. The findings were anticipated, since fungal communities can differ even over short distances (Janowski and Leski, 2023; Cook et al., 2022).

A correlation between the abundance of specific plastics and the microorganisms associated with them was investigated based on two complementary approaches: taxonomic composition analysis and cross-referencing the identified genera with published literature reporting their associations with plastic-degrading capabilities. Site-specific genera were assigned to locations based on the forest plot analysis: β (log-scale change) for KR or KZ relative to the reference group C, with Wald 95% CI and detected MP. The forest plots are presented in Supplementary Materials, Fig. S4 for both bacterial rRNA and fungal ITS. Additionally, exceptions in high relative abundance are observed in the samples. The site-specific plastic-degradation-associated genera and detected MP are presented in Table 1.

In the C location, PA was detected at a higher apparent abundance; thus, the presence of *Arthrobacter* might be related. Other genera might be associated with detected PE and PET MP. In the KR site, PA, PE, and PP MP were abundant, suggesting a possible connection between PA and *Agromyces*. None of the fungal genera were site-specific to the KR location. The KZ had the highest apparent abundance of PE, PP, PET, PS, and PVC, as well contained PA, and a higher number of site-specific plastic-degradation-associated genera. *Malassezia* (associated with PVC utilization (El-Dash et al., 2023)) is usually detected in the microbiome of animal skin; the presence of this yeast at the KZ1.T (the landfill operating site) reveals a distinct yet still poorly understood genetic profile of the landfill. *Bacillus* and *Pseudomonas* have the highest numbers of species detected as plastic utilizers and were abundant across all tested locations (Suresh et al., 2025; Bacha et al., 2023; Wilkes and Aristilde, 2017).

The KZ and KR locations showed the greatest similarity in the abundance of plastic-associated genera. Since PA, PE, and PP MP were

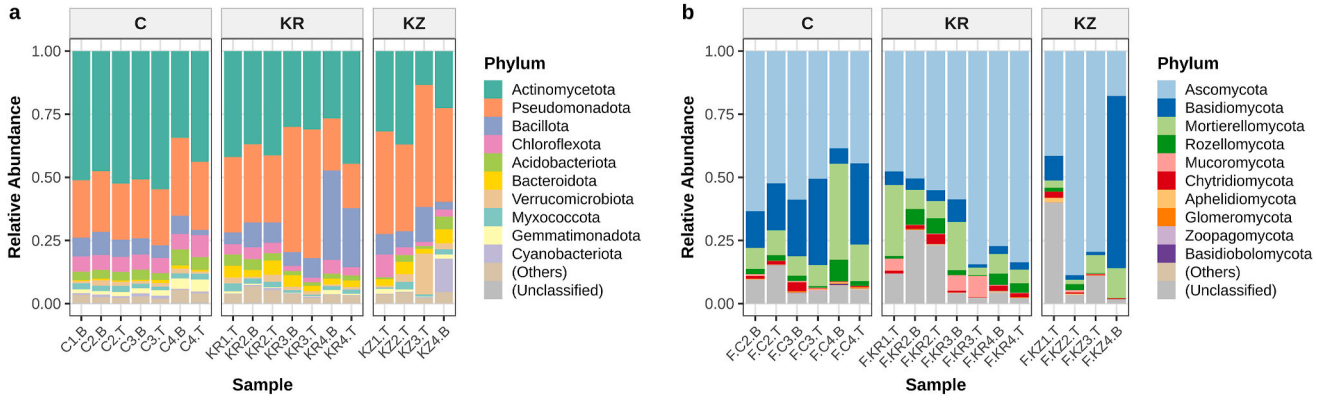

Fig. 3. Taxonomic composition of phyla-level based on bacterial 16S rRNA (a) and ITS (b) ASV in studied locations: grassland (C), closed Kariotiškės landfill (KR), and operating Kazokiškės landfill (KZ). Results are represented as the 10 most abundant phyla according to relative abundance.

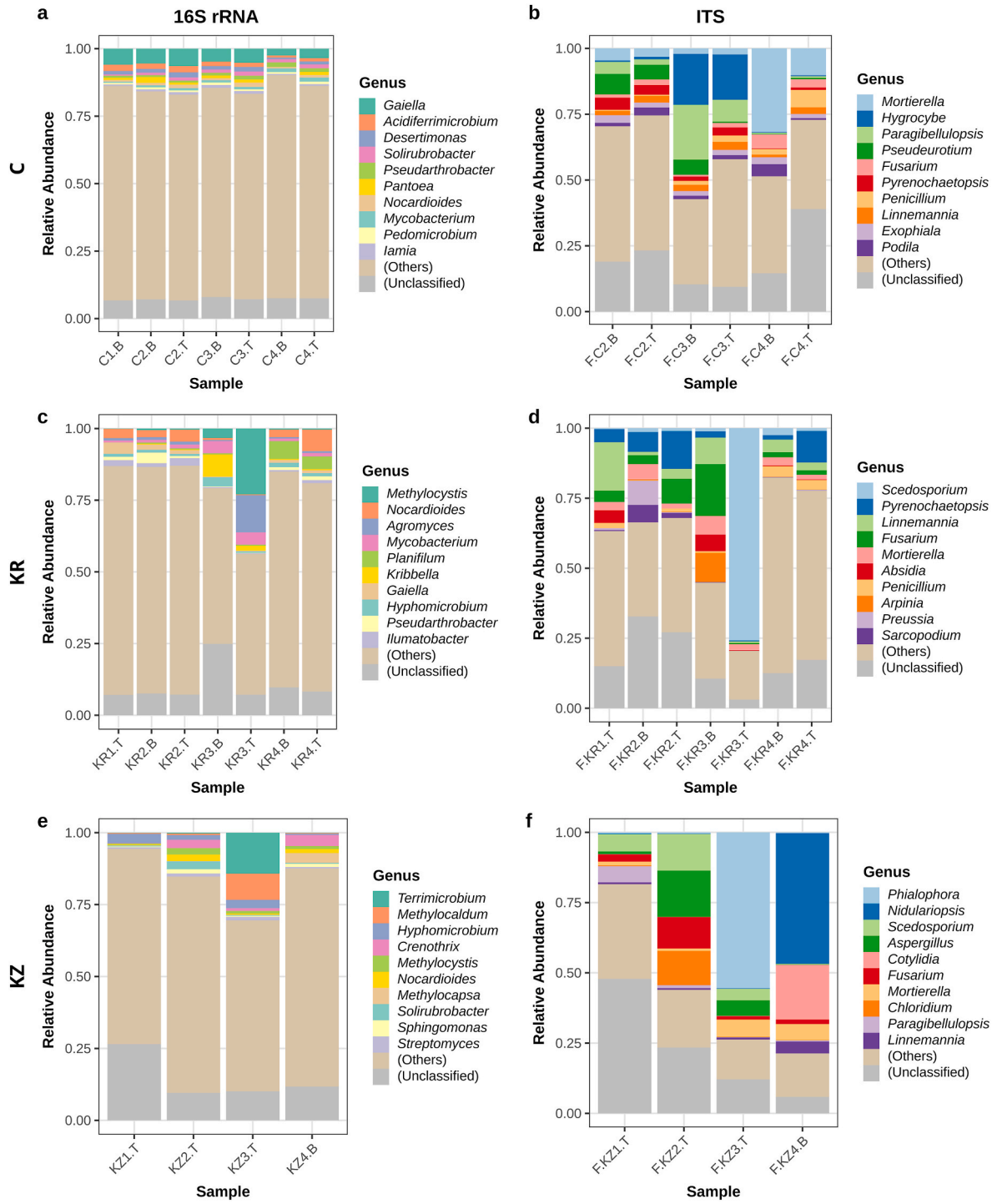

Fig. 4. Taxonomic composition of genus level in studied locations: grassland (C), closed Kariotiškės landfill (KR), and operating Kazokiškės landfill (KZ) based on bacterial 16S rRNA in C (a), KR (c), KZ (e), and ITS in C (b), KR (d), KZ (f) ASV. Results are represented as the 10 most abundant genera according to relative abundance.

detected in both locations, the abundance of plastic-associated *Mesorhizobium*, *Scedosporium*, *Microbacterium*, *Alternaria*, and *Pseudoxanthomonas* might be related. The least similar locations were KZ and C.

Overall, the study suggests a gradient of microbial adaptation: from a moderately plastic-adapted community in the C location, through a closed landfill, to a diverse, plastic-associated enriched microbial assemblage in an operating landfill. However, microbial community composition in soil is affected by a multitude of factors beyond MP presence, including organic matter content, pH, heavy metal concentrations, soil texture and type, water availability, salinity, and temperature. These factors independently influence the abundance of certain genera, making it difficult to attribute their presence to MP.

Measured organic matter content differed among sites (median values – 14.9% in KR, 8.8% in C, and 3.8% in KZ), whereas pH was similar (median values – 6.9 in C, 7.0 in KR, and 7.4 in KZ), except for the alkaline outlier KZ1.T (pH 10.27). Among the tested samples, a few samples exhibited higher organic matter contents: KR1.B (23.72%), KR3.T (26.08%), KR3.B (23.36%), and KR4.T (17.34%). *Agromyces*,

Table 1
Site-specific genera detected in the samples and associated with detected plastic biodegradation based on the literature. Site-specific genera were assigned to the location and marked as "+" based on the forest plot analysis: β (log-scale change) of KR or KZ relative to the reference group C, with a Wald 95% CI. Exceptions in high relative abundances in the samples are also observed.

Genus		Associated plastic degradation	Site-specific to			References
			C	KR	KZ	
Lysinibacillus		**PE**, **PP**, PS, PU	+			(Raczyńska et al., 2024; Lin et al., 2023; Jeon et al., 2021)
Paenibacillus		PLA, **PE**, PS	+			(Tiwari et al., 2025; Kumar et al., 2021; Bacha et al., 2023)
Acinetobacter		PU, **PE**	+			(Maroof et al., 2021; Howard et al., 2012)
Arthrobacter		**PA**	+			Bell et al. (2024)
Dactylonectria		**PET**	+			Brinch-Pedersen et al. (2024)
Agromyces		**PA**		13% in KR3.T		Bell et al. (2024)
Rhizobium		**PE**			2.7% in KZ3.T	Wu et al. (2025)
Methylocaldum		**PE, PS, PP**			0.6% in KZ2.T and 9.2% in KZ3.T	Kumar et al. (2024)
Brevundimonas		**PS**			0.55% in KZ3.T	Tang et al. (2023)
Malassezia		**PVC**			0.2% in KZ1.T	El-Dash et al. (2023)
Aspergillus	*A. terreus*	**PE**			16.4% in KZ2.T	(Sangale et al., 2019; Zahra et al., 2010)
	A. fumigatus	PU, **PE, PVC**			4.8% in KZ3.T	(El-Dash et al., 2023; Temporiti et al., 2022)
	A. flavus	PU, **PE**			0.22% in KZ1.T	Bacha et al. (2023)
Alternaria		**PE**, PU		+	+ (1.3% in KZ3.T)	(Okal et al., 2023; Matsumiya et al., 2010)
Scedosporium		PU, **PE**		75.78% in KR3.T	13.01% in KZ2.T	Ibrahim et al. (2024)
Microbacterium		PS, **PE**		1.1%, 1.6%, and 1.5% in KR1.T, KR2.T, and KR4.T, respectively	1.3% in KZ2.T	(Kumar et al., 2021; Rajandas et al., 2012)
Pseudoxanthomonas		**PA**	<0.01% in C3.T and C4.B	+ (>0.04% in all samples)	+ (>0.01% in all samples)	Sasanami et al. (2022)
Mesorhizobium		PLA, **PE**		+	+	Muangchinda et al. (2025)
Trichoderma		**PE**, PU, PVC		1.8% in both KR2.T and KR2.B		(Temporiti et al., 2022; Srikanth et al., 2022)

Bolded MPs indicate plastics detected at locations associated with site-specific genera associated with plastic degradation. KZ – operating Kazokiškės landfill, KR – closed Kariotiškės landfill, C – grassland.

enriched in KR3.T, is associated with decaying organic matter. Among genera whose abundance was associated with soil organic matter after accounting for site group (Supplementary Materials, Fig. S5), some correlations with plastic-degradation-associated genera could be seen: *Pseudoxanthomonas*, *Mesorhizobium*, and *Scedosporium* were associated with higher organic matter content, but they were also detected at the KZ location, which had the lowest measured organic matter content. Among the genera whose abundance was associated with pH after accounting for site group, one was associated with plastic degradation (Supplementary Materials, Fig. S6): *Malassezia* was associated with a higher pH value, despite the genus being slightly acidophilic. This suggests that the relative abundance of *Pseudoxanthomonas*, *Mesorhizobium*, *Scedosporium*, and *Malassezia* may be driven by other factors rather than pH and organic matter. No known alkaliphilic microorganisms were dominant in the outlier KZ1.T sample (pH = 10.27).

The soil type does not vary substantially across samples, except for the clayey C4.T and C4.B. Both samples share a taxonomic profile similar to that of the remaining C samples. Organic matter content, soil type, and pH values for the samples are presented in the Supplementary Materials, File S1. As for temperature, all tested locations are within 80 km of each other, have altitudes that vary by up to 60 m, and experienced similar weather conditions; therefore, weather is unlikely to be a factor. Additionally, the operating landfill community composition was influenced by methane produced during the decomposition of organic matter. This leads to a higher abundance of methanotrophs, such as *Methylocaldum*, *Crenothrix*, *Methylocystis*, and *Methylocapsa*.

Since the analyses were performed on bulk soil, the associations between microbial taxa and specific polymers remain uncertain. Nonetheless, the genus-level associations as plastic degraders remain partial, as the ability to degrade has been attributed to species within the genus that were previously associated with plastic degradation. Additional analyses of the plastisphere and biodegradation would be necessary to validate the proposed associations between plastic abundance and the detected genera.

3.2.5. Taxonomic composition of Archaea

Members of the Archaea domain were found in all samples studied (Fig. 5). The total amount of ASV reads of *Archaea* was 14,822, with a mean abundance with 95% CI of 1539 ± 1337 in C samples, 365 ± 301 in KR, and 373 ± 968 in KZ (the lowest count in KZ group was 33). Only 7 phyla were detected. Thermoproteota was found across the studied locations and was relatively abundant in the C samples. Halobacteriota, Thermoplasmatota, and Methanobacteriota had higher relative abundance in the KR and KZ groups. 4 reads of Iainarchaeota were detected in KR3.B and KZ4.B samples, and 2 reads of Micrarchaeota in some of the KR samples; Nanoarchaeota was found in all groups. The family Nitrososphaeraceae was the most abundant and was found in all tested groups.

As far as is known, the Nitrososphaeraceae family is associated with the plastisphere. Among the plastic-degradation-associated Archaea, the *Methanobacterium* genus was found in all tested locations, and *Methanosarcina* was detected mostly in the KR group (Liu et al., 2023).

It is believed that the consortium in biofilm form is required for complete biodegradation of MP due to syntrophy and complementary enzyme production, thereby broadening the range of targeted chemical linkages. Furthermore, microorganisms can induce one another's enzyme expression through quorum sensing or by relieving catabolite repression. The ability to degrade plastics might start with fungi through surface alteration and infiltration into the particles. As a result, bacteria and/or enzymes can penetrate deeper into the polymer (Shokunbi et al., 2025); e.g., motile bacteria can disperse more deeply using fungal highways (Kohlmeier et al., 2005), reaching more of the polymer's surface and accessing a greater number of susceptible chemical bonds. Furthermore, fungi can create anoxic microsites in biofilms, thus

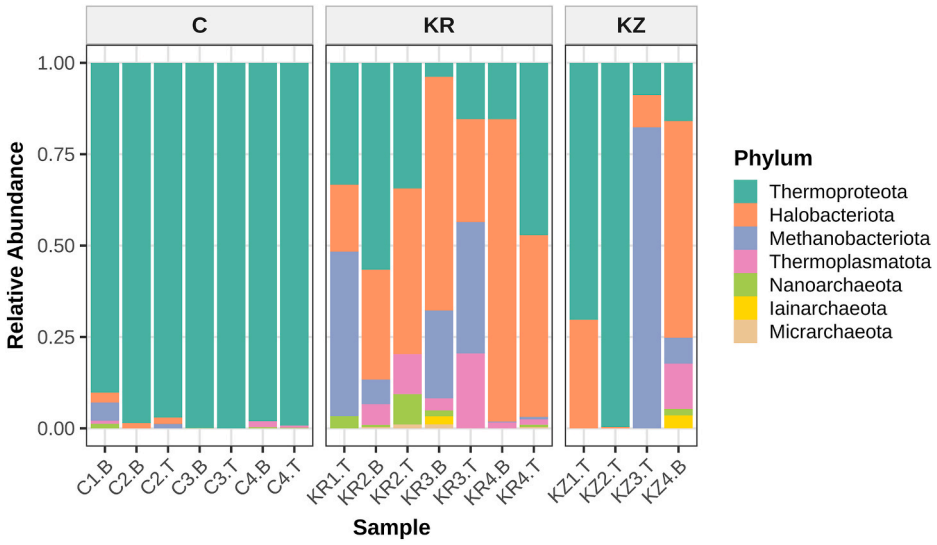

Fig. 5. Taxonomic composition of Archaea phyla in studied locations: grassland (C), closed Kariotiškės landfill (KR), and operating Kazokiškės landfill (KZ) based on archaeal 16S rRNA ASV. Results are represented as the most abundant phyla according to relative abundance.

Table 2
Results on the detected genes associated with microplastic degradation and their possible microbial origins in KZ, KR, and C. The identity threshold was 81.43%, and the coverage threshold was 93.3%.

	Alkane hydroxylases	*Two-component styrene monooxygenase*	*Cutinase from F. solani*	Phl7 hydrolase	*L. fusiformis urethanase*
C1.B	*Pedobacter* sp.; uub				
C2.T	*Pedobacter* sp.; *Pimelobacter* (synonym of *Nocardioides*) *simplex*	*Mesorhizobium* sp.; *Rhodococcus* sp.; *Variovorax* spp.			
C2.B	Actinomycetota phylum bacterium; *Pedobacter* sp.	*Variovorax beijingensis; Mesorhizobium* sp.			
C3.T	*Stenotrophomonas* sp.; *Pedobacter* sp.; *Nocardioides cavernae;* Bradyrhizobiaceae bacterium	*Variovorax gossypii*; *Rhodococcus* sp.			*Lysinibacillus capsica or Lysinibacillus fusiformis*
C3.B	uub	*Variovorax* sp.; *Mesorhizobium soli*			
C4.B	Acetobacteraceae bacterium MS302e; *Agrobacterium* sp. MS189b; uub	*Rhizobium* sp.; *Variovorax* spp.	*F. solani*		
KR1.T	*Nocardioides seonyuensis*; *Marmoricola* sp.				
KR1.B	*Nocardioides* sp.; uub				
KR2.T	*Bacillus* sp. MS243e; *Nocardioides* sp. MS274e; *Rhodococcus* sp. MS103a; uub	Pseudomonadota bacterium; *Variovorax guangxiensis*			
KR2.B	*Nocardioides* sp.	*Mesorhizobium* sp.			
KR3.T	*Mycobacterium agri*; *Pseudomonas veronii* or *Pseudomonas panacis*; uub			Cutinase 1/2 from *T. fusca; T. cellulosilytica*	
KR3.B	Acetobacteraceae bacterium; *Stenotrophomonas* sp.; *Agrobacterium* sp.; uub				
KR4.T	*Nocardioides cavernae*; uncultured *Nocardioides* sp.; *Planomonospora venezuelensis*; *Bacillus* sp. MS243e; *Nocardioides* sp. MS274e; uub				
KR4.B	Bradyrhizobiaceae bacterium MS228e; *Mycobacterium thermoresistibile*; *Mycobacterium kumamotonensis*; *Bacillus* sp. MS243e; *Nocardioides* sp. MS274e; *Marmoricola* sp. URHB0036; uub				
KZ1.T	*Mycolicibacter* (synonym of *Mycobacterium*) *sinensis*	*Variovorax paradoxus*			
KZ2.T	*Nocardioides* sp.				
KZ3.T	*Pedobacter* sp.; uub				
KZ4.T	*Nocardioides* sp.				
KZ4.B	*Marmoricola* sp. URHB0036; uub				
KZ5.B	uub	*Glaciimonas soli*; *Methylomicrobium lacus*.			

KZ – operating Kazokiškės landfill, KR – closed Kariotiškės landfill, C – grassland, uub – unknown uncultured bacterium.

fostering anaerobic bacteria and Archaea (Xiong et al., 2022). This, along with the fact that layers of biofilm can create lower oxygen niches, might explain the high abundance of *Terrimicrobium* and *Methanobacterium* (Andreides et al., 2022; Qiu et al., 2014).

Among enzymes associated with biodegradation, monooxygenases and laccases require O_2 as an electron acceptor (Suresh et al., 2025; Feng et al., 2024), indicating that the microenvironment should contain oxygen for biodegradation. Alkane 1-monooxygenases have iron in the active center (Rojo, 2009). The uptake of iron by cells could be increased by consortium siderophores (iron chelators) (Schalk, 2025), produced by microorganisms of genera such as *Pseudomonas*, *Mycobacterium* (Schalk, 2025), *Streptomyces*, *Bacillus* (Ahmed and Holmström, 2014), *Penicillium* (Holinsworth and Martin, 2009), and *Aspergillus* (Xie et al., 2024). Similarly, laccases have copper in their active center (Gałązka et al., 2023). The availability of copper can be affected by copper-binding molecules, methanobactins, produced mainly by methanotrophs (Kim et al., 2005), such as *Methylocystis*, *Methylocaldum*, and *Methylocapsa*. Additionally, microorganisms can produce organic or inorganic acids to solubilize metals, e.g., *A. niger*, which produces citric acid, or *Penicillium simplicissimum*, which produces gluconic acid (Chen and Wang, 2019; Anjum et al., 2010; Amiri et al., 2011). Peroxidases, the other enzymes associated with plastic biodegradation, use hydrogen peroxide as an oxidizing agent (Temporiti et al., 2022). Hydrogen peroxide could be produced by the activity of aromatic alcohol oxidase, which was identified in *Aspergillus*, *Fusarium*, and *Streptomyces* (Urlacher and Koschorreck, 2021), or the activity of aryl-alcohol oxidases, which could be produced by *Aspergillus* (Cinca-Fernando et al., 2025).

3.3. Screening of genes associated with plastic degradation

The biodegradation of MP is influenced by the composition of enzymes and is a complex process. Studies show that the greater the amount of plastic in the environment, the higher the abundance of genes responsible for its biodegradation (Zrimec et al., 2021; Qian et al., 2024; Alam et al., 2025). The set of primers used to analyze the samples in this study targeted genes encoding enzymes associated with activity against PET, PU, PE, and the PS monomer (styrene). The functional gene amplicons were obtained, and their possible microbial origins, based on BLASTx of detected genes, are presented in Table 2.

Analysis of the C location amplicons revealed a variety of AHs. All studied samples yielded amplicons assigned to AH, including C1.T (not shown in Table 2), whose amplicon originated from an unknown uncultured bacterium. AHs degrade naturally occurring alkane hydrocarbons under aerobic conditions. Due to its similar structure to natural substrates, AH can potentially attack PE. The terminal group is converted to 1-alkanol by the AH system (alkane monooxygenase, rubredoxin, and rubredoxin reductase) as a primary step. 1-alkanol might then be oxidized, making it more available for further degradation and mineralization by AHs systems harboring microorganisms. Since AH varies among microorganisms, the degradation capabilities of these enzymes should be further evaluated using expression systems. Increased expression of AH is additionally associated with the degradation of PS (Wang et al., 2024).

Two-component SMO genes were detected in all tested C samples, except C1.T and C1.B. Styrene occurs in the environment through both natural and anthropogenic pathways. One pathway is the depolymerization of PS. The vinyl chain of styrene is oxidized, and the resulting styrene oxide undergoes β-oxidation (Othman et al., 2021). Although PS MP were not detected at the C location, the potential for inclusion in the PS biodegradation pathway remains, given the highest prevalence of SMO genes among the tested locations.

The amplicon of the urethanase gene was obtained only in the C3.T sample, possibly originating from *Lysinibacillus* genus (identity 100%, E-value = 0), for which the primer set was designed, while the cutinase genes' amplicon, which probably originated from *F. solani* (identity 100%, E-value = 8.94×10^{-32}), was detected only in C4.B. PU plastic was not detected in C locations; thus, the detection of the urethanase gene may be associated with the presence of urethane or its relatives, such as acrylamide (Liu et al., 2025), while both could form during the fermentation of organic matter. On the other hand, detected PET could be hydrolyzed by the obtained cutinase at the ester bonds. The cleavage of PET by cutinases, harbored by, for example, *Fusarium* or *Thermobifida*, produces mono(2-hydroxyethyl)terephthalate (MHET), bis (2-hydroxyethyl)terephthalic acid (BHET), which later can be metabolized to terephthalic acid (TPA) and ethylene glycol (Roberts et al., 2020; Gupta et al., 2025). BHET could be utilized by *Bacillus* (Roberts et al., 2020), TPA and ethylene glycol by *Pseudomonas* (Roberts et al., 2020) and *Aspergillus* (Isobe, 1995) species until full mineralization.

The diversity of detected AH genes in the KR samples was the highest among the locations studied, indicating the greatest potential for PE biodegradation. As discussed for the C group, studies using expression systems should be conducted to fully evaluate biodegradation capabilities. Analysis of amplicons obtained by targeting the *T. fusca* cutinase 1 gene revealed high sequence similarity between the KR4.T amplicon and a serine hydrolase from *Thermocrispum agreste*, as well as an α/β hydrolase of *Mycobacterium* sp. ITM-2016-00318, which may be relevant since PET-active cutinases are classified as α/β serine hydrolases (Sun, 2024). It should be noted that these may be new, previously uncharacterized enzymes with potential activity against PET. Thus, further analysis of the biodegradation abilities should be conducted. Analysis of amplicons obtained by PCR targeting *phl7* showed that the amplicon from sample KR3.T had significant hits to cutinase from either *T. fusca* or *Thermobifida cellulosilytica* (identity 100, E-value = 0 for both cases). The 5′ and 3′ ends of the *phl7* gene are homologous to the *cut1/2* genes of *T. fusca* and *T. cellulosilytica*, allowing annealing. Both cutinases, from *T. fusca* and *T. cellulosilytica*, have been shown to hydrolyze ester bonds in PET (Herrero Acero et al., 2011). However, within the KR group, the *Thermobifida* genus was detected only in KR4.B (0.013%) by 16S rRNA analysis, suggesting that bacteria may be present in other parts of the landfill at low abundance and therefore not readily detectable. Only the KR2.T and KR2.B samples harbored SMO genes, indicating a lower potential of the KR site to biodegrade styrene and possibly PS than the C group. Based on FTIR analysis, no PET or PS microparticles were detected in the KR location; the abundance of the genes may be associated with the presence of cutin and styrene, the original substrates of cutinases and SMOs. The Kariotiškės landfill is covered with a layer of soil, but the large amount of waste beneath it appears to affect the composition of the soil microbiota, possibly through upward migration of the microbiome and MP, as well as horizontal gene transfer.

Only alkane and styrene monooxygenase genes were detected in samples from the open Kazokiškės landfill, indicating the potential of the microbiota to utilize PE and styrene (possibly involved in PS utilization). KZ5.T contained the AH gene, possibly from an unknown uncultured bacterium (not shown in Table 2). No cutinase genes were detected, despite the presence of PET MP. It was expected that the KZ location would show the widest representation of the studied genes, as samples were collected from the operating landfill; however, the detected genes were the least abundant among the three studied locations. The total number of obtained functional gene variants associated with degradation differed significantly (Kruskal–Wallis H = 9.03; p = 0.011). The highest diversity was observed at the C location and the lowest at KZ, which had the highest MP load. The Dunn post hoc analysis (Holm-adjusted) indicated a significant difference between C and KZ (p = 0.015), and between KZ and KR (p = 0.034), whereas the difference between KR and C was not significant (p = 0.620). The observed genetype diversity was not significant and was driven by high richness of AH and SMO genes: H = 4.02; p = 0.134. Nonetheless, the C group contained four detected gene types, the KR group – three, and the KZ group – two. These results oppose previous findings; it can be assumed that, because the landfill is active, new waste containing labile organic matter is constantly delivered there: the biowaste fraction constitutes 30–40% of the total municipal solid waste in EU countries (Stylianou et al.,

2020). This plentiful biowaste is a readily available carbon source for microorganisms, dominating microbial metabolic pathways and potentially limiting the niches where microorganisms capable of degrading more complex substrates, such as plastics, can establish their presence. Additionally, the presence of more readily degradable carbon sources may repress genes involved in MP degradation or their horizontal transfer; inhibitory compounds in the operating landfill (e.g., heavy metals) may constrain degrader taxa and/or gene expression.

A wide diversity of alkane monooxygenase genes was observed across locations, with the highest variation in KR samples. This finding correlates with other studies across different sites, where high diversity was also observed, including natural environments such as soil, freshwater, and sea (Heiss-Blanquet et al., 2005; Nie et al., 2014) and anthropogenically impacted areas, such as landfills (MacLean et al., 2024; Kumar et al., 2021; Karthikeyan et al., 2021; Whyte et al., 2002). Genes encoding PETase or MHETase from *I. sakaiensis*, LCC, PueA lipase from *P. chlororaphis*, R7 laccase-like multicopper oxidase from *R. opacus*, *Rhodococcus* spp. SMO, or *R. erythropolis* MP_{50} amidase were not detected. Because no positive controls were used and detection was based on known genes, unknown and undetected genes might have been missed. Since functional-gene detection relied on PCR with primers targeting known genes, several methodological factors may have influenced false-negative results: primer mismatch in divergent taxa, low target-gene abundance within the complex metagenome, PCR inhibition, for example, by humic acid or heavy metals extracted from the samples, and DNA quality and extraction efficiency variation across the different samples. Furthermore, the targeted amplicon approach can not directly link functional genes to their microbial origins in the studied soil communities, so alternative origins cannot be excluded. Spearman's rank correlation and the Benjamini–Hochberg FDR correction did not reveal a significant correlation between gene variant richness and pH or organic matter content.

In summary, the analyses of the three studied locations: KZ (operating landfill), KR (closed landfill), and C (grassland), reveal complex interactions between MP contamination profiles, taxonomic composition, and functional gene representation that collectively reflect site-specific adaptations. At the C location, a moderately diverse plastic-associated community, including *Lysinibacillus*, *Paenibacillus*, *Arthrobacter*, *Dactylonectria*, and *Acinetobacter*, was functionally supported by the detection of the broadest SMO gene prevalence, alongside a high prevalence of AHs genes and a cutinase gene, indicating potential for PA, PE, and PET biodegradation consistent with the MP detected there. The KR location, despite being closed, had the highest detected AHs gene diversity among all locations, as well as cutinases with possible PET-degrading activity. Along with the presence of *Agromyces*, *Trichoderma*, *Scedosporium*, *Pseudoxanthomonas,* and *Microbacterium* genera, which are potential plastic degraders, the results aligned with putative PA and PE MP degradation. Additionally, the taxonomic composition shows stronger similarity between KR and KZ than between KR and C. This analysis suggests that, following closure, as organic matter becomes depleted, the KR microbiota adapts to utilize more recalcitrant substrates, such as plastics. Paradoxically, the KZ location had the greatest MP diversity and abundance and the richest assemblage of plastic-associated genera, as determined by taxonomic composition analysis, including *Rhizobium*, *Methylocaldum*, *Malassezia*, *Aspergillus*, *Scedosporium*, *Pseudoxanthomonas*, and *Microbacterium*, but the targeted plastic-degradation-associated gene representation was the narrowest, limited to detected AH and SMO genes. Microbial population in soil is affected by multiple factors, including MP presence, but this effect cannot be separated from other differences: organic matter content, heavy metal concentrations, soil texture and type, water availability, salinity, vegetation, and pH, all of which may independently drive both taxonomic abundance and functional gene distribution.

Furthermore, because all analyses were performed on bulk soil rather than the plastisphere, the inferred associations between specific taxa, functional genes, and polymer types are limited to a genetic perspective. Further plastisphere taxonomic and proteomic analyses, enzyme expression studies, and biodegradation tests would be essential to validate the proposed degradation capacities and ecological roles, as well as the proposed associations between plastic abundance and the detected genera.

4. Conclusions

This study demonstrates the site-specific distribution of MP, microbial community composition, and biodegradation-associated gene profiles across artificial and natural environments in Lithuania, including the operating Kazokiškės landfill, the closed Kariotiškės landfill, and a natural grassland. Contrary to conventional assumptions, the operating Kazokiškės landfill site harbored a microbiome population enriched in plastic-associated genera but exhibited the narrowest detected functional gene representation, likely due to suppression of plastic-degrading pathways by constant fresh, labile organic matter from waste. Interestingly, the closed KR landfill showed the highest detected AHs gene diversity, along with the detection of SMOs and cutinases, suggesting microbial adaptation to more recalcitrant carbon sources. The similarity in microbiome composition between KZ and KR locations supports the idea that waste beneath the closed landfill influences the overlying topsoil microbiome. The grassland site harbored plastic-degradation-associated taxa, the greatest variety of SMOs, along with AHs, cutinases, and urethanases, indicating that non-landfill soils are an underappreciated reservoir of plastic-degradation potential and should not be overlooked.

Since analyses were performed on bulk soil and limited to genetic inference, the putative associations between identified taxa that previously were associated with plastic degradation, detected functional genes, and specific polymers should be further tested. Studies of plastisphere-targeted metagenomics, proteomics, and biodegradation assays are needed to validate the degradation capacities and associations identified in this study.

CRediT authorship contribution statement

Gintarė Jakštienė: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alena Koida:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Justinas Kavoliūnas:** Visualization, Validation, Software, Investigation, Formal analysis. **Sonata Pleskytė:** Visualization, Validation, Investigation. **Ingrida Prigodina Lukošienė:** Project administration, Investigation. **Eglė Lastauskienė:** Writing – review & editing, Supervision, Project administration, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.129265.

Data availability

Data will be made available on request.

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