



## Comparison of plant and bacterial communities between a subtropical landfill topsoil 15 years after restoration and a natural area



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### ABSTRACT

Engineered sanitary landfills are becoming more and more common worldwide. Ecosystem restoration of capped sanitary landfills is essential to restore the disturbed environment. Comparing plant communities, as well as bacterial communities, in landfills and natural areas, offers an efficient way to assess the restoration status. However, such studies on the restored engineered landfills are limited. Here we present an ecological restoration case in an engineered landfill in a subtropical region. Part of the South East New Territories (SENT) landfill in Hong Kong was capped and restored, by using 16 plant species growing on top of the final cover soil, during 1997–1999. In 2014, plant survey and soil properties analyses were conducted in a restored site (AT) and a natural site (CT, an undisturbed area, serving as a control). The similarity between the biota communities (i.e., plant and soil bacteria) of the two sites was assessed. Plant and soil bacterial communities at AT were significantly different ( $R = 1$ ,  $P < 0.01$ , ANOSIM) from those at CT. A lower plant diversity but a higher soil bacterial diversity were observed at AT. The soil bacterial community structure was potentially driven by soil pH, moisture content, cation exchange capacity (CEC), N, and P. The engineered landfill had not been restored to an ecosystem similar to the natural environment 15 years after restoration. Establishing similar soil properties in the landfill topsoil would be important to achieve a bacterial community similar to the natural area. This study can also offer a quick and inexpensive method for landfill engineers to assess the bacterial restoration of man-made ecosystems using plant and soil properties rather than DNA analyzing techniques.

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## 1. Introduction

Landfilling is one of the major measures adopted for managing solid waste worldwide. Non-sanitary landfills, common in the past, were constructed using several soil layers beneath and on top of the waste to prevent contamination of the environment (USEPA, 1993; Qian et al., 2002). However, leachate and landfill gas can

migrate and discharge to the environment, leading to groundwater contamination and greenhouse gas emission. On the contrary, the sanitary landfill, can minimize leachate and gas emission by applying a liner system (USEPA, 1993).

Restoration of closed landfills is essential to compensate for ecosystem disturbances, minimize adverse effects on the environment and render it safe for further use. In order to assess the status of a landfill restoration, a control area (undisturbed natural area) could be used for comparison with the restored area, in terms of plant and bacterial diversity, communities structure, abundance and similarity (SER, 2004; Perillo et al., 2009; Orsi et al., 2011). Developing countries, following economic developments worldwide, begin to seek for advanced and budgeted measures for waste management. More sanitary landfills can be expected (Hoornweg and Bhada-Tata, 2012) with the aim of successful restoration. Since

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specific limits for rainfall infiltration, landfill gas emission and slope angle are available (USEPA, 1993), evaluating the performance in preventing rainfall infiltration, landfill gas emission and slope stability are relatively manageable. However, evaluating the ecological success and ecosystem function are difficult and critical.

Previous studies focused more on the restoration process in non-sanitary landfills which were subjected to the influence of landfill gas and therefore searching for plants with methane tolerance seemed to be important (Chan et al., 1997, 1998; Marchiol et al., 2000). The ecological performance of a non-sanitary landfills was investigated (Chan et al., 1997), and the results showed that higher plant coverage, plant diversity, and microbial activities were observed at landfill sites, compared with those at reference sites. It was also pointed out that non-sanitary landfills could support ecological succession to typical and natural forests (Kim et al., 2004). Other studies on landfill restoration were also conducted at non-sanitary landfills (Biederman and Whisenant, 2009; Carrington and Diaz, 2011; Kim and Lee, 2005a, 2005b; Rawlinson et al., 2004), however, with more focus on the effects of soil manipulation, by adding wood chips (Biederman and Whisenant, 2009) and compost (Carrington and Diaz, 2011) as amendments, in affecting plant growth and development.

However, studies on the ecological performance of engineered sanitary landfills are scarce. In arid or semi-arid regions, a capillary barrier is applied as a landfill capping system (Barnswell and Dwyer, 2012). On the contrary, in humid regions (e.g., Hong Kong) sanitary landfills incorporate a geomembrane (HDPE) to prevent water percolation and landfill gas emission (Chan and Wong, 1998; Wong et al., 2015). A high degree of soil compaction (90–95%) has also been applied for slope stability (Fredlund and Rahardjo, 1993; Ng and Menzies, 2007). Therefore, sanitary landfills can ensure safety and minimum environmental impacts, but they change the soil water storage status and soil structure, compared with non-sanitary landfills and natural areas. In natural or man-made terrestrial ecosystems, the nutrient cycle (e.g., carbon, nitrogen and sulfur etc.) is mainly regulated by plants and associated microorganisms (e.g., bacteria for nitrogen fixation, nitrification and denitrification) (Bormann and Likens, 1967; Kertesz and Frossard, 2015; Parton et al., 2015; Schmidt et al., 2011). Briefly, plants capture carbon and nutrients from the atmosphere and soil respectively. These would then be transferred to the rhizosphere (via root exudates or litter) and utilized by bacteria (e.g., decomposition and mineralization). The nutrients are released back to the soil and hence available for the plants (Schulze and Mooney, 1994). Plants and bacteria are crucial components that related to the ecological performance of restored landfills.

Soil microbial communities in the topsoil layer serve as essential bio-indicators to assess the ecological performance of the soil environment (Morris and Blackwood, 2015). Most studies focused on bacteria in the refuse itself or the topsoil in non-sanitary landfills (Semrau, 2011). However, with promising performance, sanitary landfills are widely used nowadays. In this case, the ecological performance of sanitary landfills is the interest of both ecologists and environmental engineers. In addition to the safety and pollution control aspects, it is necessary to investigate how this emerging man-made ecosystem will behave, in terms of the plant and bacterial communities structures. Studies comparing the similarity of bacterial communities between a sanitary landfill and a natural area, as a measure to assess the ecological performance, are scarce.

Here, we present a restoration case of a sanitary landfill in a subtropical region. The South East New Territories (SENT) landfill in Hong Kong covers a total area of over 100 ha. It began accepting waste in 1994 and was designed to handle municipal solid waste for two decades (HKEPD, 2014). In 1997, phase I of the landfill

was saturated with municipal solid waste and capped with a cover system. Sixteen plant species, including seven exotic species (as pioneer species) and nine native species, were used for the restoration (Chen et al., 2015). Plants were allowed to grow for two years (1998–1999) prior to the continuing monitoring (2000–2012). It was found that the plant communities between the restored landfill and natural area were significantly different. This leads to the current study which focuses on the soil bacterial community in the restored sanitary landfill (containing geomembrane in the final cover system). It was hypothesized that the soil bacterial community in the restored landfill would be similar to the nearby natural area. The objectives of the present study were to (1) investigate the similarity of soil bacterial communities between the restored sanitary landfill and the natural area and (2) explore the feasibility of applying soil properties and plant growth parameters to assess the ecological performance of the soil bacterial community and offer a quick and inexpensive method for landfill restoration management.

## 2. Materials and methods

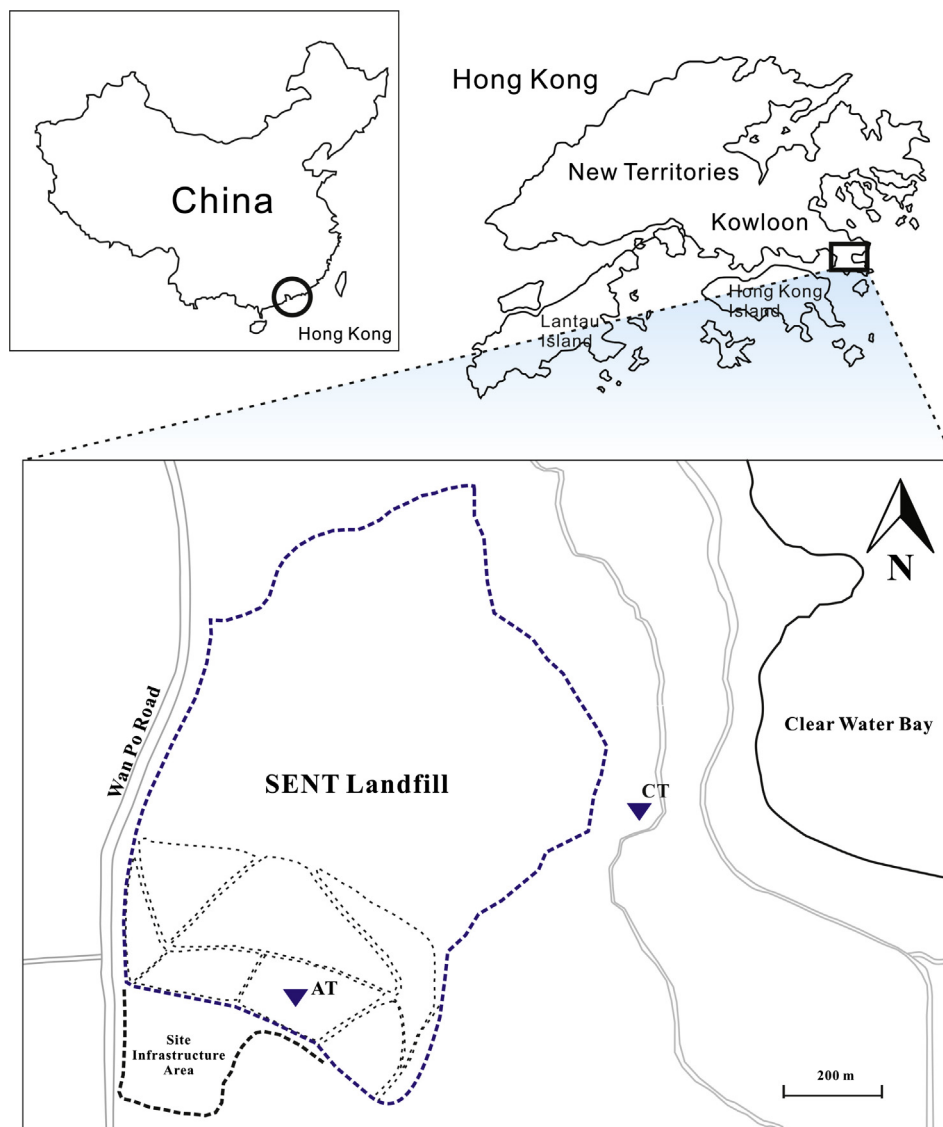
### 2.1. Study sites

One site (AT, 22°16'35.4"N 114°16'40.2"E) within the SENT landfill, capped and restored in 1997 (the earliest-restored area), was selected for study of the soil bacterial community. The landfill final capping system included the final intermediate cover, cushion geotextile, geomembrane, geonet, filtration geotextile and general cover layer (HKEPD, 2014). In detail, leachate migration to the bottom soil and rainfall infiltration into the waste are minimized by implementing the liner system (hydraulic barrier layers). Landfill gas emission is controlled using the gas extraction system with perforated pipes/wells placed within the refuse. For the final cover, there are three layers of soil (total depth: 1.5 m) above the high-density polyethylene (HDPE) membrane and the geocomposite draining layer: (a) 300 mm of screened compacted construction and demolition (C&D) fines, incorporated with different soils and/or organic matter, (b) 900 mm of lightly compacted C&D waste, and (c) 300 mm of final topsoil (completely decomposed granite and volcanic soil), incorporated with horticultural soil in planting pits with a hydro-seeding cover (Urbis Ltd, 1996; Chan and Wong, 1998) (Fig. A1). The other site (CT, 22°16'48.6"N 114°17'07.8"E), located approximately 150 m away from the east boundary of the landfill, served as a control (Fig. 1).

### 2.2. Soil sampling

According to our previous study on more other sites (four sites in total, area of each site: 100 m<sup>2</sup>) within the landfill (Wong et al., 2016), similar to site AT, the most abundant plant species were *Acacia* and *Leucaena* at other sites. Site AT was the earliest site restored (in 1997), and has been subjected to the longest succession period among all sites. Essential soil properties (i.e., moisture content, cation exchange capacity, nitrogen and phosphorus) that might affect the bacterial community were similar among all sites (including AT, represented as site C in Wong et al. (2016)). Therefore, AT could serve as a representative site for studying the landfill restoration, in order to obtain the initial data to investigate the similarity between AT and CT.

Soil sampling was undertaken in July 2014, representing the summer season in the region. A line transect (25 m) was randomly placed at each site (AT and CT, approximately with area of 45 × 23 m<sup>2</sup> and 50 × 20 m<sup>2</sup>, respectively). After removing litter from the soil surface, soil samples at depths between 5 and 10 cm were collected, with three replicates at each 5 m interval along the transect. Five individual soil samples were collected,



**Fig. 1.** Map showing the study sites (AT and CT) at SENT landfill which is located in the southeast of Kowloon, Hong Kong. Site AT is within and near the south boundary of SENT landfill, while CT is approximately 150 m away from the east boundary.

mixed and put in a 50 ml conical centrifuge tube (50 g soil). The soils collected from AT were labeled as Aa, Ab and Ac, while those from CT were labeled as Ca, Cb and Cc. The samples were subsequently stored in an ice box, transported to the laboratory within 2 h and stored at  $-80^{\circ}\text{C}$  prior to DNA extraction.

### 2.3. Terminal restriction length polymorphism (T-RFLP) analysis

The soil in each tube was mixed well and homogenized in a mortar with a pestle. Total DNA was extracted from 0.25 g subsamples of each soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA). Three replicated extractions were conducted for each soil sample (e.g., three extractions for sample Aa were labeled as Aa1, Aa2 and Aa3).

To investigate the similarity of soil bacterial communities in different soils, the 16S rRNA gene was amplified using polymerase chain reaction (PCR). Taq PCR Master Mix Kit (Qiagen, Germany) was used for PCR with forward primer FAM-8f (5'-AGAGTTT GATCCTGGCTCAG-3') (Edwards et al., 1989) and reverse primer 1492r (5'-TACCTTGTTACGACTT-3') (Wilson et al., 1990). The forward primer 8f was labeled with FAM at the 5' end. The final

concentration of the reaction mix (50  $\mu\text{l}$ ) contained 0.025 U  $\mu\text{l}^{-1}$  of polymerase,  $1 \times$  QIAGEN PCR buffer with 1.5 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$  of each dNTP, and 0.5  $\mu\text{M}$  of each primer. The thermal cycling was conducted in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) in the following conditions: 5 min of initial denaturation at  $95^{\circ}\text{C}$ ; 35 cycles of 1 min denaturation at  $94^{\circ}\text{C}$ , 1 min annealing at  $42^{\circ}\text{C}$ , 1.5 min elongation at  $72^{\circ}\text{C}$ ; and a 7 min final elongation at  $72^{\circ}\text{C}$ . The PCR products were loaded on 1% agarose gels [Sigma-Aldrich, USA; stained with SYBR Safe (0.1  $\mu\text{l ml}^{-1}$ ) (Life Technologies, Carlsbad, USA)] with  $1 \times$  sodium borate buffer (Brody and Kern, 2004) at 220 V for electrophoresis. The gels were visualized using a Gel Documentation System (Bio-Rad Laboratories, Hercules, USA).

The gels containing the target PCR products ( $\sim 1500$  bp) were excised and the DNA was extracted using a PureLink Quick Gel Extraction Kit (Life Technologies, Carlsbad, USA). Isopropanol (1 gel volume) was added after the gels were dissolved for optimal DNA yields. Each extraction of the PCR product was digested with the MspI (HpaII) restriction enzyme (Thermo Scientific, Waltham, USA) in a 20- $\mu\text{l}$  reaction system containing 150 ng of purified PCR products in Tris-HCl buffer (10 mM, pH 8.5), 2  $\mu\text{l}$  of  $10 \times$  Buffer

Tango, 0.5  $\mu$ l of restriction enzyme, and brought to 20  $\mu$ l using nuclease-free water. The reaction mixtures were incubated at 37 °C for 3 h and 80 °C for another 20 min to inactivate the restriction enzyme. Subsequently, the digested DNA fragments were analyzed using a bioanalyzer (2100 Bioanalyzer, Agilent Technologies, Santa Clara, USA) with GeneScan 500 ROX dye Size Standard (35–500 bp) (Life Technologies, Carlsbad, USA).

#### 2.4. Soil physical and chemical properties analyses

Subsamples of soils were used to analyze the soil properties. The soil samples were air-dried for at least one week, sieved through a 0.2 mm mesh and kept at 4 °C in a refrigerator before further analyses.

Soil pH (pH meter, 420A 1990, Orion Research Inc.), electrical conductivity (EC) (electrical conductivity meter, LF 330/SET, WTW), cation exchange capacity (CEC) (Unbuffered Salt Extraction Method), total organic carbon (TOC) (Walkley and Black Method) and organic matter (OM) (Loss-On-Ignition Method) were determined using the methods described by Sparks et al. (1996).

Additional fresh soil samples were also collected to determine the bulk density (BD) and moisture content (MC) by driving core samplers (diameter: 10 cm) into the soil (three replicates), following the methods described by Klute et al. (1994).

For total nitrogen (N), 0.5 g of sieved soil was digested using the Kjeldahl method (concentrated sulfuric acid), followed by the Molybdenum-blue method. For total phosphorus (P), 0.5 g of sieved soil was digested using the semi-micro Kjeldahl method, followed by the Indophenol-blue method. Extractable ammoniacal-N was extracted by potassium chloride and followed by the Molybdenum-blue method, while extractable P was extracted using hydrogen bicarbonate and followed by the Indophenol-blue method using a spectrophotometer (UV-1601, Shimadzu, Japan). All methods followed those described in Sparks et al. (1996).

For total potassium (K), copper (Cu), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn), 0.5 g of sieved soil was digested by concentrated hydrofluoric acid and nitrite acid (Page et al., 1982). The residue was dissolved in water, diluted, and analyzed using atomic absorption spectrophotometry (AAS) analysis (SpectrAA 220FS, Varian). For extractable K, Cu, Fe, Mn, Pb and Zn, 0.5 g of sieved soil was extracted using  $\text{NH}_4\text{Cl}$  solution and followed by atomic absorption spectrophotometry (AAS) analysis (SpectrAA 220FS, Varian) (Sparks et al., 1996).

#### 2.5. Plant survey

A belt transect of 25 m long was randomly placed at each site. All the plants along the transect and within 20 cm perpendicular from the transect line were recorded and identified at the species level. The plant coordinates, canopy width, height, basal diameter, and health status of the plants were also recorded.

#### 2.6. Data analysis

T-RFLP profiles were obtained using the PeakScanner™ software (Version 1.0, ABI, United Kingdom). Terminal restriction fragments (TRFs) smaller than 35 bp or larger than 680 bp were excluded. Data were further analyzed using the T-REX platform (Culman et al., 2009) for noise reduction (Abdo et al., 2006) and binning (i.e. TRF alignment) (Smith et al., 2005). A Bray-Curtis similarity matrix was constructed and visualized using hierarchical cluster analysis. Analysis of similarity (ANOSIM, 999 permutations, non-parametric) was performed to analyze the similarity of TRFs between different sites, using PRIMER version 6 (Clarke and Gorley, 2006). Jaccard's index for the coefficient of similarity of

plant species between the two sites was calculated (Legendre and Legendre, 1998). Canonical correspondence analysis (CCA, unimodal response function assumed) was conducted using Canoco 4.5 following the descriptions by Lepš and Šmilauer (2003) and Zheng et al. (2013). Edaphic variables included in the CCA were selected by judging the variance inflation factors (VIF) which were calculated to detect the multicollinearity during regression analysis (Braak and Verdonschot, 1995; Ramette, 2007). The T-RFLP profiles were analyzed, using T-RFLP (PAT+) in MiCA 3 (Shyu et al., 2007) to obtain the plausible bacterial community structure.

All statistical tests were performed with SPSS 16.0 software. Levene's test was conducted to check the homogeneity of the data. Normality of the data was checked using Shapiro-Wilk test. The Independent-Samples T Test was used to compare differences in soil properties between different sites, while for the non-normally distributed data, Mann-Whitney *U* test (nonparametric) was used. For relative comparison, the Shannon-Weaver diversity index presenting the diversity for plants ( $H'_p$ ) and 16S rRNA gene TRFs ( $H'_{TRFs}$ ) were calculated according to Dombois and Ellenberg (1974). To calculate Shannon-Weaver diversity index, the following equation was used:

$$H' = - \sum_{i=1}^s P_i \ln(P_i)$$

where

$$P_i = \frac{\text{Number of individuals of species (or TRF) } i}{\text{Total number of individuals of all species (or TRFs)}}$$

### 3. Results and discussion

#### 3.1. Soil properties

Significant differences ( $P < 0.05$ ) between AT and CT were observed in soil properties including pH, EC, CEC, moisture content, total N, total P, extractable P, total Fe, extractable Fe, extractable Mn, extractable Pb, total Zn and extractable Zn (Table 1). Higher pH, CEC, total N, extractable P, extractable Fe, extractable Mn, extractable Pb, total Zn and extractable Zn were observed in soil from AT, whereas there was lower moisture content, total P and total Fe, when compared with soil from CT.

Soil pH plays a significant role in shaping the soil bacterial community (Fierer and Jackson, 2006). Soil pH is the best predictor of soil bacterial community composition rather than other edaphic variables such as latitude, mean average temperature, soil moisture deficit, organic carbon, and C:N ratio (Fierer and Jackson, 2006). In our study, the pH of the soil collected from AT was  $4.98 \pm 0.48$ , compared with  $3.41 \pm 0.07$  from CT (Table 1). The highest soil bacterial diversity (presented as the Shannon-Weaver diversity index) has an expected pH value of around 7 (Fierer and Jackson, 2006).

#### 3.2. Plant diversity and richness

During the first two years (1998–1999), Wong et al. (2015) found that exotic species (i.e. three *Acacia* species and two *Eucalyptus* species) grew significantly better than native species which had high mortality rates of >90% (i.e. *Castanopsis fissa*, *Gordonia axillaris*, *Machilus thunbergii* and *Schima superba*). Other species, such as *Tristania conferta*, *Alnus formosana*, *Celtis sinensis*, *Cinnamomum camphora*, *Ficus superba*, *Schefflera heptaphylla* and *Quercus edithae* performed poorly, in terms of mortality rate, apical height, canopy diameter, and basal diameter.

In 2014, the plant species recorded in AT and CT both contained exotic species and native species (Table 2). *Acacia auriculiformis*

**Table 1**  
Soil properties of the samples collected from SENT landfill (AT) and control site (CT).

Soil properties	Site	
	AT	CT
pH**	4.98 ± 0.48	3.41 ± 0.07
EC* (ms cm <sup>-1</sup> )	180 ± 36.1	129.8 ± 22.7
Bulk density (g cm <sup>-3</sup> )	0.81 ± 0.22	0.87 ± 0.07
CEC*	8.83 ± 1.23	7.30 ± 0.71
Moisture content (%)**	1.66 ± 0.08	2.21 ± 0.15
Total organic carbon (%)	14.4 ± 1.18	14.5 ± 0.52
Organic matter (%)	8.34 ± 0.69	8.43 ± 0.3
Total N*	420 ± 65.8	292 ± 11.7
Extractable N	7.14 ± 1.39	9.36 ± 1.65
Total P*	5.64 ± 3.16	18.0 ± 6.21
Extractable P**	0.54 ± 0.12	0.09 ± 0.02
Total K	3929 ± 275	4302 ± 575
Extractable K	16.7 ± 0.44	15.8 ± 0.80
Total Cu	25.4 ± 17.9	22.6 ± 5.27
Extractable Cu	8.39 ± 4.26	5.80 ± 2.14
Total Fe**	14,479 ± 937	20,380 ± 2567
Extractable Fe**	203.6 ± 17.2	172.7 ± 3.16
Total Mn	531 ± 42.1	573 ± 29.7
Extractable Mn**	67.9 ± 8.69	50.4 ± 6.63
Total Pb	148.3 ± 18.8	92.7 ± 50.8
Extractable Pb**	83.2 ± 15.1	53.6 ± 7.70
Total Zn**	138 ± 28.8	30.9 ± 6.21
Extractable Zn**	63.9 ± 6.24	4.22 ± 1.08

Soil properties with significant difference between the two sites are highlighted with \*\*\*\* ( $P < 0.05$ ) and \*\*\*\*\* ( $P < 0.01$ ) (independent-samples T test or Mann-Whitney U test). Means of bulk density, total P, extractable P, total Cu and total Mn were compared using Mann-Whitney U test (nonparametric). All tests were conducted at the significance level of 0.05. Details of the T test results are shown in Table A1. The unit from Total N to Extractable Zn are mg kg<sup>-1</sup>. Data are mean ± S.D. (n = 5). For bulk density, n = 3.

and *Acacia confusa* were exotic species recorded in both AT and CT, while *Alpinia galanga* and *Schefflera heptaphylla* were the two native species observed in both sites. *A. confusa* (relative density: 27.8%), *Leucaena leucocephala* (22.2%) and *A. auriculiformis* (16.7%) were the three dominant species in AT, while *Alpinia galanga* (27.8%), *A. confusa* (19.4%), and *Mallotus paniculatus* (13.9%) were the three dominant species in CT. More native species (8 out of 10) were recorded in CT compared with those in AT (4 out of 7).

**Table 2**  
Plant diversity and richness of site AT and CT.

Plant species	Total canopy cover (m)	No. of intervals contain this species	No. of individual	Relative density (%)	Relative frequency (%)	Relative dominance (%)	Importance value (%)
<b>AT</b>							
<b>Melia azedarach</b>	0.6	1	1	5.56	6.25	2.88	4.90
<i>Acacia auriculiformis</i> <sup>a</sup>	6.0	2	3	16.7	12.5	28.9	19.3
<i>Acacia confusa</i> <sup>a</sup>	8.0	5	5	27.8	31.3	38.5	32.5
<b>Alpinia galanga</b>	0.9	1	2	11.1	6.25	4.33	7.23
<i>Leucaena leucocephala</i> <sup>a</sup>	3.8	4	4	22.2	25.0	18.3	21.8
<b>Lygodium japonicum</b>	0.2	1	1	5.56	6.25	0.96	4.26
<b>Schefflera heptaphylla</b>	1.3	2	2	11.1	12.5	6.25	9.95
Total 7 species	20.8	16	18				
<b>CT</b>							
<i>Acacia auriculiformis</i> <sup>a</sup>	2.0	1	1	2.78	3.45	4.56	3.59
<i>Acacia confusa</i> <sup>a</sup>	14.5	6	7	19.4	20.7	33.0	24.4
<b>Alpinia galanga</b>	12.9	7	10	27.8	24.1	29.4	27.1
<b>Celtis sinensis</b>	3.1	3	4	11.1	10.3	7.06	9.51
<b>Ficus hispida</b>	0.8	1	1	2.78	3.45	1.82	2.68
<b>Macaranga tanarius</b>	1.8	2	3	8.33	6.90	4.10	6.44
<b>Mallotus paniculatus</b>	5.2	5	5	13.9	17.2	11.9	14.3
<b>Schefflera heptaphylla</b>	1.3	1	2	5.56	3.45	2.96	3.99
<b>Smilax sp.</b>	0.3	1	1	2.78	3.45	0.68	2.30
<b>Psychotria rubra</b>	2.0	2	2	5.56	6.90	4.56	5.67
Total 10 species	43.9	29	36				

Species names in bold are native species.

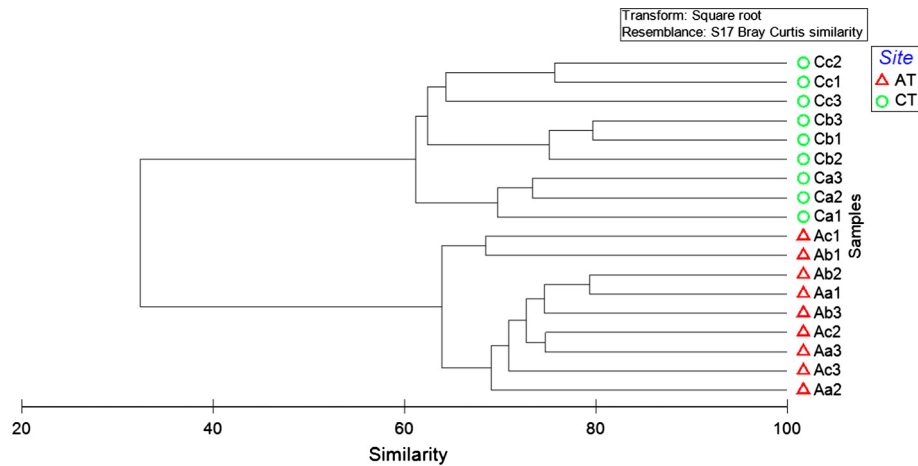
<sup>a</sup> Legume species.

The Jaccard's coefficient of similarity of the plant communities between AT and CT was 0.31, a result in line with our previous findings (Chen et al., 2015) that these two sites possessed different plant communities. Pioneer species (mostly exotic) were used for restoration, as these species, such as *A. confusa* and *L. leucocephala*, can grow faster and are more tolerant to the harsh environment (Chan et al., 1998; Wong et al., 2015). However, these species later compete with the native species in such a stressed environment (mainly compacted soil), leading to a relatively lower diversity and density of native species (Table 2). It was demonstrated that *A. confusa* outperformed *Litsea glutinosa* (native species), when subjected to unfavorable soil conditions, such as drought and compaction (Liang et al., 1999). Pioneer species are able to revegetate disturbed lands faster and prevent soil erosion. However, this practice may later lower the diversity of species, in particular native species, which may potentially affect the food web. This was observed both in our previous (Chen et al., 2015) and the present study.

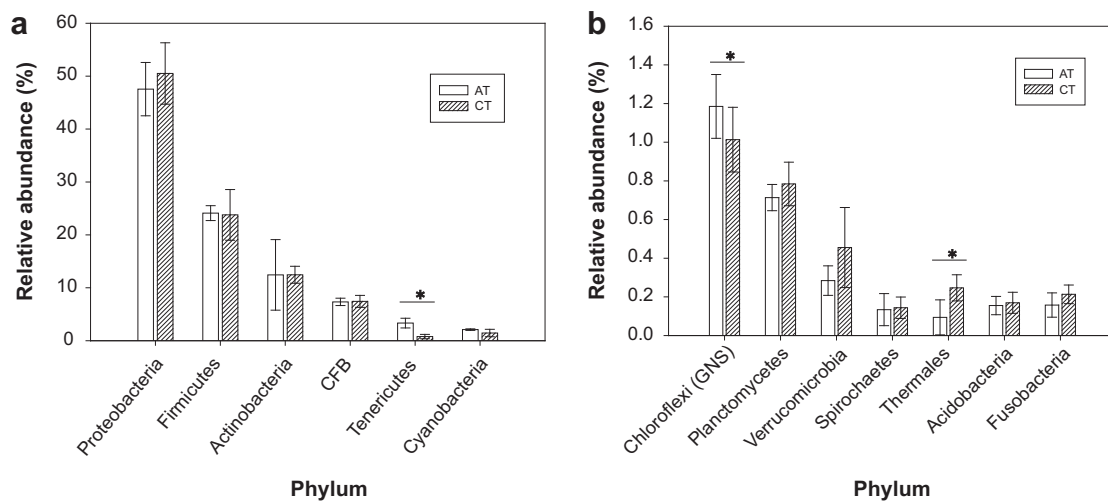
### 3.3. The similarity of soil bacterial communities

The soil collected from AT possessed a soil bacterial community significantly different from that in the soil from CT ( $R = 1$ ,  $P < 0.01$ , ANOSIM) (Fig. 2). Fig. 3 shows the plausible major phyla composition derived from the soil collected at AT and CT by using MiCA. The main factors in controlling soil bacterial richness and diversity are the soil properties (such as pH, total N and extractable P), plant richness and diversity. It was clear that soils from AT and CT possessed different profiles of TRFs (Fig. 2). The similarity within samples collected in the same site was 60% or above. TRFs obtained from CT were more heterogeneous than those from AT.

At site AT, 18 plant individuals (including the same and different species) were found along the transect, while 38 plant individuals were recorded for CT. The  $H_p$  in AT (1.80) was lower than that in CT (2.02), while the  $H_{TRFs}$  in AT (4.20) was higher than that in CT (3.20). Although the 16S rRNA gene TRFs diversity underestimates the true soil bacterial diversity (Blackwood et al., 2007; Orcutt et al., 2009), relative comparison is valid for investigating the relative difference. Our results indicated that higher plant diversity



**Fig. 2.** Hierarchical cluster analysis comparing different T-RFL profiles obtained from different DNA derived from different soil samples collected at site AT and CT. The soils collected from AT were labeled as Aa, Ab and Ac, while the soils from CT were labeled as Ca, Cb and Cc, each with three replicates labeled as 1, 2 and 3.



**Fig. 3.** The major phyla composition derived from the soil collected at AT and CT by using MiCA. About 60% of the accession number (both AT and CT) derived from MiCA are classified as uncultured bacteria without the phylum identification. The phyla that could not be identified in GenBank (NCBI) were excluded. Other minor phyla are shown in Table A2. Stars indicate significant different between sites at the significant level of 0.05 (T test). Data are mean  $\pm$  S.D. (n = 9).

did not necessarily impose a higher soil bacterial diversity. A similar conclusion was drawn by Fierer and Jackson (2006), showing that there was no clear relationship between plant diversity and soil bacterial diversity. It has been shown that the microbial community biomass increased with greater plant diversity (Zak et al., 2003), and increasing plant community richness significantly altered soil bacterial community composition and was negatively correlated with bacterial diversity (Schlatter et al., 2015). This is in line with the present results in which lower plant richness (7 vs. 10 species) but higher bacterial diversity (4.20 vs. 3.20 of  $H'_{TRF}$ ) were observed in the restored landfill site. The pH value in AT ( $4.98 \pm 0.48$ ) was significantly higher than that in CT ( $3.41 \pm 0.07$ ). This is probably the essential factor in controlling the soil bacterial community structure. A higher pH value in AT exerted a higher  $H'_{TRFs}$  value (4.20, higher TRFs diversity and richness), compared with that in CT ( $H'_{TRFs} = 3.20$ ), which is in line with the study by Fierer and Jackson (2006) in which pH values closer to 7 showed an increased phylotype diversity.

Another recent study attempted to investigate the factors controlling the soil bacterial diversity, and it was shown that microbial parameters were significantly related with soil moisture content, soil pH, organic matter and sulfate (Van Horn et al., 2013).

However, the magnitude and even the direction of the relationships were different from sample to sample (Van Horn et al., 2013). More concrete conclusions could not be drawn with regard to pinpointing the main edaphic factors in controlling soil bacterial diversity. Furthermore, the range of soil pH measured was 7.0–10.0, which was out of the range of the present study (3.41–4.98). Another study also showed that the bacterial diversity was correlated with soil pH (pH = 4.0–6.5,  $R = 0.458$ ,  $P = 0.024$ ) (Shen et al., 2013).

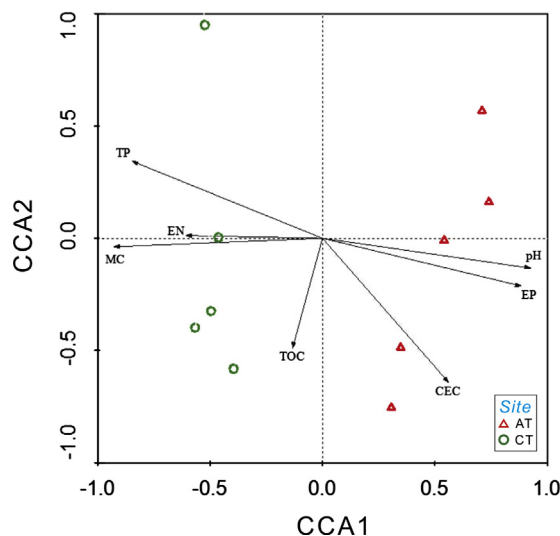
#### 3.4. Potential driving factors on soil bacterial communities

Soil bacterial community structure is closely linked to soil and vegetation properties (Cong et al., 2015). Different plant species and communities possess distinct canopy covers, rooting depths, litter quality/quantity (Zak et al., 2003; Garbeva et al., 2006). These factors are linked to distinct symbionts (rhizobium and mycorrhizae) and decomposers (saprophytic fungi) which are associated with different bacteria. The higher canopy cover in CT (43.9%) than AT (20.8%, Table 2) could be one of the reasons that led to higher soil moisture content in CT (Table 1). Soil moisture is a major factor that influences the bacterial structure (Bell et al., 2009). Soil

extractable N is a main nutrient for ecosystem processes such as N leaching, mineralization and plant uptake (Ros et al., 2009), and subsequently affect bacterial community structure. Total and extractable P are always the limiting factors for plant growth, thus mycorrhizal symbiosis occurs (Smith and Read, 2008; Bonfante and Genre, 2010). Soil TOC, which is positively correlated with organic matter, including fresh, decomposed or semi-decomposed plant litter, animal residue, root exudates, and living/dead organisms, represents the main source for bacterial consumption (Parton et al., 2015). TOC also affects the nutrient holding capacity (CEC, which correlates with EC). Furthermore, soil pH is considered as one of the most important factors influencing bacterial diversity (Fierer and Jackson, 2006; Rousk et al., 2010). Therefore, such soil properties were included in canonical correspondence analysis (CCA) which explored the factors governing the bacterial community.

In our study, the potential factors controlling the soil bacterial community included soil pH, extractable P and CEC for bacteria in AT; and extractable N, total P and moisture content for bacteria in CT, based on corresponding arrows direction and magnitude shown in Fig. 4, suggesting that they are important for explaining the variation between the microbial communities from the two studied sites. The bacterial TRFs were separated into two groups (Fig. 2) showing that the soil properties potentially affect the soil bacterial community (Fig. 4). Another study also pointed out that the factors controlling bacterial community are soil moisture content, pH and organic matter (Van Horn et al., 2013).

TRFs derived from AT were positively correlated with pH ( $P < 0.05$ , Fig. 4). Similar observations were made by Zheng et al. (2013) who showed that pH was one of the major contributions to the archaeal community composition. Another study (Colombo et al., 2016) also pointed out that, rather than fertilization, irrigation (i.e., moisture content) had a stronger effect on bacterial communities. Due to slope stabilization and water infiltration minimization, it is expected that the soil moisture content in the landfill site (AT) would be lower than in the natural area (Table 1), due to the higher soil compaction rate and lower level of water storage. It is noted that soil moisture content was one of the contributing factors controlling the bacterial community structure in CT (Fig. 4).



**Fig. 4.** Biplot of canonical correspondence analysis (CCA) for the relationship between soil primary properties (a) and other properties (b) ( $n = 5$ ) and TRFs composition derived from 16S rRNA gene of soil bacteria in site AT and CT. **CEC:** cation exchange capacity; **MC:** moisture content; **TOC:** total organic carbon; **EN:** extractable N; **TP:** total P; **EP:** extractable P.

In addition to soil moisture content, another study also showed that total N, C and P were important factors in controlling the bacterial communities in different treatments (agricultural soil vs. non-agricultural soil) (Bissett et al., 2011). It is still essential to understand how these factors affect the soil bacterial diversity and abundance and the subsequent effects on nutrient cycling. An attempt was made to compare soil bacterial community succession patterns between restored mined and non-mined sites (Banning et al., 2011). The results showed that, after 14 years of development, the soil bacterial community became stable and unchanged, compared with the 18-year-old rehabilitation soil (re-landscaping by seeding native species including  $N_2$ -fixing legumes and diammonium phosphate amendment). However, the bacterial community structure still differed from that in the non-mined site (principal coordinate analysis). Our results also showed that the bacterial community in the restored landfill site (presented as TRFs) was significantly different from that in the undisturbed area 15 years after restoration. This can be expected due to the several key soil properties, including pH, N, P and moisture content, were different between the two sites (Table 1). To assess the similarity of the soil bacterial communities derived from different sites, measuring several key soil properties (e.g., pH, MC, OM, CEC, N, and P) would be important to understand the patterns and general trend of these communities.

It is noted that the concentrations of some metals, especially Zn and Pb, were significantly higher ( $P < 0.05$ ) in soil from AT than those in CT (Table 1). The levels of metals contained in the present study were within the global range (Duxbury, 1981). The effects of extractable metal concentrations on bacterial growth are more significant than their total concentrations in soil (Saeki et al., 2002). Thus, it is essential to focus on extractable Zn and Pb. In addition, the Zn concentration obtained in the present study ( $63.9 \pm 6.24 \text{ mg kg}^{-1}$ , Table 1) was below the concentration ( $\sim 130 \text{ mg kg}^{-1}$ ) that starts to affect the tolerance level of bacteria growth (Diaz-Ravina and Baath, 1996). For extractable Pb, a concentration of  $\sim 400 \text{ mg kg}^{-1}$  in six types of soil did not show significant effects on the operational taxonomic units (OTUs), which indicated that there was limited effect on the change of bacterial community due to Pb addition (Lazzaro et al., 2006). The extractable Pb obtained in our study was  $83.2 \pm 15.1 \text{ mg kg}^{-1}$  which was much lower than  $400 \text{ mg kg}^{-1}$ , thus the concentrations of both Zn and Pb in soil may have limited effects on the bacterial community in the restored landfill site.

It is possible that the current restoration status is in transition. However, based on information available, ecosystems can change over time, even in the undisturbed areas, due to changes of edaphic properties, notably pH (Chen et al., 2015) which is one of the main factors affecting bacterial diversity (Fierer and Jackson, 2006). A study also suggested that the microbial community structure is altered by environmental changes (Cong et al., 2015).

Using the sequencing-based method will probably result in the two sites possessing different key bacterial species, in which it is still difficult to explain or determine which species composition is preferred. A fundamental question should be asked: What is the preferred species composition for establishing a stable and diversified food web in the restored area which could become a self-supporting ecosystem (Urbanska et al., 2000; SER, 2004) with more support from the microbes, in terms of nutrient cycling (in particular carbon and nitrogen) (Cong et al., 2015). Within this direction, our study focused on the diversity and interaction of higher plants and bacteria of the restored landfill site compared with the natural area. The two studied ecosystems, i.e. the “restored ecosystem” and the “natural ecosystem”, developed in different ways, which implies that the current restoration practice needs to be reconsidered. These results can serve as a cooperative reference for engineers and ecologists for the restoration of

man-made ecosystems (e.g., completed landfills). A better restoration plan might be developed by adjusting the essential soil properties and recruiting more native species, although exotic species can still be used with great care. For example, based on our previous (Chen et al., 2015; Wong et al., 2016) and the present studies, *A. auriculiformis* and *A. mangium* are less aggressive exotic (pioneer) species compared with *A. confusa* and *L. leucocephala*. These species could be introduced to create a suitable environment (nitrogen fixation by legumes and create shelters to deal with dry and windy conditions) for the establishment of native species. At the later stage, exotic species should be removed or thinned, to further promote the growth and establishment of native species.

Further studies focusing on the bacterial species composition and abundance are necessary to assess the performance and their specific functional role in nutrient cycling (i.e., C, N, P, and S cycling) in the restored landfill. The diversity of the functional genes involved in biogeochemical carbon, nitrogen, phosphorus, and sulfur cycling could also be examined to understand the functional diversity of the existing bacteria in restored landfill sites. Soil amendment can be considered to provide an optimized soil condition (e.g., neutral pH) to support the diversity and ecological function of the soil bacteria. The ecological performance of the control area (natural environment without disturbance) should be further evaluated as detrimental environmental conditions (i.e., acidic soil) may impose negative impacts. Our previous study showed that soil pH in the natural area decreased from 5.2 to 3.2 during 2000–2012 (Chen et al., 2015). Acid rain on forest could affect the organic carbon accumulation by suppressing litter decomposition, soil respiration and microbial biomass (Wu et al., 2016).

#### 4. Conclusions

Fifteen years after restoration, a sanitary landfill in Hong Kong was unable to be restored to an ecosystem similar to the natural area, in terms of both plant and soil bacterial community structures. The higher plant diversity does not necessarily represent a higher soil bacterial diversity. The common use of fast-growing exotic species for initial revegetation works should be reconsidered, due to the consequences in competing with native species later on. To achieve similar soil bacterial communities between man-made and natural sites, there seems to be a need to establish similar soil properties, especially pH, MC, TOC, CEC, N, and P. Future studies should be conducted to analyze the structure and quantity of bacteria and fungi, and their association with soil parameters (or other potential parameters, e.g., soil aggregation) at different depths of the landfill topsoil, in order to obtain a fuller picture of microbial communities.

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.wasman.2016.08.015>.

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