Microbial Mediation of Carbon, Nitrogen, and Sulfur Cycles During Solid Waste Decomposition

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Abstract
Landfills are a unique “terrestrial ecosystem” and serve as a significant carbon sink. Microorganisms convert biodegradable substances in municipal solid waste (MSW) to CH4, CO2, and microbial biomass, consisting of the carbon cycling in landfills. Microbial-mediated N and S cycles are also the important biogeochemical process during MSW decomposition, resulting in N2O and H2S emission, respectively. Meanwhile, microbial-mediated N and S cycles affect carbon cycling. How microbial community structure and function respond to C, N, and S cycling during solid waste decomposition, however, are not well-characterized. Here, we show the response of bacterial and archaeal community structure and functions to C, N, and S cycling during solid waste decomposition in a long-term (265 days) operation laboratory-scale bioreactor through 16S rRNA-based pyrosequencing and metagenomics analysis. Bacterial and archaeal community composition varied during solid waste decomposition. Aerobic respiration was the main pathway for CO2 emission, while anaerobic C fixation was the main pathway in carbon fixation. Methanogenesis and denitrification increased during solid waste decomposition, suggesting increasing CH4 and N2O emission. In contrast, fermentation decreased along solid waste decomposition. Interestingly, Clostridiales were abundant and showed potential for several pathways in C, N, and S cycling. Archaea were involved in many pathways of C and N cycles. There is a shift between bacteria and archaea involvement in N2 fixation along solid waste decomposition that bacteria Clostridiales and Bacteroidales were initially dominant and then Methanosarcinales increased and became dominant in methanogenic phase. These results provide extensive microbial mediation of C, N, and S cycling profiles during solid waste decomposition.

Keywords Landfill · Solid waste decomposition · Carbon, nitrogen, and sulfur microbial cycles · Microbial mediation

Introduction
Terrestrial ecosystem is a significant carbon sink and plays a crucial role in global climate-feedbacks. Microbial-mediated soil carbon dynamics is one of the most important processes in global carbon cycling [1, 2]. An understanding of soil microbial community structure and function and their influence on land–atmosphere carbon exchanges is crucial to assess terrestrial carbon cycle-climate feedbacks.

Landfill is a unique “terrestrial ecosystem,” containing a large amount of biodegradable substances in municipal solid waste (MSW). In 2014, USA, EU, and China co-produced 6.3 billion tons of MSW [3]. With global urbanization, more and more MSW is produced. Sanitary landfill is one of the major methods for MSW disposal worldwide. Subsequently, size of this special “terrestrial ecosystems” is expanding. Consortia of microbial community converts biodegradable substances in MSW to methane, carbon dioxide, and microbial biomass, consisting of carbon cycling in landfill [4]. Landfill is also the third largest anthropogenic methane sources after wetland and paddy, producing approximately 75 Tg methane per year during 2000–2009 [5]. In addition, landfill is one of the important anthropogenic N2O sources. Microorganisms
mediate biogeochemical cycles of C, N, and S and play a central role in landfill carbon dynamics and greenhouse gas emission, but their response to solid waste decomposition has not been well-characterized. This issue related to “what is the importance of spatial and temporal variation in microbial community structure and function to key environmental processes and geochemical cycles?” is also one of the 50 important research questions in microbial ecology [6].

Solid waste decomposition in landfill is a key microbial-mediated environmental process. Most previous studies on microbial-mediated C cycle focus on the linkage of microbial community composition and the methane production. Studies on microbial-mediated N cycle are mainly involved in nitrification and denitrification processes [7]. In contrast, the study on microbial-mediated S cycle is limited. Fei et al. investigated the methanogen community variation in methane production stage and found that Methanobacteriaceae is the abundant methanogen [8]. Christopher A et al. assessed bacterial and archaeal population during methane production, and results showed that bacterial and archaeal community expressed specific character related to methane yield [9]. In addition, clear microbial community composition succession was observed during solid waste decomposition in laboratory-scale reactors [7, 10]. The results suggested that microbial community structure responded to the methane metabolism. Yang and Song also investigated the nitrification and denitrification during MSW decomposition using functional genes and found that nitrification and denitrification mainly occurred in early aerobic phase and methanogenic phase, respectively [7]. Overall, up to now, the information on microbial community structure and function, especially the function during MSW decomposition, is still limited. This gap is mainly due to the lack of feasible technology to analyze microbial community function. Emerging technologies such as Geochip and metagenomics enable us to identify this microbial complexity and link their structure and function to carbon dynamics [11, 12].

Here, we used 16S rRNA-based pyrosequencing and metagenomics approaches to analyze the response of microbial community structure and functions to C, N, and S cycling during solid waste decomposition in a laboratory-scale bioreactor. Bioreactor can eliminate many of the confounding variables (e.g., solid waste heterogeneity) [13] that make such a proof of principle experiment difficult to perform and interpret. Solid waste decomposition is a long-term event, which endures several decades. Therefore, it is difficult to obtain representative samples for solid waste decomposition process in landfill sites. Bioreactor can simulate the solid waste decomposition in a short term (several months), presenting the microbial activity on C, N, and S cycling during solid waste decomposition [14]. This study can provide fundamental knowledge for carbon dynamics and greenhouse gas emission and provide a microbiome blueprint in landfills.

**Materials and Methods**

**Solid Waste Samples**

Solid waste samples were collected from a 265-day operation laboratory-scale bioreactor. Bioreactor construction, operation, and solid waste decomposition phases separation have been published somewhere [15]. In short, fresh solid waste sample (100 kg) was obtained from Changshengqiao (CSQ) landfill, Chongqing China. After pretreatment, 16.4 kg fine solid waste (contained 57.1% food waste, 16.8% plastic, 13.4% paper, 9.1% branches and leaves, and 3.6% fabric and cloth based on wet mass) was packed into the bioreactor. The average particle density of packed solid waste was 1000 kg dm−3. Leachate recirculation was employed to accelerate solid waste decomposition [4]. During 265-day operation, physiochemical parameters of solid waste (upper, middle, and lower layers; each layer is 20 cm high) and leachate were monitored monthly and weekly, respectively. Accordingly, solid waste decomposition was separated into aerobic phase (AP), anaerobic acid phase (ACP), and methanogenic phase (MP) based on the variation of pH and BOD5/COD ratio, which are key factors in determination solid waste decomposition phases [16, 17]. There are several models for solid waste decomposition phases separation. Among them, three phases (AP, ACP, and MP) model is one of the most classic one [16]. AP occurs in a very short term (usually 1–2 weeks) in landfill to consumed oxygen mixed in the void space of solid waste. In ACP, microbial community decomposes macromolecular matter to small molecular acid such as carboxylic acid. In MP, methanogenesis process occurs.

**DNA Extraction and Illumina MiSeq Sequencing and Data Analysis**

To obtain complete information of microbial community structure and function, the solid waste samples were taken from upper, middle, and lower layers and well mixed to generate a composite sample. The mixed solid waste sample was used for 16S rRNA pyrosequencing and metagenomic analysis.

DNA for 16S rRNA pyrosequencing of triplicate solid waste samples was extracted using previously reported method [18], which is design for solid waste DNA extraction. The bacterial and archaeal community composition were analyzed by Illumina MiSeq sequencing using reported primers 338F-806R [19] and Arch334F-Arch915R [20], respectively. After amplification with high-fidelity Taq
polymerase (Invitrogen, USA), equal quantities of three PCR products per sample were pooled. Mixed DNA was then purified with the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA), and quantified using the NanoDrop2000. Amplicons were sequenced at the Illumina MiSeq platform of Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China).

The raw pyrosequencing data were deposited in the NCBI Sequence Read Archive (Accession number: PRJNA389232 for bacteria, PRJNA411954 for archaea). The data treatment was followed previously published procedure [21]. Generally, we obtained 655,805 and 589,719 valid sequences with an average length of 442 and 526 bp for bacterial and archaeal community, respectively. Each sample had an average of 36,434 ± 4953 bacterial sequence and 36,857 ± 4815 archaeal sequence (Table 1).

**Genomic DNA Extraction, Library Construction, and Metagenomics Sequencing**

Community genomic DNA, library construction and metagenomics sequencing were performed according to previously published protocols [15]. Briefly, community genomic DNA was extracted from solid waste samples using the EZNA® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) and quantified with NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, Delaware, USA). Afterwards, it was examined with the 1% agarose gels electrophoresis. DNA was fragmented to about 300 bp for paired-end library construction. Paired-end sequencing was performed on Illumina HiSeq4000 platform (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using HiSeq 3000/4000 PE Cluster Kit and HiSeq 3000/4000 SBS Kits. Raw metagenomics datasets have been deposited into NCBI Sequence Read Achieve database (Accession Number: PRJNA412592). The related sequence analysis was performed in Majorbio Bio-Pharm data analysis platform.

**Sequence Quality Control and Genome Assembly**

Sequence quality control and genome assembly were performed according to previously published protocols [15]. Briefly, after 3′ and 5′ end of sequence and low-quality sequence (length < 50 bp; quality value < 20; having N bases) were removed; the short reads were assembled using De bruijn-graph-based assembler SOAPdenovo (http://soap.genomics.org.cn, Version 1.06). Scaffolds with a length over 500 bp were retained for statistical tests after K-mers, varying from 1/3 to 2/3 of reads length were tested. The best K-mer, which yielded the minimum scaffold number and the maximum value of N50 and N90, was chosen. Scaffolds with a length over 500 bp were extracted and broken into contigs without gaps. Contigs were used for further gene prediction and annotation.

**Gene Prediction, Microbial Structural, and Functional Annotation**

Gene prediction, microbial structural, and functional annotation were performed according to previously published protocols [15]. MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/) was used to predict open reading frames (ORFs). The predicted ORFs with length being or over 100 bp were retrieved and translated to amino acid sequences using the NCBI translation table. Identical reads were removed using CD-HIT [22]. KEGG (Kyoto Encyclopedia of Genes and Genomes) and Orthologs (KO) databases were used for taxonomic and functional annotation. JCVI Metagenomics reports (http://jcvi.org/metarep) were used for analysis and comparative metagenomics [23]. KO annotation was used for functional analysis and KO counts were normalized according to the length of the read and the length of

|                          | AP  | ACP | MP  |
|--------------------------|-----|-----|-----|
| Total number of metagenomic reads | 27,263,846 | 26,665,854 | 34,516,474 |
| Metagenomic taxonomically assigned reads | 99.7% | 98.9% | 99.9% |
| Bacteria (Metagenomic data set) | 98.6% | 97.7% | 95.4% |
| Archaea (Metagenomic data set) | 0.1% | 1.1% | 4.5% |
| Fungi (Metagenomic data set) | 0.90% | 0.06% | 0.01% |
| Reads of key genes in C cycles | 19,491 | 18,499 | 17,319 |
| Reads of key genes in N cycles | 52,139 | 46,898 | 70,481 |
| Reads of key genes in S cycles | 3708 | 8309 | 16,183 |
| Bacteria (16S rRNA) | 100,831 | 120,502 | 113,294 |
| Archaea (16S rRNA) | 95,321 | 122,957 | 101,937 |

16S rRNA based pyrosequencing reads for bacteria and archaea, n = 3. AP aerobic phase, ACP anaerobic acid phase, MP methanogenic phase
the target gene [24]. The functional analyses focused on the C, N, and S cycling.

**Result**

**Physiochemical Parameters Variation During Solid Waste Decomposition**

Solid waste physiochemical parameters varied according to the solid waste decomposition, and were consistent with previously reported solid waste chemistry characteristics for solid waste decomposition [14, 25]. The concentrations of organic matter (OM), BDM % (ratio of biodegradable matter (BDM)), TN, and nitrate in the end of the operation were much lower than that in initial (Fig. S1), suggesting that solid waste decomposition occurred. Although these parameters differed at upper, middle, and lower depths due to heterogeneity of solid waste [13] and different moisture contents caused by recirculation leachate flow path [26], their variation showed similar trend. Notably, the concentrations of these parameters in upper layer in late period (229 to 260 days) were lower than those in middle and lower layers. This is because a portion of cover soil intruded into the upper layer owing to solid waste settlement [16]. The concentration of the related physiochemical parameters in cover soil (OM: 5.85 g/kg; TN: 36.1 mg/kg) is much lower than that in solid waste (OM: 97.5 g/kg and TN: 6.8 g/kg, lowest value of fresh solid waste). Therefore, the changes of these parameters in upper layer were not compared with others in regard to OM, BDM %, and TN. In sum, there are 13–28%, 56–63%, and 27–49% loss of OM, BDM %, and TN in the reaction. Because landfill cover soil played a crucial role in methane oxidation [27, 28], the cover soil intruded solid waste sample in MP was also pooled with other solid waste samples in this phase for microbial community structure and function analysis.

**Taxonomic Composition During Solid Waste Decomposition**

Most metagenomic reads (98.9–99.9%) were taxonomically assigned to bacteria, archaea, and fungi (Table 1). As expected, bacteria numerically dominated the genetic composition of the microbial communities in all three solid waste decomposition phases (99.7% in AP, 98.9% in ACP and 95.4% in MP). Archaea in MP (4.5%) is higher than that in AP (0.1%) and ACP (1.1%). This is because methanogen is expected to be abundant in methanogenic phase to convert methane metabolic precursors to methane. Fungi in AP are higher than that in ACP and MP and their composition is shown in Fig. S2. Fungi, the primary organic matter decomposer, are supposed to decompose macromolecular matter (lignin and cellulose) to micromolecular matter on the early stage of solid waste decomposition [29].

The most abundant bacteria and archaea found during solid waste decomposition on the basis of both 16S rRNA and metagenomic sequencing were Proteobacteria, Firmicutes, and Bacteroidetes, Methanomicrobiales, and Methanosarcinales (Fig. 1), which is consistent with results of previous studies on microbial community composition analysis in landfill using high throughout sequencing technique [9, 21, 30]. In addition, high abundance of archaea E2 (54% in ACP) was found in 16S rRNA-based pyrosequencing, whereas high abundance of Methanobacteriales (14% in ACP) and Methanomassiliicoccales (13% in AP) was found in metagenomic data set (Fig. 1). Our previous study on methanogens succession during MSW decomposition also observed the obligate anaerobic methanogens in AP [31]. However, their ecological function in aerobic condition remains largely unknown. These differences can be partly explained by PCR biases in the 16S rRNA-based approach [32, 33]. Comparative analysis of metagenomic and 16S rRNA-based pyrosequencing on taxonomic diversity and structure of prokaryotic communities using Tara Oceans global expedition data set showed that metagenomic analysis may provide more realistic estimates of community richness and evenness than 16S rRNA-based pyrosequencing analysis by overcoming PCR biases [33].

Interestingly, metagenomic analysis revealed that Methanomassiliicoccales existed during solid waste decomposition. Genomics analysis indicated that Methanomassiliicoccales genomes encode a truncated methanogenesis pathway via H$_2$-dependent methylotrophic methanogenesis [34]. Previous studies have detected acetoclastic and hydrogenotrophic methanogens in landfill but have not detected methylotrophic methanogenesis. This observation of possible H$_2$-dependent methylotrophic methanogenesis provides previous unidentified methane production pathway in landfill. Halophile Haloferacales [35] was also found in metagenomic analysis in this study, likely due to high salt content (70–7700 mg/L) in landfill [16, 30].

**KEGG Modules Distribution in Bacteria and Archaea**

The three most abundant bacterial phyla (Proteobacteria, Firmicutes, and Bacteroidetes) and archaeal orders (Methanosarcinales, Methanomicrobiales, and Methanobacteriales) were picked for analysis of shared KEGG pathway modules. There are a total of 905 and 488 KEGG pathway modules for bacterial and archaeal communities, respectively. Among them, 350 and 167 KEGG pathway modules exist in one, two, or in all the three phyla or orders, respectively (Fig. S3, Table S1 and S2). Approximately 2/3 models for bacteria and archaea were left, suggesting potential of function during solid waste decomposition is complex.
The three most abundant bacterial phyla *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* shared 178 modules, suggesting that these modules represent core metabolic pathways during solid waste decomposition. The functional roles of these 178 modules were mostly assigned to carbohydrate metabolism, amino acid, nucleotide biosynthesis, and for transportation of metal ions and small molecule metabolites (e.g., glycine, phosphate, molybdate). Phyla *Proteobacteria* and *Firmicutes* shared 62 modules, including modules implicated in sugar and amino acid transport system, two-component regulatory system for nitrogen regulation and nitrate respiration, PTS system, and sugar (glucose, maltose N-acetylglucosamine) specific II component. Phyla *Proteobacteria* and *Bacteroidetes* shared 7 modules, including modules implicated in two-component regulatory system (C4-dicarboxylate transport and alginate production), biosynthesis (F420, lignin, and C19/C18-steroid hormone), and nicotinate degradation. Phyla *Firmicutes* and *Bacteroidetes* shared 8 modules, including modules implicated in two-component regulatory system (multicellular behavior control and cell wall stress response), teichoic acid transport system, and phosphatidylcholine (PC) biosynthesis. Phylum *Proteobacteria* had 57 unique modules, higher than *Firmicutes* (37 modules) and *Bacteroidetes* (1 modules), which accords to the relative abundance of these phyla.

The three most abundant archaeal orders, *Methanomicrobiales*, *Methanobacteriales*, and *Methanosarcinales*, shared 107 modules. The functional roles of the shared modules were mostly assigned to carbohydrate metabolism, amino acid, nucleotide biosynthesis and methanogenesis, anaerobic C fixation, nitrogen fixation, and transport systems. *Methanomicrobiales* and *Methanobacteriales* shared 2 modules with functions of key enzyme in carbonate metabolism (succinate dehydrogenase and fumarate reductase). *Methanosarcinales* and *Methanobacteriales* shared 5 modules with functions of one carbon oxidation in citrate cycle, biosynthesis (N-glycan precursor, phosphatidylethanolamine), ferredoxin, oxidoreductase, and GABA (gamma-Aminobutyrate) shunt. *Methanosarcinales* and *Methanobacteriales* shared 20 modules with functions of transport system (amino acid and metal) and biosynthesis (nucleotide sugar and trehalose). Modules assigned to assimilatory sulfate reduction were also present in the shared modules. Individually, *Methanosarcinales* had 23 unique modules, higher than *Methanobacteriales* (5 modules) and *Methanomicrobiales* (4 modules).
C, N, and S Cycling During Solid Waste Decomposition

For the carbon cycling, the potential for Calvin cycle, anaerobic C fixation, fermentation, and CO respiration were detected in all three phases (Table 2, Fig. 2 and Table S3 and S4; Figs. 2, 3, and 4 were modified based on the analysis of marker genes described by Llorens-Mares et al., 2015 [36]). Among them, aerobic respiration, anaerobic C fixation, and fermentation were dominant, accounting for 98.6%, 97%, and 80.7% of selected carbon functional reads in AP, ACP, and MP, respectively. In MP, the most abundant pathway was aerobic respiration and anaerobic C fixation. Aerobic methane oxidation only occurred in MP with very low abundance (0.0033%). Calvin cycle occurred in ACP and MP, both with low abundance. The relative abundance of methanogenesis pathways increased along solid waste decomposition from none detection in AP to 1.4% in ACP and to 8.1% in MP, which is accord with the solid waste decomposition phase development that methane production mainly occurred in methanogenesis phase [13]. MP had higher relative abundance of CO respiration than AP and ACP. In contrast, the relative abundance of fermentation decreased along solid waste decomposition as expectation. AP and ACP had higher anaerobic C fixation than MP.

Various orders of bacteria and archaea were involved in carbon cycling. For anaerobic carbon fixation, Clostridiales (35% and 35%) and Bacteroidales (27% and 14%) were mainly involved in AP and ACP and Clostridiales (24%) and Methanosarcinales (33%) were involved in MP. Interestingly, Methanosarcinales appeared in ACP with low abundance (2%) and became abundant in MP. Clostridiales was predominantly involved in fermentation, representing 34%, 51%, and 93% of totals in AP, ACP, and MP, respectively. In addition, Propionibacteriales (27%), Corynecbacteriales (13%) and Lactobacillales (11%) were also dominant in AP. Propionibacteriales (15%) and Corynecbacteriales (19%) were dominant in MP. Spirochaetales, Burkholderiales, and Clostridiales were the largest contributors in aerobic CO oxidation and their abundance varied dramatically in AP, ACP, and MP. Spirochaetales (88% and 59%) and Burkholderiales (10% and 90%) were predominantly involved in aerobic CO oxidation in AP and MP, but Spirochaetales (59%) and Clostridiales (38%) were dominant in ACP. For Calvin cycle, Methanomicrobiales (75% and 30%) were mainly involved in AP and MP, whereas Alteromonadales (93%) became important in ACP. Rhizobiales (49%) and Rhodobacteriales (20%) were also important in MP. For aerobic respiration, Rhodobacteriales (20%), Sphingobacteriales (18%), Bacillales (17%), and Rhizobiales (14%) were main contributors in AP; Rhodobacteriales (70%) and Bacillales (15%) were dominant in ACP; and Pseudomonadales (66%), Bacillales (16%), and Burkholderiales (15%) were abundant in MP. For Methanogenesis, Methanosarcinales (47%), Methanomicrobiales (25%), and Methanobacteria (28%) were main contributors for Methanogenesis in ACP, whereas Methanosarcinales

| Cycle   | Step                               | AP (%) | ACP (%) | MP (%) |
|---------|------------------------------------|--------|---------|--------|
| Carbon  | Aerobic C fixation*(Calvin cycle)  | ND     | 0.7     | 0.5    |
|         | Aerobic CH4 oxidation              | ND     | ND      | 0.0033 |
|         | Aerobic respiration                | 8.0    | 24.4    | 59.5   |
|         | Anaerobic C fixation               | 55.2   | 49.9    | 19.7   |
|         | CO oxidation                       | 1.4    | 1.0     | 10.5   |
|         | Fermentation                       | 35.4   | 22.7    | 1.5    |
|         | Methanogenesis                     | ND     | 1.4     | 8.1    |
| Nitrogen| Ammonification                     | 3.3    | 3.5     | 0.8    |
|         | Denitrification                    | 2.6    | 4.6     | 10.4   |
|         | Nitrate reduction + Nitrite oxidation | 6.9 | 2.3     | 9.5    |
|         | Nitrate reduction                  | 0.1    | 2.3     | 4.7    |
|         | Nitrogen assimilation              | 40.4   | 61.0    | 31.1   |
|         | Nitrogen fixation                  | 2.7    | 1.7     | 1.2    |
|         | Nitrogen mineralization            | 44.0   | 24.7    | 42.3   |
| Sulfur  | Assimilatory sulfate reduction     | 40.6   | 11.3    | 35.9   |
|         | Dissimilatory sulfate reduction and sulfide oxidation | 4.3 | 1.4 | 0.4 |
|         | Sulfur mineralization              | 54.2   | 71.5    | 63.5   |
|         | Polysulfide reduction              | 1.7    | 16.1    | 0.4    |

ND not detected
**Fig. 2** Genetic potential for carbon cycling pathways during solid waste decomposition using a combination of normalized marker genes. Arrow size proportional to the potential flux of the carbon pathways (100% value, see Supplementary Table S3). Dotted lines: not detected marked genes but putative presence of the pathway (see main text). Relative abundances (> 10%) for the main microbes potentially driving each conversion step are shown. *AP* aerobic phase, *ACP* anaerobic acid phase, *MP* methanogenic phase.
Fig. 3 Genetic potential for nitrogen cycling pathways during solid waste decomposition using a combination of normalized marker genes. Arrow size proportional to the potential flux of the carbon pathways (100% value, see Supplementary Table S3). Dotted lines: not detected marked genes but putative presence of the pathway (see main text). Relative abundances (> 10%) for the main microbes potentially driving each conversion step are shown. AP aerobic phase, ACP anaerobic acid phase, MP methanogenic phase.

**AP**

- **Ammonification**
  - Rhizobiales 24%
  - Clostridiales 24%
- **Denitrification**
  - Pseudomonadales 41%
  - Methanosaetales 20%
- **Nitrification**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**Nitrogen assimilation**

- **Nitrogen assimilation**
  - Clostridiales 29%
  - Bacteroidales 23%

**Nitrogen reduction**

- **Nitrogen reduction**
  - Methanobacterales 17%
  - Methanosaetales 5%

**Nitrogen fixation**

- **Nitrogen fixation**
  - Methanosaetales 17%
  - Methanobacterales 5%

- **N2 fixation**
  - Methanosaetales 89%
  - Methanobacterales 7%

**NO2^-**

- **NO2^-**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**NH4^+**

- **NH4^+**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**NO3^-**

- **NO3^-**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**Organic nitrogen**

- **Organic nitrogen**
  - Clostridiales 29%
  - Bacteroidales 23%

**AP**

- **Ammonification**
  - Rhizobiales 24%
  - Clostridiales 24%
- **Denitrification**
  - Pseudomonadales 41%
  - Methanosaetales 20%
- **Nitrification**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**Nitrogen assimilation**

- **Nitrogen assimilation**
  - Clostridiales 29%
  - Bacteroidales 23%

**Nitrogen reduction**

- **Nitrogen reduction**
  - Methanobacterales 17%
  - Methanosaetales 5%

**Nitrogen fixation**

- **Nitrogen fixation**
  - Methanosaetales 17%
  - Methanobacterales 5%

- **N2 fixation**
  - Methanosaetales 89%
  - Methanobacterales 7%

**NO2^-**

- **NO2^-**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**NH4^+**

- **NH4^+**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**NO3^-**

- **NO3^-**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**Organic nitrogen**

- **Organic nitrogen**
  - Clostridiales 29%
  - Bacteroidales 23%

**MP**

- **Ammonification**
  - Rhizobiales 24%
  - Clostridiales 17%
- **Denitrification**
  - Pseudomonadales 41%
  - Methanosaetales 20%
- **Nitrification**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**Nitrogen assimilation**

- **Nitrogen assimilation**
  - Pseudomonadales 74%
  - Methanosaetales 3%

**Nitrogen reduction**

- **Nitrogen reduction**
  - Methanobacterales 17%
  - Methanosaetales 5%

**Nitrogen fixation**

- **Nitrogen fixation**
  - Methanosaetales 89%
  - Methanobacterales 7%

**NO2^-**

- **NO2^-**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**NH4^+**

- **NH4^+**
  - Nitrosomonadales 54%
  - Bacteroidales 14%

**NO3^-**

- **NO3^-**
  - Nitrosomonadales 54%
  - Bacteroidales 14%
Fig. 4 Genetic potential for sulfur cycling pathways during solid waste decomposition using a combination of normalized marker genes. Arrow size proportional to the potential flux of the carbon pathways (100% value, see Supplementary Table S3). Dotted lines: not detected marked genes but putative presence of the pathway (see main text). Relative abundances (>10%) for the main microbes potentially driving each conversion step are shown. AP aerobic phase, ACP anaerobic acid phase, MP methanogenic phase.
(83%) was predominantly in Methanogenesis in MP. Aerobic methane oxidation only occurred in MP and 100% Corynebacteriales were involved in this pathway.

For the nitrogen cycling, most of the detected genes catalyzed nitrogen assimilation and nitrogen mineralization, which are involved in all three phases of solid waste decomposition, representing 84.4%, 85.7%, and 73.4% of selected nitrogen functional reads in AP, ACP, and MP, respectively (Table 2, Fig. 3, and Table S3 and S4). These two pathways were mainly accomplished with Pseudomonadales, Rhodobacterales, Bacillales, and Clostridiales. Specifically, low abundance of Methanosarcinales (1%) and Methanomicrobiales (1%) were also involved in mineralization in ACP, whereas 3% Methanosarcinales were involved in this pathway in MP. Methanomicrobiales (1%) and Methanobacterales (1%) were involved in nitrogen assimilation in MP. There is an increasing trend for denitrification along solid waste decomposition, from 2.6% in AP to 4.6% in ACP, and to 10.4% in MP, by Rhodobacterales, Pseudomonadales, and Propionibacterales. Nitrate reduction had similar trend as denitrification and the main player were Pseudomonadales, Alteromonadales, and Eggerthellales. Eggerthellales (53%) was abundant in AP. Alteromonadales (71%) and Eggerthellales (28%) were dominant in ACP and Pseudomonadales (100%) was predominant in MP. Ammonification and nitrogen fixation were detected in all three phases of solid waste decomposition with relative low abundance, 0.8–3.5% and 1.2–2.7%, respectively. Clostridiales (10%, 0%, and 42%) and Bacteroidales (86%, 30%, and 40%) were mainly involved in ammonification. Clostridiales (52%, 65%, and 4%), Bacteroidales (26%, 4%, and 0%), and Methanosarcinales (0%, 17%, and 89%) were mainly involved in nitrogen fixation. Specifically, cyanobacteria (Nostocales) were involved in this pathway in AP (5%) and ACP (5%) with low abundance. Surprisingly, Methanosarcinales (17%) and Methanomicrobiales (1%) were involved in nitrogen fixation in ACP, while Methanosarcinales became abundant in MP (89%). Potential of nitrification and anammox were not detected throughout solid waste decomposition.

For the sulfur cycling, assimilatory sulfate reduction and sulfur mineralization were the main pathways in all three phases of solid waste decomposition, accounting for 94.8%, 82.5%, and 99.4% of selected sulfur functional reads in AP, ACP, and MP, respectively (Table 2, Fig. 4, and Table S3 and S4). These two pathways were mainly involved by Rhodobacterales, Pseudomonadales, Propionibacterales, and Bacteroidales. Dissimilatory sulfate reduction and sulfide oxidation was also detected in all phases with low relative abundance, 0.4–4.3% of total, by Clostridiales (87% in AP, 99% in ACP, and 100% in MP). Polysulfide reduction had higher abundance in ACP (16.1%) than AP (1.7%) and MP (0.4%). Accordingly, the main players were Bacteroidales, Alteromonadales, and Eggerthellales.

**Discussion**

Landfill, a “baby” terrestrial ecosystems, represents a significant carbon sink with carbon biotransformation [4]. Meanwhile, landfill releases large amount of microorganism-mediated greenhouse gas (CO₂, CH₄, and N₂O). Therefore, landfill is a good example to study the biological mechanisms that regulate carbon exchanges between the belowground and atmosphere. In this study, integration of solid waste physiochemical parameters, 16S rRNA-based pyrosequencing, and metagenomics analysis, we show that the structure and potential function of microbial communities changed during solid waste decomposition in response to biodegradable substrates.

Labile carbon seems to maintain long term carbon stability and storage [12]. During solid waste decomposition, microorganism first hydrolyze cellulose and food waste to from hydrolysis products (sugars, amino acid, and fatty acid), then metabolize hydrolysis products to methane metabolic precursors (acetate and H₂), and finally convert methane metabolic precursors to methane [4]. Accordingly, microbial community composition changed according to the available biodegradable substrate. For instance, the relative abundance of Firmicutes, typical cellulose-degrading microorganisms [37], increased from 32.5 ± 2.0% in AP to 45 ± 3.2% in ACP and then decreased to 13.2 ± 1.3% in MP, suggesting its important roles in hydrolysis and fermentation. The relative abundance of Firmicutes assigned by metagenomic data set was also lower in MP (11.1%) than AP (35%) and ACP (31.1%). Both metagenomic and 16S rRNA pyrosequencing data sets showed that Methanosarcinales and Methanomicrobiales were main contributors in methanogenesis to produce methane and enrich in MP. Our previous study [38] showed that BOD₅ significantly influenced bacterial and archaeal communities structure in solid waste during solid waste decomposition. Because hydrolysis, the rate-limited step on solid waste decomposition, mainly contributes to the levels of BOD₅, it can be concluded that biodegradable substrate is a key factor in shaping microbial community structure during solid waste decomposition.

Carbon cycling during solid waste decomposition can be seen from the variation in related genes involved in various carbon cycling pathways. As expected, genes (l-lactate dehydrogenase) involved in fermentation enriched in the AP and ACP, whereas genes (mcrB and methyl coenzyme M reductase system, component A2) involved in methanogenesis occurred in ACP and became abundant in MP. This variation accords to the changes of microbial community composition during solid waste decomposition. Anaerobic C fixation (related genes 2-oxoglutarate: ferredoxin oxidoreductase subunit alpha and beta, frdA,
ATP citrate lyase, CO dehydrogenase subunit delta and gamma) was the main pathway in carbon fixation in solid waste than aerobic C fixation (Rubisco small chain and phosphoribulokinase) owing to the anoxic condition in solid waste bioreactor. Substrate quality impact on microbial assimilation of soil organic carbon that higher substrate quality leads to higher ratio of microbial carbon to soil organic carbon [2]. In this study, comparison of the loss of OM and BDM% indicated that recalcitrant carbon was stored in solid waste. OM includes liable and recalcitrant carbon, while BDM% represents biodegradable carbon. There are 56–63% biodegradable carbon (indicated by BDM%) lost, much higher than 13–28% liable and recalcitrant carbon lost (indicated by OM).

Greenhouse gas (CO$_2$, CH$_4$, and N$_2$O) emission from solid waste might be reflected by their related genes variation during solid waste decomposition. Methane emission mainly occurred in MP with abundant genes (merB and methyl coenzyme M reductase system, component A2) involved in methanogenesis. During this period, genes methane monoxygenase involved in aerobic CH$_4$ oxidation occurred by 100% Corynebacteriales. Corynebacteriales was observed in anaerobic digesters [39]. However, their function in anaerobic condition is largely unknown. Carbon dioxide emission by aerobic respiration (coxI, coxIII, coxA, and coxC) occurred across solid waste decomposition and showed an increasing trend. In AP, oxygen in void space of solid waste was consumed rapidly. In ACP and MP, aerobic respiration became strong. This is because oxygen carried by recirculation leachate was consumed, which is consistent with the status of in situ leachate recirculation in landfill that leachate recirculation improved carbon dioxide production [40]. N$_2$O emission by denitrification occurred across solid waste decomposition. The involved genes (nosB, nosC, and norZ) increased across this period, leading to the increasing nitrate lost. Oxygen diffusion into solid waste by leachate recirculation is supposed to inhibit denitrification [40]. However, denitrification activity became strong along solid waste decomposition. This is probably because strong aerobic respiration consumed diffused oxygen rapidly, making aerobic condition transiently existed.

N availability affects the carbon sink activity of terrestrial ecosystems. Microbial N$_2$ fixation increased under elevated CO$_2$ [41, 42]. A previous functional gene-based GeoChip analysis showed that the abundance of nifH genes for N$_2$ fixation increased under elevated CO$_2$ but net nitrification, net N mineralization, and the total soil N content were not significantly changed [41]. In this study, N$_2$ fixation were detected during solid waste decomposition and the relative abundance of genes (nifD, nifH, nifK, and nitrogenase) decreased along solid waste decomposition, from 2.7% in AP, to 1.7% in ACP, and to 1.2% to MP, suggesting dynamics of N$_2$ fixation. As we did not measure the changes of CO$_2$, it is difficult to relate the genes to the changes of CO$_2$. The link between genes of N$_2$ fixation and CO$_2$ emission requires further study. Nitrification was not detected in this study, which agrees with the high ammonia accumulation in the bioreactor. This can partly be explained by the “ammonia accumulation” in landfill or leachate [40, 43]. Anammox was also not detected in this study. Although anammox is assumed to exist in bioreactor landfill [44] and Anammox bacterium Candidatus Kuenenia stuttgartiensis [45] was found in aged solid waste, there is an absence of functional genes evidence.

Sulfur also affects the carbon sink activity of terrestrial ecosystems [1, 46]. A previous large-scale inter-region analysis of time series data showed that dissolve organic carbon (DOC) was controlled by the changes of sulfate [46]. High potential for assimilatory sulfate reduction (related genes: cysC, cysD, and cysN) and sulfur mineralization (related genes: cysteine dioxygenase and 3-mercaptopropyurate sulfrtransferase) by Rhodobacteraceae, Pseudomonadales, Propionibacterales, and Bacteroidales was detected during solid waste decomposition. These two pathways converted sulfate to organic sulfur and to hydrogen sulfide. Dissimilatory sulfate reduction and sulfide oxidation by Clostridiales directly convert sulfate to hydrogen sulfide, although the abundance of their genes (aprA, aprB, and dsrA) was low in this study. Polysulfide is thought to be a possible intermediate that is used by bacteria in sulfur respiration [47]. Bacterium Clostridium were able to reduce polysulfide to hydrogen sulfide and also produced acetate when lactate was supplied [48]. In this study, the gene (psrA) for polysulfide reduction was abundant in ACP, suggesting more hydrogen sulfide released in this phase. Taken together, these sulfur cycle pathways co-contribute hydrogen sulfide release in landfill. The relationship between sulfate and DOC in leachate should be investigated in the future.

Archaea are now recognized to have important roles in global C and N cycles [49]. In this study, the potential for C cycling (methanogenesis, anaerobic carbon fixation, and Calvin cycle) and N cycling (nitrogen assimilation, mineralization, and N$_2$ fixation) involved by archaea was extensively detected. Autotrophic archaea are either anaerobes or can tolerate or use oxygen only at low concentrations [50]. They can oxidize inorganic substrates (e.g., H$_2$, H$_2$S, CO, NH$_3$, etc.) and use inorganic compounds (e.g., SO$_4^{2−}$ and NO$_3^{−}$) as electron acceptors [50]. Those inorganic substrates and compounds were enriched in landfill where there is also anoxic condition, providing habitat for autotrophic archaea. Autotrophic carbon fixation is via different metabolic pathways. Key gene phoshoribulokinase and RubisCo for Calvin cycle was present in Methanomicrobiales in this study as well as others [51]. However, none of the chemolitho-autotrophic archaea seems to use this cycle for CO$_2$ fixation [50]. Genomes of Methanosaeta thermophile contains
genes encoding phosphoribulokinase and RubisCo (genomes available from the DOE joint Genome Institute website) [51], suggesting potential ability of Calvin cycle. Key genes such as 2-oxoglutaratase synthase and ATP-citrate lyase involved in reductive citric acid cycle and CO dehydrogenase for Wood-Ljungdahl pathway [50] were also present in Methanosarcinales in MP and Methanosarcinales and Methanomicrobiales in ACP in this study, suggesting that autotrophic archaea [52] play an important role in anaerobic carbon fixation under anoxic condition. Archaea is also involved in N2 fixation, including Methanosarcinales [53] and Methanomicrobiales [49]. There is a shift between bacteria and archaea involved in N2 fixation across solid waste decomposition. Bacteria (Clostridiales and Bacteroidales) were initially dominant in AP and ACP. Archaea (Methanosarcinales) abundance increased along solid waste decomposition and became dominant in methanogenic phase, reflecting the role of archaea in N2 fixation became important in methanogenesis.

Potential C, N, and S cycling pathways during solid waste decomposition coupled with bacterial and archaea groups. Specifically, Clostridiales were detected as main contributors in several pathways. Clostridiales, capable of decomposing cellulose, have been extensively detected in landfill and leachate [54–56]. In this study, Clostridiales has been assigned as potential catalyzing anaerobic carbon fixation, fermentation, aerobic CO oxidation, nitrogen assimilation, mineralization, and ammonification, N2 fixation, dissimilatory sulfate reduction, and assimilatory sulfate reduction, suggesting that Clostridiales play and important role in C, N, and S cycling in landfill.

For long-term perspective, C, N, and S cycle in landfill requires consideration of complex interactions and feedbacks that occur between microbes, plants (natural or anthropogenic plantation application after landfill closed), and their physical environment in the context of climate change. Given the expanding of landfills terrestrial ecosystem, it is urgent to understand the mechanisms by which microorganisms regulate the carbon exchange between landfill-atmosphere. However, a full understanding of C, N, and S cycle in landfill depends on the progress of microbial ecology [6]. For example, microbial function redundancy has been reported in natural environment and engineering facilities [57]. In this study, the existence of aerobic respiration and aerobic methane oxidation in methanogenesis might also because of microbial function redundancy. This means that those aerobic respiration and aerobic methane oxidation functional genes might not and/or do low expression irrespective of their high relative abundance of community. Therefore, metatranscriptomics is needed for further study on the microbial function. In addition, obligate methanogens were found to exist in aerobic phase. This is consistent with our previous studies that Methanomicrobiales and Methanosarcinales was dominant in AP [31]. How those obligate methanogens survive and what are their function under aerobic condition needs further study.

We noted that this study used bioreactor with landfill leachate recirculation to simulate MSW decomposition. Leachate recirculation is also an engineering application for landfill leachate reduction in some landfills [40]. Recirculation process inevitably brings some oxygen into landfill along with leachate, which was also observed in this study. For example, aerobic respiration and aerobic methane oxidation were abundant in methanogenesis. Therefore, well-controlled laboratory experiments are necessary for the specific microbial community structure and function study.

In conclusion, the issues addressed in this study are important to the understanding of carbon dynamics in landfill. We found that bacterial and archaeal community structure and functional genes involved in C and N fixation and greenhouse gas emission changed during solid waste decomposition. Such shifts in bacterial and archaeal community structure and function could potentially modify the direction and magnitude of landfill regulation of the rate of carbon storage and greenhouse gas emission, thereby influencing the climate change. Our findings also have practical consequence that these observed special bacterial and archaeal groups mediated C, N, and S cycles during solid waste decomposition provided direct microbiological evidence for solid waste decomposition in landfill, thereby showing clues for carbon and nitrogen management and pollution control in landfill.

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Author Contribution LS and SY conceived and designed research. YW, LS, and RZ conducted research. LS, YW, RZ, and SY contributed discussions and suggestions. YW and LS analyzed data. LS and SY wrote the manuscript. All authors read and approved the manuscript.

Declarations Ethics Approval This work does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest The authors declare no conflict of interest.

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