DNA metabarcoding of the leachate microbiota from sanitary landfill: potential for bioremediation process

Michel Rodrigo Zambrano Passarini1 · João Victor Fonseca Moreira1 · Jose Alejandro Morales Gomez1 · Rafaella Costa Bonugli-Santos1

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Abstract
Leachate generation contains a variety of toxic compounds, and is a major problem for municipal solid waste (MSW). Microbial profile knowledge is essential to new alternatives and improvements in current treatments of these effluents. In this respect, the microbial community in the leachate from the sanitary landfill of the city of Foz do Iguaçu was analyzed. The 16S rDNA metabarcoding suggested the dominance of fermenting bacteria belonging to Firmicutes phylum, followed by Proteobacteria, Bacteroidetes, and Synergistetes. The most abundant genera were Sedimentibacter, Vulcanibacillus, and Anaerovorax. However, 60% of amplicon sequence variants (ASVs) were not classified taxonomically. In addition, an expressive abundance was attributed to the superphylum known as PVC group, little studied and with unknown scientific potential. The leachate acidogenic phase was masked in the chemical and physical analyzes. Nevertheless, it was evidenced in the metabarcoding methodology. No specifically methanogenic group was detected in significant abundance. Therefore, from bacterial community identification, a bioremediation process can be designed. Enriched culture media can be developed and targeted to the recovery of specific groups which may be involved in leachate biodegradation. What is more, the results expand the knowledge of bacterial diversity, especially from the presence of unknown genera in this habitat.

Keywords Municipal solid waste · 16S rDNA · Next-generation sequencing · Prokaryote diversity · Biological treatment

Introduction
Landfill leachate is a product generated from the degradation of household and hospital organic waste. It is a dark liquid with characteristic smell and high biochemical oxygen demand, produced by rainwater infiltration in municipal solid waste (MSW) cells and organic matter degradation (Palermo and Gomes 2017). Chemical, physical and microbiological processes on MSW degradation cause the release of pollutants to leachate (Kjeldsen et al. 2002). Municipal landfill leachates must be considered wastewater with the highest environmental impact because it can infiltrate water sources for human and animal consumption and may cause a health problem due to the digestion of toxic compounds (Christensen et al. 2001; Di Iaconi et al. 2006). The leachate pollutant composition can be divided into four main groups: (1) dissolved organic matter composed of volatile fatty acids and refractory compounds similar to fulvic and humic compounds; (2) inorganic macro components formed by calcium, magnesium, sodium, potassium, ammonium, iron, manganese, chloride, sulfide, and hydrogen carbonate; (3) heavy metals composed of cadmium, chromium, copper, lead, mercury, nickel, zinc; and (4) xenobiotic organic compounds present in low concentrations (less than 1 mg L⁻¹), including aromatic hydrocarbons, phenols, aliphatic chain chlorines, pesticides, and plasticizers (Di Iaconi et al. 2006; Kjeldsen et al. 2002).

Leachate generated in landfills is collected and treated by different strategies, especially by on-site treatment and discharge (treatment system is constructed on-site or leachate is recycled on-site); on-site pretreatment followed by off-site treatment (some off-site facilities may have limits that require pretreatment; and transport and treatment off-site
In recent decades, bioreactor landfills have been developed by recirculating the leachate collected from leachate collection and removal system (LCRS) back into the landfill, reducing the amount of leachate needed to be disposed. By leachate recirculation, the moisture content of MSW can be increased, which accelerates MSW degradation and landfill stabilization processes (Feng et al. 2018).

There are different phases during the biological degradation of solid waste. It begins in a hydrolytic phase and ends in a methanogenic phase. In the hydrolytic phase, the organic fraction can be metabolized aerobically to form CO₂ and H₂O. The anaerobic phase is characterized by fermentation, which can generate organic acids and ammoniacal nitrogen. The formation of acetate and methane is carried out by the acidogenic and methanogenic phases, respectively (Adhikari et al. 2014).

The lack of soil sealing and leachate drainage cause soil infiltration in dumps and controlled landfills. The leachate is then diluted in groundwater or nearby water bodies, generating contamination (Christensen et al. 2001). Although sanitary landfills are engineered to avoid potential leaking, it may possibly occur due to bad management, low-quality building materials and degradation of the geomembrane (Palermo and Gomes 2017). Leachate presence in water bodies and soil could disturb the present microbiota due to its acid or alkaline pH and toxic compounds (Kjeldsen et al. 2002).

To reduce the landfill leachate impact, a more efficient and inexpensive treatment must be developed to mitigate the effects caused to the environment and human health. For this reason, knowledge of microbial diversity is essential for bioremediation approaches development and optimization towards leachate. Knowing the microbial diversity associated with leachate enables the isolation methodologies for recovery of the specific microorganisms present in this process, improves knowledge for the bio-augmentation techniques, or environmental enrichment could be developed to metabolize the toxic compounds present in these environments (Tedersso et al. 2015).

Next-generation sequencing enabled the whole community characterization in landfills and leachate (Santos et al. 2020; Weaver et al. 2019). Although these studies have provided insight into the composition of resident microbial populations, the assessments remain incomplete due to the complexity that these communities can present. It is especially the case of stabilized leachate that shows low biodegradation efficiency and needs more biological investigations (Kurniawan et al. 2006). Thereby, the detection of the low-abundance microbial species might reveal further valuable information about a better microbial diversity and adaptability of bacterial assemblages (Köchling et al. 2015). In this way, using 16S rRNA metabarcoding approaches, this research aimed at the microbiota characterization associated with leachate, with a recirculation system, from Foz do Iguaçu sanitary landfill for future application using culturo-omics approaches to bioremediation process.

Materials and methods

Sampling

The sample was collected on May 15, 2019, from MSW cells (reservoir 1, which receives an influx of leachate) in the municipality of Foz do Iguaçu sanitary landfill Paraná, Brazil. Approximately 500 mL of leachate were collected in a minimum depth of one meter from lagoon liquid storage, using a sterile glass bottle. The sample was transferred adequately to 50 mL sterile falcons and frozen at −80 °C for DNA preservation in the Laboratory of Biochemistry and Microbiology—UNILA. About 400 mL of leachate sent for physical–chemical analysis were kept at 4 °C.

Foz do Iguaçu sanitary landfill (25°27′47.9″ S 54°36′26.4″ W) is located in the city’s northeast region. The landfill has a total area of 389.73744 m² and has three cells where the MSW is deposited, at the time of collection. Cells 1 and 2 have six layers of MSW each, and their area is 58.38715 m² and 61.58401 m², respectively. The MSW deposit on cell 1 began in 2001 until 2010, and cell 2 from 2010 to 2017. Cell 3 operation started in July 2017, and comprises an area of 64.78063 m². Four reservoirs in the landfill receive an influx of leachate produced by MSW cells (supplementary material). The leachate was collected through the landfill drains and sent to liquid storage in a stabilization pond. It remained stored until recirculated, through pumping and sprinklers, in impervious areas within the landfill.

The sample was sent to TECLAB—Technologies in Laboratorial Analysis, for physical–chemical characterization. Levels of arsenic, cadmium, lead, copper, chrome, mercury (using atomic absorption and emission Spectroscopy), nitrates, nitrites, ammoniacal nitrogen, organic nitrogen, total nitrogen, dissolved oxygen, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and pH were measured. The analyzes were performed in triplicate according to the company’s protocol.

16S amplicon library preparation and sequencing

For the DNA extraction, about 10 mL of leachate was centrifuged at 10,000×g for 25 min. The resulting supernatant was discarded, and the pellet was resuspended in 250 µL of deionized water. According to the manufacturer’s instructions, total leachate community DNA was extracted from 10 mL of the sample using the DNeasy® PowerSoil® kit.
(QIAGEN). The DNA integrity was confirmed by electrophoresis in a 0.8% agarose gel. DNA samples were stored at −20 °C until downstream analyses. DNA amplification and sequencing were performed by BPI BIOTECNOLOGIA EPP company (Botucatu-SP). PCR reactions were executed with a final volume of 20 µL containing 10 µL of GoTaq® Colorless Master Mix 2x (Promega, USL), 0.3 µM of forward oligonucleotide, 1 µL of total genomic DNA and sterile ultrapure water. The 16S region (V3 and V4) of rRNA was amplified with the primers: 5’TCTGCTGGCACGCTACAGTGTATAGACGAGATGTGTATAAGAGACAGACCTACGGGCTACGAG-3’, and 5’-GTCTCGTGACGCTACGAGATGTGTATAAGAGACAGAcTACHVGGGTATCTAATCC-3’ (Klindworth et al. 2013). Reactions were performed in a Veriti™ Thermal Cycler (Applied Biosystems). After the amplification reaction, its efficacy was assessed through an electrophoresis with 2% agarose gel stained with UniSafe dye 0.03% (v/v). The amplicons had 600 bp in size. Indexation reaction was performed using Nextera XT Index kit (Illumina). Indexers used were N701 (TAAGGCGA) and S502 (ATAGAGAG). Generated libraries were purified using Agencourt AMPure XP (Beckman Coulter) magnets beads. Quantification of the libraries were performed using RT-PCR KAPA-KK4824 kit (Library Quantification Kit—Illumina/Universal). The equimolar DNA pool created was submitted for sequencing in an Illumina MiSeq (Illumina® Sequencing) with MiSeq Reagent Kit V2 Nano.

Bioinformatic analysis of amplicon library sequences

Overall quality inspection, filtering, denoising, chimera removal, and merging to collect amplicon sequence variants (ASV’s), were made thorough DADA2 16 s Pipeline Workflow version 1.16 (Callahan et al. 2016) implemented at R software (Ihaka and Gentleman 1996), following recommendations from the author. The “FilterAndTrim” function was used for quality filtering with options that included removal of primers, ambiguous bases (N) and sequences with less than 100 bp and an expected error (MaxEE) higher than 2. No trim was necessary, as quality for overall sequencing was above a 30 Phred score for R1 and R2 strand (see pictures below, left plot R1 strand and right plot R2 strand). After quality filtering, an error model for sequencing was built with “learnErrors” function and forward reads were dereplicated with “deref” function to collapse repeated sequences. These sequences were denoised to get a set of ASV’s, and filtered for chimera detection with “removeBimeraDenovo” function. Finally, a table with ASV’s counts and a set of denoised sequences were exported for downstream analyses.

To perform sequencing classification and taxonomic assignment, the approach of machine learning classification implemented in Qiime2 2019.10 version (Bolyen et al. 2018) alongside with SILVA database reference 138 version (Yarza et al. 2008) was used. Pre-formatted files of SILVA database for Qiime2 with 99% of similarity and 7-level rank taxonomic labels, were used for this purpose and can be found at https://docs.qiime2.org/2021.2/data-resources/. Finally, results were plotted in R software (Ihaka and Gentleman 1996), using the following packages: Phyloseq and Plotly. The rarefaction curve was performed with a sampling depth of 500 reads to capture the diversity present in sample with high read counts, but low enough to include the majority of samples. The α diversity was assessed using the Shannon diversity index ($H'$) and Simpson’s dominance index ($C$) (Brower et al. 1998).

Results and discussion

Leachate physical–chemical characterization

Leachate composition may directly influence the microbial community richness, and it is essential to understand the environment. Table 1 shows the results of the sample’s physical–chemical characterization, and the data were used to identify the biological phase of our leachate. Each phase has a direct impact on leachate composition. Young leachate has a considerable amount of biodegradable organic matter, mainly volatile fatty acids and high molecular weight compounds (Adhikari et al. 2014).

| Parameter          | Result | Unit  | Q.L. | M.A.V. |
|--------------------|--------|-------|------|--------|
| Arsenic            | <0.10  | mg L$^{-1}$ | 0.10 | 0.5    |
| Total cadmium      | 0.048  | mg L$^{-1}$ | 0.005 | 0.2    |
| Total lead         | <0.005 | mg L$^{-1}$ | 0.005 | 0.5    |
| Total copper       | 0.120  | mg L$^{-1}$ | 0.005 | 1.0    |
| Total chrome       | 0.080  | mg L$^{-1}$ | 0.005 | 1.1    |
| BOD/COD            | 0.58   | –     | –    | N.E.   |
| BOD                | 2.0740 | mg L$^{-1}$ | 2.0   | N.E.   |
| COD                | 3.5990 | mg L$^{-1}$ | 5.0   | N.E.   |
| Mercury            | <0.01  | mg L$^{-1}$ | 0.01 | 0.01   |
| Nitrates           | <1.0   | mg L$^{-1}$ | 1.0   | N.E.   |
| Nitrites           | 0.20   | mg L$^{-1}$ | 0.02 | N.E.   |
| Ammoniacal nitrogen| 486    | mg L$^{-1}$ | 0.05 | 20.0   |
| Organic nitrogen   | 638    | mg L$^{-1}$ | 0.50 | N.E.   |
| Total nitrogen     | 1.125  | mg L$^{-1}$ | 0.50 | N.E.   |
| Dissolved oxygen   | 6.6    | mg L$^{-1}$ | 0.10 | N.E.   |
| pH                 | 8.50   | –     | –    | 5–9    |

N.E. not established, Q.L. quantification limit, M.A.V. maximum allowed value for effluent discharging (CONAMA 430)
The leachate showed pH (8.52), characteristic of the methanogenic phase according to the National Environment Council (CONAMA 430). The COD level was within the standard interval of a methanogenic phase (Kjeldsen et al. 2002). On the other hand, the level of BOD was well below that reported for the methanogenic and acid phases, and the BOD/COD ratio was 0.58, characteristic of the acidic phase (Kjeldsen et al. 2002). Based on these results, it is possible to indicate that the sample had a mixed profile with traits from both acid and methanogenic phases, and good biodegradability of organic matter. These data, together with the ammoniacal nitrogen concentration, are also used for characterization of landfill leachate with age (Kjeldsen et al. 2002). Except for good biodegradability (organic matter is considered biodegradable when the BOD/COD ratio is lower 5-CONAMA 430), all values indicate that our leachate is in a stabilized stage (typical in the methanogenic phase), more than 10 years (Kurniawan et al. 2006), confirming the historical data of the Foz do Iguaçu sanitary landfill. The leachate reservoirs heterogeneousness by recirculation must have caused this variation in the biodegradability and in the methanogenic phase with acidic characteristics.

Studies have shown that leachate recirculation produces stabilized leachates with good BOD/COD ratio (Top et al. 2019). Although a landfill may have the most advanced leachate management practices, recycling process, in some researches, resulted in an imbalance between acidogenesis and methanogenesis in the landfill leachate (Pohland 1980; Hernández-Berriel et al. 2010). Thus, we believe that the specific knowledge of the microbiota can be a strategy for understanding the effect of recirculation on the leachate biological stages.

Most of the nitrogen in aerobic and anaerobic landfill reactors is in the ammonia forms following the degradation of protein and amino acids (Marttinen et al. 2020). Landfill leachate treatment generally focuses on the removal of organic nitrogenous, carbonaceous matter, and ammonia nitrogen. Leachate from older landfill is rich in ammonia nitrogen due to hydrolysis and fermentation of the nitrogenous fraction of biodegradable substrates. The variation of organics and ammonia nitrogen with time may have important implications in leachate treatment, it can accelerate algae growth due to its high nutrient content, deplete dissolved oxygen in the water, and cause toxic effect in the surrounding water life (Hasar et al. 2009; Talalaj et al. 2019). Ammoniacal nitrogen concentration in our leachate was 0.66 times lower than the average value (Kjeldsen et al. 2002), a level that does not exert any adverse effect on anaerobic processes (Şan and Onay 2001). Although possibly characterized by a stabilized leachate, the efficiency in the removal of ammoniacal nitrogen is due to recirculation (Bilgili et al. 2007).

The sample’s physical–chemical characterization result in 1.125 mg L⁻¹ of total nitrogen, this value was 0.38 times lower than reported by Gabarró et al. (2013). In contrast, nitrite was detected in the present study, opposing what was reported by Gabarró et al. (2013). Nitrite and nitrate are produced during nitrification, which is the process that converts ammonia to nitrite and then to nitrate and is an important step in the global nitrogen cycle; this step most occurs aerobically (Bernhard 2010). Also, the study of Kaczorek and Ledakowicz (2006) about the kinetics of nitrogen removal from sanitary landfill leachate showed that the temporary accumulation of nitrite was also observed during the denitrification. The utilization rates of nitrate and nitrite were best fitted, taking into account the limitation by nitrogen substrates (Kaczorek and Ledakowicz 2006), as noted in our case (low concentration of total nitrogen). In the leachate treatment, the ammonium conversion to nitrite was achieved and controlled by means of the available alkalinity (Ganigué et al. 2007). These findings affirm the importance of pH for the bacterial activity of ammonia oxidizing bacteria, the maintenance of free ammonia (NH₃) at favorable levels for nitrite accumulation, and the prevention of nitrite to nitrate oxidation (Ganigué et al. 2007). This condition may justify the low ammonia value in our sample and efficient nitrogen conversion without a high percentage of nitrate. Concerning leachate recirculation and phase mixtures, according to Yang and Song (2019) nitrification and denitrification mainly occurred at aerobic and methanogenic phase, what is possibly happening in the Foz do Iguaçu system. Regarding the presence of nitrate in the sample, it is important to highlight that these chemical compounds have significant solubility and are carried to water bodies through lixiviation resulting in their contamination (Costa et al. 2019).

The metal concentrations of landfill leachate are expected to reduce as the leachate changes from acidogenic to methanogenic, concurrent with a decrease in redox potential and an increase in pH (Top et al. 2019). However, in our sample, even with high pH, the cadmium and copper have yet to be identified. The presence of these metals in the leachate is worrying, especially in accidents that can result in contamination of natural environments (Badiye et al. 2013).

**Microbial community, functional diversity and bioremediation potential**

A total of 40,705 reads were recovered from the leachate sample. After quality processing (filtering, denoising, reads merging, and chimera removal) a final set with 13,714 sequences was obtained. DNA sequences were grouped in 430 ASVs taxonomically labeled. Rarefaction curve of observed ASV richness showed that it reached saturation, indicating that the sampling effort was sufficient to reveal almost all the bacterial species present in the prokaryote.
diversity from the sample (Fig. 1). Shannon and Simpson’s index (value 5.7 and 0.99, respectively) indicated a great diversity in the sample.

Metabarcoding revealed a considered high diversity represented by 20 different phyla, 18 from Bacteria (Fig. 2A), and two from Archaea domain (Fig. 2B). Dominant phyla were Firmicutes (31.37%), followed by Proteobacteria (18.25%), Bacteroidetes (11.72%) and Synergistetes (7.39%). To Archaea domain, Euryarchaeota and Nanoarchaeota phyla had a relative frequency of 91.9 and 8.1%, respectively. Nevertheless, proper quantification of archaeal diversity and community ecology remains limited, as sequence coverage of Archaea is usually low owing to the inability of available prokaryotic primers to efficiently amplify archaeal compared to bacterial rRNA genes (Bahram et al. 2019). In this sense, throughout work, despite presenting the found archaea community, this work sought to explore the bacterial community in more detail.

These results followed the same described in the Can-deias young sanitary landfill (Köchling et al. 2015) study by 454 pyrosequencing. The authors found a high microbial diversity: the abundance was represented by Firmicutes, the most abundant phylum followed by Proteobacteria, Bacteroidetes, Spirochaetes, Synergistetes, and Tenericutes phylum. Slight differences from our work may occur due to the landfill age gap of six years. On the other hand, newer than our treatment system, the research of Khanh Son landfill, also using new generation sequencing, had as dominant phyla: Proteobacteria, Firmicutes, Bacteroidetes, and Euryarchaeota (Dao et al. 2016). Like this geographic and climate variations, especially related to the average temperatures, of landfill sites also have a decisive role in microbial composition.

The recent work performed by Yang and Song (2019) reviewed microbial diversity studies based on the new generation sequencing (Illumina MiSeq) in microbial populations involved in the anaerobic acid phase, intermediate phase between the anaerobic phase and the methanogenic phase, that act to convert hydrolysis substrate to methane metabolic precursors. The authors concluded that Proteobacteria, Firmicutes, and Bacteroidetes were the dominant representatives, with variations between the relative abundance as described above. Their work showed that the phylum Firmicutes was highly representative of the anaerobic acid and aerobic phases. In contrast, the phylum Proteobacteria was very rare in the anaerobic acid phases, and the relative abundance of Bacteroidetes increased from aerobic to anaerobic acid phases. Comparing with our relative abundance, it is possible to indicate that due to recirculation, possibly the anaerobic acid phase may be suppressed in the treatment system of the city of Foz do Iguacu, considerable information aimed at improving the biological treatment.

In this sense, the effect of leachate recirculation was evaluated in the anaerobic digestion, using a bioreactor system, in the study of Luo and Wong (2019). The pH values of recirculated leachate were adjusted to 6.0 and 7.5, lower than that detected in the present study. Despite different conditions, the taxonomic profile of relative abundance identified was very similar to our work. Among them, Firmicutes was the dominant phylum during the whole anaerobic digestion process. Also, under the conditions of the study, the authors identified that the recirculation provides a straightforward approach to enhance the hydrolysis efficiency and, at the same time, biochemical balance between metabolic products and microorganisms in leachate to enhance methanogenic reaction.

Analyzing the sequences obtained only in the group of bacteria, 52.1% have not been identified at the genera level (Fig. 3). This result is impacting and shows how the diversity of this extreme environment is poorly known. In addition, all ASVs with an abundance greater than 3% are among the unidentified group. The genus classification was possible from the abundance 2.68% (genus Sedimentibacter). To the greatest abundances, it was not possible to identify the genus. In addition, the ASV with greater abundance, 9.5%, has no identification at the phylum level, that is, a highly significant ASV at work, does not have any taxonomic information. Analyzing this sequence individually (Blast—NCBI) a similarity was detected with PVC group (supplement material). PVC group is a superphylum of bacteria named after
its three important members, Planctomycetes, Verrucomicrobia, and Chlamydiae.

The vast majority of the dominant diversity found was anaerobic fermenting bacteria affiliated to Firmicutes phylum and Clostridia class. Firmicutes phylum comprises many Gram-positive bacteria, including pathogenic free-living bacteria, which have a great ecological diversity (Brinkmann et al. 2017). Representatives from this group such as Sedimentibacter genus (relative abundance of 2.68%, Fig. 3), can contribute to the degradation of organic matter and xenobiotic compounds as well as ferment a wide variety of recalcitrant organic compounds (Maphosa et al. 2012; Xu et al. 2020).

Furthermore, from the Firmicutes phylum, the genera Vulcanibacillus (2.51%) and Anaerovorax (1.91%) stood out. Vulcanibacillus is a genus of bacteria from the family of Bacillaceae with one known species (Vulcanibacillus modesticaldus). This anaerobic thermophilic bacterium was isolated from deep-sea hydrothermal vents. Its morphology is rod-shaped, immobile, Gram-positive, chemoorganotrophic, and able to form spores. Its growth temperature was 37–60 °C and pH 6–8.5. This genus is capable of using nitrate as an electron acceptor in the oxidation of carbohydrates, proteins and organic acids (L’Haridon et al. 2006). Representatives of this genus have recently been discovered, especially in the soil. St Clair et al. (2020) detected representatives in samples of soil microbiome of a Los Angeles urban farm. Based on the literature, there are no reports of this genus in leachate (isolation or independent culture methods). A recent work highlights the potential bioremediation of this genus based on an enrichment culture (Ngivprom et al. 2021), and this allows new perspectives for the treatment of leachate, never before evaluated. In addition, the ASV to Vulcanibacillus genus showed 94% similarity with V. modesticaldus (Blast—NCBI), highlighting an unknown diversity with potential for isolation.

What is more, only one species of the genus Anaerovorax has been characterized so far, the strict anaerobic chemoorganotrophic bacteria Anaerovorax odorimutans. The structure of the cell wall is similar to Gram-positive bacteria (without an outer layer), but its staining occurs as Gram-negative. It was isolated from anoxic freshwater bodies and
marine sediments. It does not use inorganic electron acceptors and does not form spores (Vos et al. 2011). They are characterized by the fermentation of putrescine, an organic molecule that forms in rotting meat and gives it a characteristic odor, contingent with the odor of leachate (Matthies et al. 2000).

Proteobacteria, the second phyla with the highest percentage of relative abundance (Fig. 2), is the greatest Gram-negative phylum, divided into nine classes. It also contains many free-living genera and nitrifying bacteria. The *Thiophaeococcus* and *Pseudomonas* genera showed relative abundance of 1.88 and 1.55%, respectively (Fig. 3). To *Thiophaeococcus* genus, so far only two species have been known isolated from mangrove (Kumar et al. 2008) and lagoon (Divyasree et al 2014). The genus was considered truly marine to Divyasree et al (2014) showing obligate phototrophy. This ASV showed high similarity (>99%) with two sequences deposited at the NCBI, both uncultured anaerobic bacterium clone still unpublished. Our work can be the turning point in the environments colonized by this genus. *Pseudomonas* genera are vastly employed on contaminated water bodies denitrification (Yang et al. 2019), and together with *Thauera* (Fig. 3) are capable of performing denitrification using nitrate or nitrite as electron acceptors. *Pseudomonas aeruginosa* was described as resistant to cadmium, and it can absorb and accumulate it through a stress response (Chellaiah 2018). Despite the high level of cadmium (0.048 mg L$^{-1}$), its concentration did not surpass the maximum value established by CONAMA. Cadmium toxicity alters the cellular division, apoptosis processes and cellular respiration, inhibiting the mitochondria (Rahimza-deh et al. 2017). The toxicity of leachate against cadmium concentration should be investigated. Thus, in future studies, if *Pseudomonas* and *Thauera* is isolated, it can be explored for further culturomics studies.

Regarding Bacteroidetes phylum, only the genus *Proteiphilum*, an exclusively proteolytic genus (Krieg et al. 2010), was identified (Fig. 3). Its abundance suggests a role in the
degradation of proteins present in the leachate and nitrification process. It is a genus of bacteria already known in urban waste treatment systems, in young and older systems (Yang and Song 2019).

The presence of sulfate-reducing bacteria indicates that the reductive pathway of the sulfur cycle may be present and in use (Long et al. 2016). Sulfur-reducing genera detected were Desulfobulbus, Desulfomicrobium, and Desulfovibrio, belonging to Proteobacteria phylum, and Desulfibacter and Desulfuviacillus, belonging to Firmicutes phylum (Fig. 3).

Our data showed many ASVs related to the phylum Synergistetes (Fig. 2, abundance of 7.4%), but with a low taxonomic identification level. The Thermovirga genus was the most abundant (Fig. 3, abundance of 0.61%). Synergistetes is a recently recognized phylum of anaerobic bacteria that inhabit a majority of anaerobic environments, including animal gastrointestinal tracts, soil, oil wells, and wastewater treatment (Hugenholtz et al. 2009). This phylum was identified from anaerobic membrane bioreactor treating landfill leachate, participating in the anaerobic degradation process (Mnif et al. 2012), representing one of the central communities in the urban waste leaching system.

Almost half of the sample diversity had less than 1% of the frequency (Fig. 3). This low-abundance diversity may work as a microorganism pool, and as the leachate composition changes, they become dominant (Wittebolle et al. 2008). Analysis performed in different microbiomes (rhizosphere of different perennial grassland species) showed that low-abundance bacteria action presented specific responses to a given metabolic process (Dawson et al. 2017). However, high bacterial diversity levels presume that several taxons of different perennial grassland species) showed that low-abundance bacteria action presented specific responses to a given metabolic process (Dawson et al. 2017). However, high bacterial diversity levels presume that several taxons are redundant concerning the function (Rivett and Bell 2008).

In the study of Yang and Song (2009), Prevotella and Sphingobacterium were more abundant at the methanogenic phase. In addition, although the chemical–physical parameters indicate a methanogenic phase profile in our leachate, the metabarcoding showed the absence of this group in our work. As the leachate reservoirs receive an influx of all MSW cells, methanogenic groups are likely inhibited by the leachate of cell 3 containing a high organic matter level compared to the older cells. On the contrary, it is highly likely that methanogenic groups dominate cells 1 and 2. Besides, the sample was collected in a shallow depth, which is another factor that explains the low abundance of methanogenic groups. Also, the primers used in the sequencing method are known to be non-specific for amplifying methanogens. Thus, the lower abundance may be due to methodological bias rather than ecologic reasons (Bahram et al. 2019).

Regarding the Archaea diversity (Fig. 4), despite not being the study focus, it was possible to identify the Methanosarcina, (48.39%), Methanoseta (32.26%), and Methanoculleus (11.29%). All genera were also observed in low-abundance (Köchling et al. 2015) in leachate from Candeias landfill. Cardinali-Rezende et al. (2009) identified hydrogenotrophic and methanogens archaea belonging to Methanomicrobioides order (Methanoculleus genus) from the digestion process fed wastewater sludge and organic fraction of the municipal solid waste. Methanospirillum and Methanothermobacter can be considered one of the most common genera related to the hydrogenotrophic methanogens organism (produced methane from hydrogen and carbon dioxide), found in anaerobic digesters. On the other hand, the genera Methanosarcina and Methanosaeta can be considered one of the most common genera to acetoclastic methanogens (produced methane from acetic acid reduction), found in anaerobic digesters (Czatkowska et al. 2020). It suggested a considerable methanogenic diversity in our sample, responsible for methane production. That corroborated the chemical data (mixed characteristics of the acidic and methanogenic phases). However, this diversity was represented by a low abundance of these genera (only 0.5% of ASVs obtained were defined in the archaea domain).

Thus, the anaerobic digestion of organic residues observed in our work can be influenced especially by the dominance of prokaryotic genera belonging to the phylum Firmicutes, with the possible involvement of acetoclastic methanogens. However, toxic contaminants in leachate, including heavy metals and organic xenobiotic compounds may have influenced the microbial composition in the samples studied (Zegzouti et al. 2020). Our study is essential, since it was possible to observe a considerable microbial community related to pathogenic taxonomic groups, indicating care with potential leaks and environmental contamination. As well as taxonomic groups possibly involved in bioremediation processes.

The use of microbial communities recovered from the landfill itself in bioremediation processes can be regarded as a useful tool in replacing conventional methods (as physical–chemical approaches). Biological treatment, including bioremediation approaches, may often be preferred due to its reliability, low operating costs, and simplicity providing several advantages about the biodegradable matter and nitrogen compound removal (Zveibil 2001).

The bacterial community can be resistant to a wide range of compounds found in leachate samples, and it may have a high potential application for its treatment (Morris et al. 2018). The majority of environmental microbiomes is not amenable to cultivation under standard laboratory growth conditions and hence remain uncharacterized. For environmental applications, such as bioremediation, it is necessary to isolate microbes performing the desired function, which may not necessarily be the fast growing or the copiotroph microbiota (Pathak et al. 2020). Therefore, from our project, a bioremediation process can be designed based on
the recent strategic known as culturomics, where enriched culture media can be developed and targeted to recover the main prokaryotic genera identified in the present study, as representatives of the *Vulcanibacillus* genus, which may be involved in leachate bioremediation processes. This strategy aims to improve cultivation efficiencies of environmental microbiota and can be coupled with the application of omics-based tools (Bodor et al. 2020).

Lastly, to our knowledge, the present study can be characterized as one of the first studies on the microbial diversity of leachate in the stabilization phase with a recirculation system. More studies are needed to understand the microbial diversity of leachate in different scenarios, with and without recirculation, and in different phases. The results emphasize the need for regular monitoring of microbiological of the main species identified to encourage future studies on the use of microbial communities recovered from leachate. Further analysis using metatranscriptomics and/or metagenomics approaches must to be done to help elucidate ecologic functionality, and allow a targeted search for taxa and/or genes that are capable of biotransforming toxic and recalcitrant compounds found in leachate.

**Conclusions**

The leachate stored in the sanitary landfill of Foz do Iguaçu showed physical–chemical conditions, from both acid and methanogenic phases. This phenomenon is reflected in the prokaryotic community, with the phylum Firmicutes’ predominance and genera from the aerobic and anaerobic phases. The aerobic group can act in the hydrolytic reactions, where the organic fraction can be metabolized aerobically to form CO₂ and H₂O. Also, bacteria from the anaerobic phase are characterized by fermentation, generating organic acids, and ammoniacal nitrogen. In addition, it was observed that the leachate acidogenic phase was masked in the chemical and physical analyzes, but was evidenced in the metabarcoding methodology. The concentration of ammoniacal nitrogen was below average, which indicates the action of the methanogenic phase. However, no specifically methanogenic group was detected in significant abundance. Several factors may have contributed to the absence of methanogenics. As one of the first studies that analyze leachate from landfill considered old and with recirculation, it is possible to conclude that these results were due to the effect of the leachate.
recirculation between the MSV systems cells. Moreover, although the physical-chemistry parameters indicate good degradability, further investigations are necessary to confirm the recirculation system efficiency.

Functionally, microorganisms involved with the carbon, nitrogen, and sulfur cycle were detected, mainly. Those groups have a crucial role in the development of bioremediation strategies towards toxic leachate compounds. As considered an extreme environment, the sample presented several microbial groups able to develop even under toxic conditions. Even though the biodegradation efficiency showed by physical-chemistry analysis, the recirculation may result in the accumulation of cations, especially cadmium. Aiming at these processes, the genera Sedimentibacter, Vulcanaebacillus, Anaeorovorax, Thiophaeococcus, and Pseudomonas can be explored in future studies for bioremediation applications. Finally, an expressive abundance was attributed to the superphylum known as PVC group, little studied and with unknown scientific potential.

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**Author contributions** MRZP: formal analysis, funding acquisition, project administration, supervision, visualization, writing—original draft, writing—review and editing. JVFM: formal analysis and methodology. RCB-S: formal analysis, writing—review and editing. FAMC: formal analysis and methodology. JAMG: formal analysis and methodology. RCB-S: formal analysis, funding acquisition, project administration, supervision, visualization, writing—original draft, writing—review and editing.

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**Availability of data and material** The datasets generated during the current study are available in the Rafaela Santos repository, https://basespace.illumina.com/projects/143361223. Reference number 2-DNA-CH-16S.

**Declarations**

**Conflict of interest** The authors declare that they have no competing interests.

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