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Mitigating CH_4 Emissions in Semi-Aerobic Landfills: Impacts of Operating Conditions on Abundance and Community Structure of Methanotrophs in Cover Soils

Huai Li[1,2], Zi-Fang Chi[1,3]*, Wen-Jing Lu[4], and Hong-Tao Wang[4]

[1]*Key Laboratory of Groundwater Resources and Environment, Ministry of Education, Jilin University, Changchun 130021, P.R. China*
[2]*School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, P.R. China*
[3]*State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (MARC), Tsinghua University, Beijing 100084, P.R. China*
[4]*School of Environment, Tsinghua University, Beijing 100084, P.R. China*

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**Corresponding author*
Phone: +86-431-88502608;
Fax: +86-431-88502608;
E-mail: chizifang@jlu.edu.cn

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Methanotrophs are the most important sink of CH_4, which is a more highly potent greenhouse gas than CO_2. Methanotrophic abundance and community diversity in cover soils from two typical semi-aerobic landfills (SALs) in China were detected using real-time polymerase chain reaction (real-time-PCR) and denaturing gradient gel electrophoresis (DGGE) based on 16S rRNA genes, respectively. Real time-PCR showed that Type I methanotrophs ranged from 1.07×10^6 to 2.34×10^7 copies/g soil and that of Type II methanotrophs from 1.51×10^7 to 1.83×10^8 copies/g soil. The ratio of Type II to Type I methanotrophic copy numbers ranged from 5.61 to 21.89, indicating that Type II methanotrophs dominated in SAL. DGGE revealed that Type I methanotrophs responded more sensitively to the environment, changing as the community structure varied with different soil types and locations. *Methylobacter*, *Methylosarcina*, and *Methylomicrobium* for Type I, and *Methylocystis* for Type II were most prevalent in the SAL cover layer. Abundant interflow O_2 with high CH_4 concentration in SALs is the reason for the higher population density of methanotrophs and the higher enrichment of Type II methanotrophs compared with anaerobic landfills and other ecosystems, which proved a conclusion that increasing the oxygen supply in a landfill cover layer would greatly improve CH_4 mitigation.

Keywords: Cover soil, denaturing gradient gel electrophoresis (DGGE), methanotrophs, real-time polymerase chain reaction (RT-PCR), semi-aerobic landfill (SAL)

Introduction

Methane (CH_4) is the second largest contributor after carbon dioxide (CO_2) to global warming, with a global warming potential 25 times higher than that of CO_2 [36]. Meanwhile, landfills are considered as one of the major sources of methane emission, accounting for 1.5%–15% of the global methane sources [40]. Consequently, aerobic methane oxidation by methanotrophic bacteria, which are Gram-negative, that use methane as the sole carbon and energy source, becomes one of the most important sink of CH_4. In addition, anaerobic methane oxidation by methanotrophic Archaea driven by sulfate or nitrite has been discovered [37], but the efficiency of methanotrophic Archaea would be at least 1 order of magnitude lower than the rates of aerobic methane oxidation [16]. Therefore, methanotrophic Archaea was proven to be less important in CH_4 uptake in landfill covers where a large quantity of aerobic methanotrophic bacteria exist. Thus, the methanotrophs studied in the cover layer in this paper are aerobic methantrophic bacteria.

A number of researchers estimated that 10% to 100% of the CH_4 generated in landfills is oxidized by methanotrophs [9, 13]. Moreover, several studies reported that landfills act as sinks of CH_4 rather than as sources [7, 8, 33]. Therefore,

stimulating the activities of such bacteria in landfill cover soils could possibly reduce the emission of CH_4 from landfills, especially in landfills where active gas collection is not required.

Further studies found that there are many factors affecting the activity of methanotrophs, including pH [21], concentration of CH_4 and O_2 [8, 38], moisture [32], temperature [10, 39], NH_4-N [27, 32], and Cu^{2+} [15]. Among these factors, the availability of CH_4 and O_2 are the most important ones that determine the growth and the type of methanotrophs because they are the substrates for methanotrophs. Traditionally, methanotrophs are classified into two general groups (Type I and II) based on several characteristics, such as cell morphology, membrane arrangement, carbon assimilation pathway, and predominant phospholipid fatty acids. Type I methanotrophs, which belong to Proteobacteria, are composed of *Methlyomonas, Methylococcus, Methylomicrobium, Methylosarcina*, *Methylosphaera, Methylothermus, Methylosoma, Methylohalobius, Methylocaldum,* and *Methylobacter* [21, 31]. Type I methanotrophs can be further divided into two different groups, Type Ia and Ib; the latter is composed of *Methylococcus, Methylocaldum,* and *Methylothermus*, whereas the others are Type Ia methanotrophs [6]. Genera that are members of Type II methanotrophs belong to α-Proteobacteria, including *Methylosinus, Methylocella, Methylocapsa,* and *Methylocystis* [21].

The semi-aerobic landfill (SAL) is a technology of Japanese origin. With properly engineered designs, ambient air naturally flows into the waste body through leachate collection pipes and consequently enhances the waste stabilization processes and leachate quality. Previous estimates suggest that more than 50% of the area in an SAL is aerated as a result of continuous ambient air flow. SAL would therefore be an ideal environment for the growth of methanotrophs because of the interflow of CH_4 and O_2 in the same region (especially around the venting pipes). Researchers hypothesize that the community structure of methanotrophs in the cover layer of SALs is different from that in sanitary landfills and other habitats because of the special design and therefore CH_4 emissions would be mitigated comparing to traditional landfills, but few studies investigated methanotrophic communities in SALs especially in China.

In the present study, the abundance and community structure of methanotrophs from cover soils of two SALs in China were investigated using real-time polymerase chain reaction (real-time-PCR) and denaturing gradient gel electrophoresis (DGGE), respectively. The purpose of this study was to understand the changes in methanotroph communities and abundance in SAL cover soils that may help in designing better management practices for methane emission mitigation in SALs.

Materials and Methods

Landfills and Landfill Gas Measurement

Soil samples were collected from two SAL cover layers. One is Weifang SAL (23°23′N, 103°23′E) located in Shandong Province, Bohai Sea Region, central Shandong Peninsula. Another is Loudi SAL (27°42′N, 111°59′E) located in central Hunan Province, at the central section of the Yangtze River.

Weifang landfill was put into service in September 2002, having a refuse treatment capacity of 550 t/day and a total volume of 8×10^6 m^3. Loudi landfill was operated in November 2007, having a refuse treatment capacity of 300 t/day and a total volume of 7.7×10^6 m^3.

Before soil sampling, the component and concentration of landfill gases, including CH_4, CO_2, and O_2, around the perforated vertical venting pipe of the two landfills were measured using an infrared gas analyzer (X-am7000; Drager, German).

Sampling

Samples were collected from the cover layers (10–30 cm in depth) of the two landfills. The cover layer soil of Weifang landfill is brown clay, whereas that of Loudi landfill is red clay. Sampling was conducted around the perforated vertical venting pipes, which are connected to the leachate drainage pipe; ambient air flows through these venting pipes into the waste body. At the same time, the perforated venting pipes act as outlets of landfill gas produced from the waste body; thus, it is an ideal area for invigorating methanotrophs because of the abundant interflow of CH_4 and O_2. The locations where sampling was done are listed in Table 1, and the basic characteristics of soil samples, including pH, moisture content, organic matter, NH_4-N, and Cu^{2+}, were measured in accordance with a standard method (GB7830-7892-87). Each sample was a mix of three parallel samples (n = 3) with the same distance around the perforated vertical venting pipe.

Table 1. Locations of samplings in Weifang and Loudi landfills.

	Weifang landfill			Loudi landfill		
Samples No.	W1	W2	W3	L1	L2	L3
Distance from perforated pipe (m)	0	3	15	0	3	15

Molecular Biology Study of Methanotrophs in Cover Layers of SALs

DNA extraction. DNA extraction of the samples was performed using the FastDNA SPIN Kit for Soil (MP Biotechnology, USA) in accordance with the manufacturer protocol. The product from the DNA extraction was verified by electrophoresis in 1% agarose. The resultant DNA was stored at −20°C for further analysis of methanotrophs.

Real-Time-PCR for Quantitative Analysis of Methanotrophs. Two forward primers MB10γ and MB9α (Table 2), as well as their common reverse primer 533r (Table 2), were used to determine the 16S rRNA gene copy numbers of Type I and Type II methanotrophs, respectively [23]. The DNA extracts were used as template.

Protocol for real-time-PCR. Real-time-PCR was performed on an iCycler iQ5 thermocycler (Bio-Rad, USA). Amplification was performed using SYBR Premix Ex Taq (Takara, Japan). Each PCR with a final volume of 25 µl contained 12.5 µl of SYBR Premix Ex Taq (2×), 10 µl of F Primer (10 pmol/µl), and 10 µl of R Primer (10 pmol/µl), as well as approximately 20 ng of purified template DNA.

The real-time-PCR protocol for both target groups was as follows: 95°C for 4 min followed by 41 cycles of 1 min at 94°C and 2 min at 58°C. In each real-time-PCR amplification, a melting-curve analysis was performed following the aforementioned real-time-PCR protocol to confirm the PCR product specificity by measuring fluorescence continuously as the temperature was increased from 55°C to 95°C, with 0.5°C increments every cycle from 55°C to 95°C to generate the dissociation curve.

Data analysis was performed using iCycler software (version 1.0.1384.0 CR). The parameter Ct (threshold cycle) was determined as the cycle number at which a statistically significant increase in the reported fluorescence was detected.

Standard curve for real-time-PCR. The primer pairs MB10γ/533r and MB9α/533r were applied to amplify Type I and Type II methanotroph-specific 16S rRNA gene fragments from the environmental DNA. The amplification products were purified with a QIAquick PCR purification kit (Qiagen, Germany). The products were cloned using the pGEM-T easy vector system (Promega, USA). Subsequently, the products were transformed into competent *Escherichia coli* (JM109) (Takara, China), screened for positive clone, and prepared as plasmid DNA. The identity of the cloned insert was confirmed by direct sequencing of the plasmid, which was performed using the universal sequencing primer T7/SP6 (SinoGenoMax Co., China). Plasmid DNA was extracted from transformed recombinant cells using a Wizard Plus SV minipreps DNA purification kit (Promega, USA). The concentrations of plasmid DNA were determined using a Thermo Scientific Nanodrop 2000 spectrophotometer (NanoDrop Technologies). The copy numbers of the 16S rRNA gene were calculated directly from the concentrations of the extracted plasmid DNA.

The standard curve was constructed using standard values obtained by a 10-fold serial dilution of the extract plasmid harboring the target insert. The standard curve was generated by plotting the DNA amount (plasmid copies/ml) against the Ct value exported from the iCycler iQ5 thermocycler (BioRad, USA). A semi-logarithmic plot was generated ($\log_{10}$ of the concentration of DNA versus Ct). The standard curve was calculated using the following equation:

$$y = -ax + b.$$

The efficiency of the reaction (E) was calculated by the following equation:

$$E = (10^{-1/a}) - 1.$$

Finally, the amount of DNA for unknown samples was extrapolated from the Ct value and the value obtained from the standard curve. Statistical analysis of real-time-PCR results was performed with the program SPSS 13.0 for Windows (SPSS Inc., USA).

DGGE analysis for community structure of methanotrophs. The primers MethT1bR and MethT1dF (Table 2) were used to amplify the 16S rRNA sequences of Type I methanotrophs present in environmental DNA; the 16S rRNA sequences of Type II methanotrophs were amplified using primer MethT2R and the bacteria-specific primer 27F (Table 2). A thermocycler (TC-3000;

Table 2. Methanotroph-specific primers [23, 42].

Primer	Sequence[a]	Target genera
MethT1dF	5′-CCTTCGGGMGCYGACGAGT-3′	Type I methanotrophs
MethT1bR	5′-GATTCYMTGSATGTCAAGG-3′	
27F	5′-AGAGTTTGATCMTGGCTCAG-3′	Type II methanotrophs
MethT2R	5′-CATCTCTGRCSAYCATACCGG-3′	
358F	5′-CCTACGGGAGGCAGCAG-3′	All bacteria
517R	5′-ATTACCGCGGCTGCTGG-3′	
MB10γ	5′-AAGCGGGGGATCTTCGGACC-3′	Type I methanotroph
MB9α	5′-GTTCGGAATAACTCAGGG-3′	Type II methanotroph
533r	5-TTACCGCGGCTGCTGGCAC-3′	All bacteria

[a]Y represents C or T; R represents A or G; M represents A or C; S represents C or G.

High System; Barloworld Scientific Ltd., UK) was used to perform the PCR for Type I and Type II methanotrophs.

A nested-PCR approach was adopted to profile the Type I and Type II methanotrophic communities by DGGE. The PCR amplified segaments following the above-mentioned steps were used as templates, with GC358F (with a 40-bp GC clamp added to primer 358F's 5′ end) and 517R used as primers (Table 2).

The nested-PCR products were analyzed on 1% agarose gels to confirm the presence of a single amplification of the expected size. DGGE analysis was conducted using a DCode system (Bio-Rad Laboratories, USA). Samples of PCR product (30 μl) were loaded onto 6.5% (w/w) polyacrylamide gels in 1× TAE buffer. The polyacrylamide was a denaturant gradient from 20% to 70% (for Type I sequences) and 30% to 60% (for Type II sequences). The gel was photographed with a gel photo system (GelDoc 2000; BioRad, USA). The photographs were analyzed with the BioRad Quantity One software package (Bio-Rad Laboratories).

Specific PCR-DGGE bands were manually excised from the gel, suspended in 50 μl of sterile water, and incubated overnight at 4°C. The PCR-DGGE protocol was then repeated using the excised bands as a template, until only a single band was detected. The last PCR cycle was performed without the GC-clamp attached to the forward primers 358F and 517R. The resulting PCR products were sent to SinoGenoMax Co., Ltd. (Beijing, China) for purification and nucleotide sequencing.

Phylogenetic analysis and clustering. The Basic Local Alignment Search Tool (BLAST) program was used to search the National Center for Biotechnology Information (NCBI) sequence database (http://www.ncbi.nlm.nih.gov/BLAST/) for sequence similarity.

All nucleotide sequences were aligned using the CLUSTAL X program [4]. Phylogenetic trees were constructed using the neighbor-joining method with the MEGA 5.0 software. Reference sequences were obtained from the GenBank database and were included in the phylogenetic trees for comparison. All nucleotide sequences were optimally aligned prior to tree construction.

Band patterns from PCR-DGGE fingerprints were analyzed by the unweighted pair group method with arithmetic mean using DNAMAN v. 4.1 (Lynnon Biosoft, USA). The scanned gel images were analyzed using Labwork 4.6 software (Media Cybernetics, USA). Binary sequences were generated for individual fingerprints by determining the number and position of bands compared with the total number of band positions using the Labwork software.

The relative abundance of each band was considered as a variable. Similarity matrices of methanotroph as revealed by DGGE patterns were calculated with the squared Euclidean-distance coefficient using the SPSS 13.0 software (SPSS Inc., USA).

Accession numbers for nucleotide sequences. The partial sequences of the 16S rRNA gene of methanotrophs obtained in this study are available from the NCBI database under the accession numbers HM755772–HM755776, HM755778–HM755781, HM755782–HM755786, HM755789–HM755793, and HM755796–HM755804.

Results

Characteristic of Soil Samples and Landfill Gas Components from Cover Layers

Table 3 shows the basic characteristics of the examined cover soils from Weifang and Loudi landfills. In both the SALs, there was no difference in pH (8.09–9.18) and the organic contents, which varied from 57.63 to 132 g/kg in the Weifang landfill and from 53.36 to 90.52 g/kg in the Loudi landfill. The concentration of NH_4-N in the Weifang landfill (95.38–125 mg/kg) was slightly higher than that in the Loudi landfill (40.27–91.91 mg/kg). There were remarkable differences in moisture and Cu^{2+} between the two landfills; the moisture was higher in the Loudi landfill than in the Weifang landfill because of the climate of North and South China, and Cu^{2+} was higher in Weifang landfill than in the Loudi landfill.

Table 4 shows the results of landfill gas components measured around the perforated vertical pipes of the two landfills. In both SALs, areas near the perforated pipes had more O_2 because of the special design of the SALs; air was sucked in directly or indirectly through the bottom drainage pipe and transported to the refuse through vertical venting pipes, which were connected directly to the bottom drainage pipes. Consequently, the increased O_2 caused the low CH_4 concentration around the perforated pipes.

Table 3. Basic characteristics of examined cover soils from Weifang and Loudi landfills.

	Weifang landfill			Loudi landfill		
Samples No.	W1	W2	W3	L1	L2	L3
pH[a]	8.12 ± 0.13	8.15 ± 0.24	8.74 ± 0.20	9.18 ± 0.09	8.09 ± 0.04	8.87 ± 0.11
Moisture content (%)[a]	7.02 ± 0.03	10.24 ± 0.13	12.87 ± 0.12	34.45 ± 0.20	20.25 0.11	24.95 ± 0.10
Organic content (g kg^{-1})[a]	132 ± 2.72	132 ± 3.56	57.63 ± 0.05	90.52 ± 1.30	53.36 ± 1.11	76.72 ± 2.05
NH_4-N (mg kg-1)[a]	112 ± 2.04	125 ± 0.90	95.38 ± 1.00	40.27 ± 2.46	86.85 ± 1.20	91.91 ± 0.10
Cu^{2+} (mg kg^{-1})[a]	80.95 ± 0.90	86.85 ± 1.07	41.02 ± 1.28	24.88 ± 0.35	30.48 ± .048	25.11 ± 0.25

[a]Values are given as the mean ± standard deviation (n = 3).

Table 4. Location distributions of CH_4, CO_2, and O_2 concentrations.

	Weifang landfill			Loudi landfill		
Samples No.	W1	W2	W3	L1	L2	L3
CH_4 (%)[a]	1.4 ± 0.1	20.5 ± 0.1	51.0 ± 0.3	11.2 ± 0.2	24.0 ± 0.7	39.0 ± 1.0
O_2 (%)[a]	20.5 ± 0.3	12.2 ± 0.2	1.0 ± 0.0	15.6 ± 0.4	10.9 ± 0.3	5.5 ± 0.0
CO_2 (%)[a]	8.6 ± 0.1	14.0 ± 0.1	32.0 ± 0.5	9.8 ± 0.1	14.5 ± 0.2	24.0 ± 1.2

[a]Values are given as the mean ± SD (n = 3).

Methanotrophic Abundance Based on Real-Time-PCR

Ribosomal RNA gene fragments of Type I and Type II methanotrophic bacterial populations were detected in the two landfills. One of the landfill samples (W3, which is one of the brightest bands) detected by DGGE was used to construct the standard curve for Type I and Type II methanotrophs.

The obtained R^2 values were greater than 0.99 for both standard curves. Slopes of −3.37 and −3.22 were generated for Type I and II methanotrophs, respectively, which correlated with the efficiency of the PCR (E = 98.0% and 104.4% for Type I and Type II populations, respectively).

Based on the copy numbers present in the extracted DNA samples, the 16S rRNA gene copy numbers per gram of soil were calculated using the above general linear model analysis. In general, the quantities of Type I methanotrophs in tested samples of the two landfill cover layers ranged from 10^6 to 10^7 copies/g dry soil, whereas that of Type II methanotrophs ranged from 10^7 to 10^8 copies/g of soil (Fig. 1). The average methanotroph concentrations in the Weifang landfill were 5.97×10^6 and 5.89×10^7 gene copies/g dry soil for Type I and Type II methanotrophs, respectively. In the Loudi landfill, the average methanotroph concentrations were 8.64×10^6 and 7.78×10^7 gene copies/g dry soil for Type I and Type II methanotrophs, respectively. The ratio of Type II to Type I methanotrophic copy numbers of these samples ranged from 5.61 to 21.89, indicating that Type II methanotrophs accounted for a significantly higher population of the total methanotrophs in both landfills cover layers.

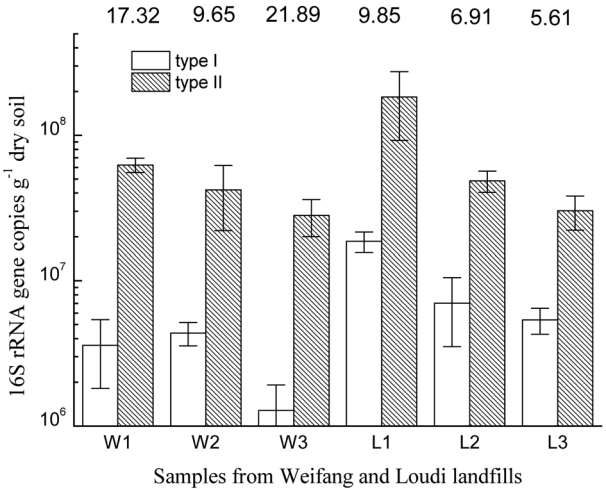

Fig. 1. Quantification of gene copy numbers of methanotrophs in Weifang and Loudi landfill cover soils.
Ratios of Type II to Type I methanotrophic copy numbers are shown above the chart.

Methanotrophic Community Structure Based on DGGE

The DGGE profile of the methanotrophic community showed some variations among samples of landfills (Figs. 2A and 2B).

The structure of the Type I methanotrophic community typically varied with different landfills and locations. In this study, there were two dominant bands, I-2 and I-9, present in all samples from the Weifang landfill, but there was no similar band in all the samples from the Loudi landfill.

In the Weifang landfill, the community diversity of Type I methanotrophs changed with location, as indicated by the disappearance of band I-6; furthermore, the I-4 and

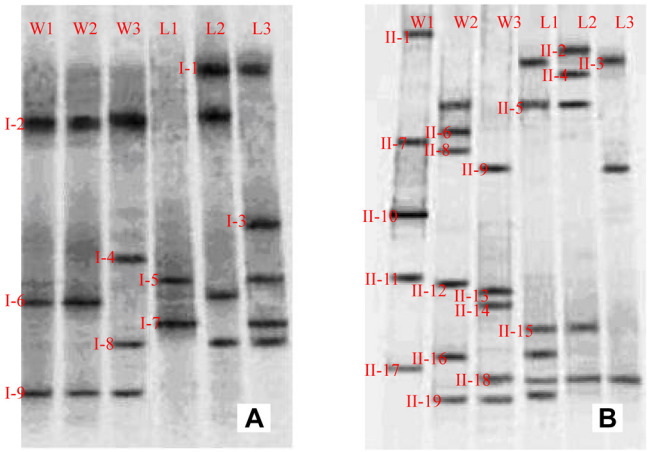

Fig. 2. Methanotrophic bacterial communities of Weifang and Loudi landfills.
(**A**) Type I; (**B**) Type II.

I-8 bands became the dominant bands in sample W3, which was 15 m away from the perforated pipes and had a low oxygen concentration (Table 4). Bands I-2 and I-6 were dominant in samples W1 and W2, which were near the perforated pipes. The community diversity of the three samples (W1, W2, and W3) increased with distance from the perforated pipes. In the Loudi landfill, the dominant bands of Type I methanotrophs were different from those of Type I methanotrophs in the Weifang landfill, and the community diversity of samples around the perforated pipes changed significantly with distance (Fig. 2A). The results indicate that the structure of Type I methanotrophs was obviously influenced by O_2 concentration, in accordance with the wide recognition that Type I methanotroph is an O_2-favoring genera.

The structure of the Type II methanotrophic community changed obviously with different locations. II-18 was the dominant band in all three samples from the Loudi landfill and was also the dominant band in sample W3 from the Weifang landfill, indicating that the genus of II-18 is a ubiquitous Type II methanotroph in the cover soils of the landfills. The results also show that there were more dominant bands near the perforated pipes where O_2 concentration and moisture content were higher but CH_4 concentration was lower (Table 4). This result indicates that O_2 is a limiting factor for Type II methanotrophs at high CH_4 concentration (>20%), although Type II is generally considered as a CH_4-favoring genera.

Cluster analysis of the DGGE was processed to examine the bacterial community structure. The results show that different locations influenced the community of Type I methanotrophs (Fig. 3A). Two major clusters corresponding to the two landfills were observed. The first cluster grouped the samples from the Loudi landfill (L1, L2, and L3). The second major cluster grouped the samples W2 and W3. W1 was separated from the two major clusters, which may have been caused by the low CH_4 concentration in this area (Table 4), indicating that Type I methanotrophs were influenced more by CH_4 under landfill conditions.

Similar to Type I methanotrophic DGGE, cluster analysis of Type II methanotrophic DGGE showed some location differences (Fig. 3B). Two major clusters corresponding to the two landfills were also observed. The first cluster grouped the samples from the Loudi landfill (L1, L2, and L3). The second major cluster grouped the samples W1 and W2. W3 was separated from the two major clusters, which may have been caused by the low O_2 concentration in this area (Table 4), indicating that Type II methanotrophs were influenced more by O_2 under landfill conditions.

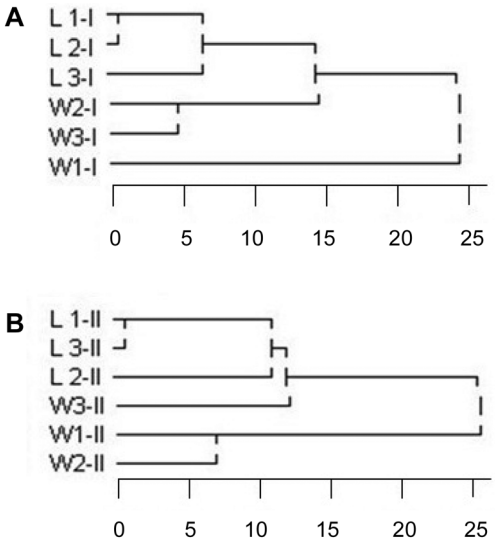

Fig. 3. Hierarchical cluster analysis results of DGGE profiles of methanotrophs.
(**A**) Type I; (**B**) Type II.

Phylogenetic Analysis of Type I and II Methanotrophic Populations

In total, 28 bands were excised and sequenced from denaturing gels containing the 16S rRNA gene fragments of Type I and Type II methanotrophs. The results reveal that there were different Type I and Type II methanotrophic populations in the tested samples (Figs. 4A and 4B). The detected Type I populations were related to several methanotrophic genera, including *Methylobacter*, *Methylomicrobium*, *Methylosarcina*, an unknown Type I methanotroph, and an uncultured bacterium gene. *Methylobacter*, *Methylomicrobium*, and *Methylosarcina* were the most prevalent genera in the two landfills. *Marinobactor* (I-9, HM755781; Fig. 4A) was detected in the Weifang landfill only.

Phylogenetic analysis showed that the most commonly detected Type II methanotrophs were *Methylocystis*. Other Type II methanotrophs detected were *Methylosinus* and *Methylobacterium* (Fig. 4B).

Discussion

Understanding the ecology of methanotrophs in landfills of different locations is important for controlling methane emission from landfills. The present study showed that operating conditions affect the quantity and structure of Type I and Type II methanotrophic populations.

Methanotrophs are influenced by geographical locations with different climates. Weifang has a warm, temperate

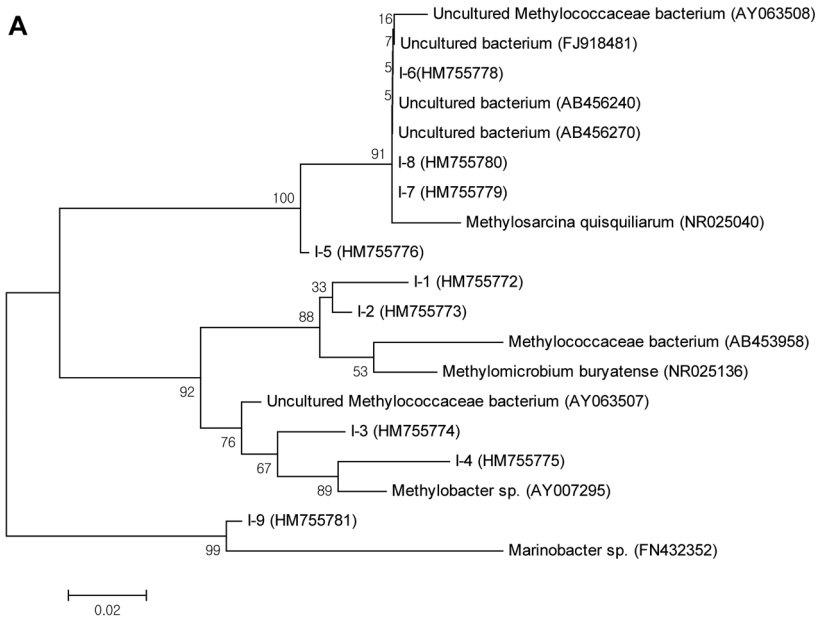

Fig. 4. Neighbor-joining tree depicting phylogenetic relationships of methanotrophic bacteria, detected by PCR-DGGE, of Weifang and Loudi landfills.
(**A**) Type I; (**B**) Type II.

zone semi-moist continental monsoon climate, with a mean annual precipitation of 600–800 mm and a mean annual temperature of 12.3°C, whereas the Loudi region has a central–north subtropical moist monsoon climate, with a mean annual precipitation of 1,200–1,700 mm and a mean annual temperature of 16.8°C. There is evidence that Type I methanotrophs are superior to Type II strains at low temperatures (<10°C). The growth of Type I methanotrophs at low temperatures and of Type II at elevated temperatures was reported for different landfill cover soils [10, 28, 32].

Although Type II methanotrophs are more ubiquitous and are acclimated to the environment, the results of this study show that there were some differences in terms of abundance and community structure of Type II methanotrophs in both landfills, which vary in location and climate. This effect is in agreement with previous findings that Type II methanotrophs outcompete Type I methanotrophs under similar landfill conditions [20]. However, for other different land utilization patterns, including degradation, farming, and restoration, Type I would outcompete Type II methanotrophs, mainly because of low CH_4 concentrations.

Landfill operation and management affect the condition and physiochemical characters of waste and cover soil; consequently, they affect the ecology of the total microbial community and methanotrophs. Type I and Type II methanotrophs were more abundant in areas near the venting pipes because of the special conditions near the pipes. The community structures of Type I methanotrophs obviously changed as the distance became farther from the perforated pipes compared with those of Type II methanotrophs. This trend was observed because Type I methanotrophs grow faster than Type II, and thus Type I methanotrophs are more sensitive to the environment, such as landfill gas concentrations [2].

The methanotrophs were more abundant in the Loudi landfill than in the Weifang landfill, where the temperature was higher and moisture was more suitable (18%–25%) [10, 11, 41].

NH_4-N suppressed CH_4 oxidation, but Type I methanotrophs were a little more resistant to NH_4-N [11, 12, 25, 32]. In this study, we confirmed the effect. The abundance of methanotrophs in the Loudi landfill, which has lower concentrations of NH_4-N, was much more than in the Weifang landfill, and the ratios of Type II/Type I were lower in the Loudi landfill than in the Weifang Landfill.

Type I methanotrophs were resistant to a high concentration of Cu^{2+}, which adjusts the ratio of *sMMO* to *pMMO*; *sMMO* is mostly present in Type II methanotrophs [3, 5, 17, 18, 29, 30, 35]. This study confirmed this effect. Type I methanotrophs were more abundant in the Loudi landfill, where the

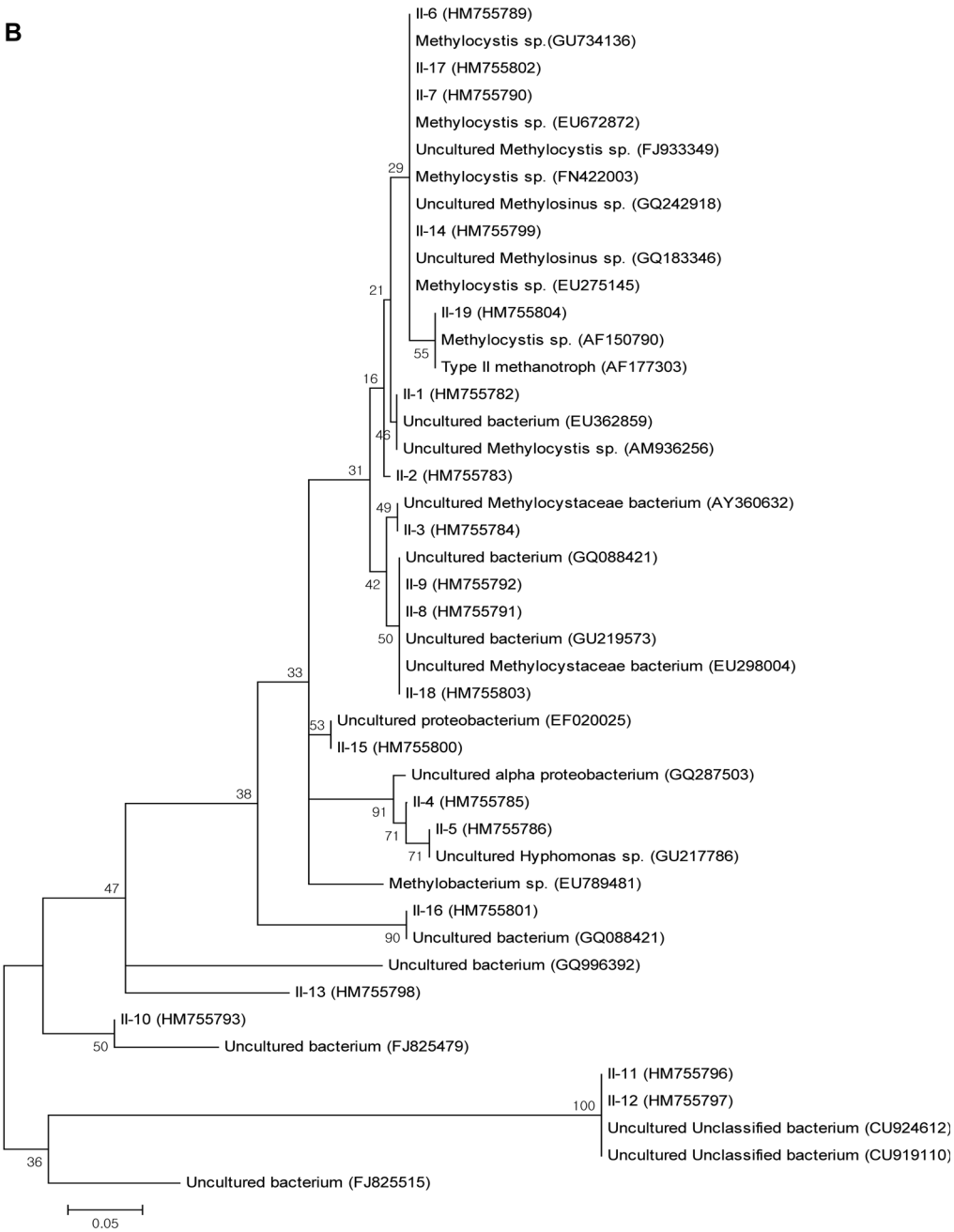

Fig. 4. Continued.

concentration of Cu^{2+} was lower than in the Weifang landfill (Table 3 and Fig. 1).

Although the characteristics of soil would affect the structure and abundance of methanotrophs, from the trend in Fig. 1, the abundance of methanotrophs were influenced much more by oxygen and methane concentrations than the characteristics of the soil.

In the Weifang landfill, which has a longer operation time (operated in September 2002), the methanotrophs were more enriched than those in the Loudi landfill, which was put into service in November 2007. This observation is also evidenced by a simpler structure of Type I methanotrophs in the Weifang landfill than that in the Loudi landfill. These phenomena are in accordance with the findings of other studies [24].

As the operation time extended, the landfill conditions became stable, the O_2 concentration decreased, and the CH_4 concentration increased. At high ratios of CH_4 to O_2, Type I

methanotrophs responded rapidly and with pronounced shifts in population structure. On the other hand, Type II methanotrophs were apparently more abundant, always present, and exhibited a largely stable population structure; moreover, they became active as the operation time progressed and contributed to CH_4 oxidation activity under high CH_4 mixing ratios.

In this study, high methanotrophic population densities were detected, indicating enhanced growth of methanotrophs in SALs. The copy numbers of methanotrophs (10^6–10^8) detected were obviously higher than those of rice soil (between 10^6 and 10^7 [34]), forest soil (between 10^6 and 10^7 [26]), and sanitary landfills soils (between 10^6 and 10^7 [19]), but close to that of landfill biocovers (about 10^8 [1]). The results suggest that SALs have a relatively higher concentration of CH_4 and O_2 than other ecosystems, such as paddy or forest soils, so as to enhance the capacity of methane oxidation in landfill cover soils by increasing the population size of methanotrophic bacteria. The results indicate that SALs have higher ratios of O_2 to CH_4 than sanitary landfills, resulting in larger populations of methanotrophs and a higher methane oxidation efficiency in SALs.

A change in O_2 to CH_4 ratio also changes the structure of Type I and Type II methanotrophs. Previous studies that used cultivation-dependent and DGGE techniques to investigate the methanotrophic bacterial community structure detected Type I populations (mainly *Methylobacter* sp. and *Methylomicrobium* sp.) and Type II populations (mainly *Methylocystis* sp. and *Methylosinus* sp.) in typical sanitary landfill cover soils [42]. However, the present study showed that *Methylobacter* sp., *Methylosarcina* sp., and *Methylomicrobium* sp. dominated the Type I methanotrophs, and *Methylocystis* sp. dominated the Type II methanotrophs in cover soils of typical SALs in China. The phenomena indicated that a higher ratio of O_2 to CH_4 would enhance more genera of Type I methanotrophs, but Type II methanotrophs would be domesticated to homogenous genera.

In this study, substantial numbers of methanotrophs (>10^6 16S rRNA gene copies/g dry soil) were detected in all samples by real-time-PCR. The ratio of Type II to Type I methanotrophic copy numbers ranged from 5.61 to 21.89. This number was higher in the Weifang landfill than in the Loudi landfill, indicating that lower concentrations of Cu^{2+} (Table 3) and longer landfilling time washed out Type I methanotrophs. The ratio of Type II to Type I methanotrophic copy numbers in other habitats, such as rice paddy, was under 0.5 [43], and was under 3 in sanitary landfill cover soils [22]. These results indicate that higher concentrations of CH_4 and high ratios of O_2 to CH_4 under special landfill conditions, such as those of SALs, favor the acclimation of Type II methanotrophs instead of Type I methanotrophs.

Although the data show a significant domination of Type II methanotrophs over Type I methanotrophs in both landfills, there were no statistically significant differences in terms of Type II population between the two landfills, despite their differences in location, climate, and soil types.

The main conclusions can be drawn as follows: i) the abundance of Type I methanotrophs ranged from 1.07×10^6 to 2.34×10^7 copies/g soil, whereas that of Type II methanotrophs ranged from 1.51×10^7 to 1.83×10^8 copies/g soil in cover soils from two SALs in China; ii) Type II methanotrophs dominated in all samples compared with Type II methanotrophs, despite the differences between the two landfills in terms of climate, locations, and soil types, as evidenced by the high ratio of Type II to Type I methanotrophic copy numbers (from 5.61 to 21.89); iii) the results prove the hypothesis that SALs provide a special environment for methanotrophs, wherein there is a high concentration of CH_4 and abundant interflow of O_2, especially around the vertical venting pipes. Thus, we can arrive at a conclusion that increasing the oxygen supply would greatly improve CH_4 oxidation in landfill cover soils of China (such as the biocover equipped with a novel passive air diffusion system [14]), even in SALs that have a higher O_2 concentration than sanitary landfills.

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