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Next-generation sequencing showing potential leachate influence on bacterial communities around a landfill in China

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1   **Next-generation sequencing showing potential leachate**

2   **influence on bacterial communities around a landfill in**

3   **China**

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21 **ABSTRACT**

22 The impact of contaminated leachate on groundwater from landfills is well known but specific

23 effects on bacterial consortia are less well-studied. Bacterial communities in landfill and an

24 urban site located in Suzhou, China were studied using Illumina high-throughput sequencing. A

25 total number of 153944 good quality reads were produced and sequences assigned to 6388

26 operational taxonomic units (OTUs). Bacterial consortia consisted of up to 16 phyla including

27 *Proteobacteria* (31.9 to 94.9% at landfill, 25.1 to 43.3% at urban sites), *Actinobacteria* (0 to

28 28.7% at landfill, 9.9 to 34.3% at urban sites), *Bacteroidetes* (1.4 to 25.6% at landfill, 5.6 to

29 7.8% at urban sites), *Chloroflexi* (0.4 to 26.5% at urban sites only) and unclassified bacteria.

30 *Pseudomonas* was the dominant (67-93%) genus in landfill leachate. Arsenic concentrations in

31 landfill raw leachate (RL) (1.11×10^3 µg/L) and fresh leachate (FL2) (1.78×10^3 µg/L), and

32 mercury concentrations in RL (10.9 µg/L) and FL2 (7.37 µg/L) were higher than Chinese State

33 Environmental Protection Administration (SEPA) standards for leachate in landfills. Shannon

34 diversity index and Chao 1 richness estimate showed RL and FL2 lacked richness and diversity

35 when compared with other samples. This is consistent with stresses imposed by elevated arsenic

36 and mercury and has implications for ecological site remediation by bioremediation or natural

37 attenuation.

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39 **Keywords** Landfill, leachate, bacterial diversity, *Pseudomonas*, Arsenic.

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INTRODUCTION

Municipal landfill waste compositions can range from food wastes to high-strength detergents, solvents and pharmacological products comprising a broad spectrum of xenobiotic and recalcitrant toxic compounds with potential harmful ecological impacts (Köchling et al., 2015, Song et al., 2015a). Although modern landfills in well-regulated economies are highly engineered and monitored, older or informal (unplanned, uncontrolled) landfills worldwide are sources of leachate which, unless correctly collected and treated, can cause serious reductions in the quality of water bodies and groundwater sources (Li et al., 2014, Zhang et al., 2013a). Previous studies have indicated a diverse range of heavy metal concentrations in leachates (Song et al., 2015b, Zhang et al., 2013a). Heavy metals have been previously shown to directly influence the bacterial community composition of various environments (Muller et al., 2001, Vishnivetskaya et al., 2011, Sandaa et al., 1999, Mor et al., 2006, Yao et al., 2017). Long term studies have shown a strong influence of mercury towards the bacterial community of a river basin and soil (Muller et al., 2001).

To study complex microbial ecosystems such as leachate, molecular techniques have several advantages over culture-based techniques as they allow the analysis of uncultured organisms and provide higher resolution measurements closer to the complete microbial profile (Staley et al., 2011). Analysing the microbial community around a landfill can potentially determine whether the leachate is being transported through the landfill liner into the natural soil and groundwater, via changes in the diversity and composition of bacterial consortia as different species are more or less tolerant of elevated pollutant concentrations (Wang et al., 2017, El-Salam and Abu-Zuid, 2015, Vukanti et al., 2009).

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Previous studies on heavy metal influence towards microbial communities were performed using PCR-DGGE and GS 454 FLX pyrosequencing (Muller et al., 2001, Yao et al., 2017, Vishnivetskaya et al., 2011). Next generation sequencing (NGS) methods can assist in the identification of very rare taxa in the landfill samples (Köchling et al., 2015, Song et al., 2015a). NGS provides efficient, multiple level details of the operational taxonomical units (OTUs), richness and diversity, so it can be used to identify both similarities and differences between sites. Furthermore, the rapidity and portability of NGS methods and apparatus, for example, Nanopore (Oxford Nanopore Technologies, Oxford, UK) mean that sequencing of microbial consortia now presents a potentially rapid, low-cost option for the detection of leachate impacts on natural groundwater consortia and hence mapping of contaminant plumes based on ecological, rather than chemical, indicators (Brown et al., 2017).

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Understanding the environmental conditions and bacterial community is of upmost importance when it comes to cleaning up the contaminants by employing techniques such as biodegradation. It is a microbial process that degrade contaminants found in the environment. Over the past 20 years, in-situ biodegradation has successfully been applied to various environments with different level of degrading abilities depending on the bacteria (Meckenstock et al., 2015). The process requires careful identification of the degrading bacteria prior to implementation. Generally, constant monitoring of the microbial activity is also required to ensure constant and consistent microbial activity over time. For example, Adetutu et al. (2015) utilised biostimulation (BS), biostimulation-bioaugmentation (BS-BA) and monitored natural attenuation (MNA) approaches to bioremediate groundwater polluted with trichloroethene (TCE). Next-generation

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92   sequencing was an effective technique to study the microbial community dynamics throughout

93   while performing the dechlorination process.

94

95   In the present work, we investigated the potential for NGS to identify potential impacts on soil

96   and groundwater bacterial communities due to heavy metal-rich landfill leachate in a conurbation

97   in Suzhou, Jiangsu province, China. The objectives of this study were i) to characterize the

98   composition of the bacterial communities of a selected landfill (leachate, soil and groundwater)

99   and a non-landfill site in same conurbation, hereby referred to as "urban" (soil and groundwater);

100   ii) to compare the unique and dominant bacterial taxa among the landfill and urban samples; and

101   iii) to investigate and compare the bacterial diversity and heavy metal concentration of the soil

102   and groundwater samples from a landfill and urban site. The study not only adds to the

103   knowledge in respect of leachate impacts on subsurface consortia under urban areas, but assesses

104   the potential of NGS for rapid monitoring of environmental impacts from landfills, and has

105   implications for the design and implementation of biological remediation options such as natural

106   attenuation or *in situ* microbially-induced carbonate precipitation.

107

108   **MATERIALS AND METHODS**

109   **Sample locations**

110   The selected landfill (located at 31°14'18.31"N 120°33'3.09"E) began operation in 1993 and

111   receives about 1,500 tons/day of household wastes and industrial wastes from the Suzhou

112   conurbation. A new landfill was constructed in 2006 on the surface of the older landfill (Rong et

113   al., 2011). The urban site samples were collected from an area that was previously used for

114   agriculture prior to reclamation for industrial development. The two sites are approximately 27

5

115 km from each other. The two sites are approximately 27 km from each other. Suzhou is situated

116 on top of a 200 m deep sequence of Quaternary sediments. The depth of drift reduces to 0m

117 directly to the West and South West of the City (Jiangsu Provincial Bureau of Geological and

118 Mineral Exploration, 1984). At depth the bedrock is composed of Devonian quartzite and shales

119 of the Wutong Formation, the sandstones shales and quartzites of the Maoshan Group and zones

120 of Carboniferous limestone (the karstic features of which are known commercially as Taihu

121 Stone, exposed at Dongting Mountain and in Linwu Cave) which forms the hills to the south and

122 west of the city. This sequence is intruded by the Suzhou Granite which is exposed to the West

123 of the city centre. The variable erosive bedrock surface, has been infilled by alluvial and

124 lacustrine sediments of the lower flood plains of the Yangtze River. The subsurface materials

125 vary from clays to silty sands (Shi et al., 2012). The structure of the quaternary strata below

126 ground varies at the very large scale, due to the movement of the rivers and changes in the extent

127 and location of the lakes with time. However, the extent of variation has been limited by the

128 volume of materials being deposited within a geologically short period of time. Some of the

129 silty/sandy subsurface zones are a result of reworking of loess by the Yangtze River. The silty

130 sands have sufficient porosity to act as aquifer materials (Ma et al., 2011). Pumping works from

131 these aquifers have caused the collapse of their porous structure resulting in approximately 1 m

132 of settlement across the region increasing to 1.4m towards city centres, and reducing to 0m

133 towards the locations of large permanent lakes (Shi et al., 2012). Details regarding Suzhou

134 landfill construction and waste were briefly discussed by Rong et al. (2011).

135

136 The landfill sampling comprised of two leachates, soil from three different locations around the

137 landfill (samples LS1, LS2 and LS3) and one groundwater from the landfill monitoring well

138   (samples BHGW) (Table 1). Leachate samples were either fresh (FL2, collected from an outlet

139   pipe that runs beneath the landfill) or raw leachate (RL, sampled from a leachate pond). Soil

140   samples were collected using a Spiral auger at 30cm depth. The first soil location was near the

141   leachate pond; the second was close to agricultural land on the boundary of the site; and the third

142   soil location was close to the groundwater monitoring borehole. The groundwater was collected

143   at an approximate depth of 4 meters using a hand-held slow flow peristaltic pump. The samples

144   were collected from well below the groundwater surface such that any residual floating matter

145   would not be collected. Groundwater and leachate were collected in sterile high density

146   polyethylene plastic bottles and soil samples were collected in a sterile plastic zip lock bags and

147   transported to the laboratory under ambient temperature conditions, then stored in a cold room

148   (4°C) prior to analysis.

149

150   To contrast the bacterial community from the landfill, soil (samples USS1 and USSur1) and

151   groundwater (samples USGW) samples were collected from the urban site. Two samples from

152   the two different locations in an urban area were selected for the soil sampling which were 200

153   meters apart. The groundwater borehole was chosen for the groundwater sampling. Ground water

154   was collected at a depth of 4 meters. The first location of the soil sampling was located closer to

155   the urban site groundwater and the second location of the soil sample was an isolated location.

156

157   **Physicochemical analysis of soil and water samples**

158   The following heavy metals were analysed for all samples: mercury (Hg), arsenic (As), cadmium

159   (Cd), copper (Cu), lead (Pb), zinc (Zn) and chromium (Cr). The heavy metals were analyzed at

160   Tsingcheng Environment Company in Suzhou, China. Mercury and arsenic were analysed using

161   Atomic Fluorescence Spectroscopy (AFS 2100, Haiguang Instruments Co. Ltd); zinc, lead and

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162   copper were analysed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP

163   710, Agilent Technologies); cadmium was analysed using graphite furnace-Atomic Absorption

164   Spectroscopy (240Z, Agilent technologies) and chromium was analysed using Flame-Atomic

165   Absorption Spectroscopy (ICP 710, Agilent technologies). The pH of soil, groundwater and

166   leachate samples was measured using a Suntex® TS 3000 pH/Temp portable probe in the

167   Department of Environmental Science at XJTLU. The samples were stored at +4°C prior to

168   analysis.

169

170   **Preparation and extraction of DNA from soil, leachate and groundwater samples**

171   ***Preparation of samples for DNA extraction***

172   One liter of groundwater was filtered on a 0.22 μm pore size polycarbonate membrane filter

173   (Millipore, USA) using a vacuum pump. Samples were filtered and the filters were placed in

174   sterile Petri dishes and stored at -20°C until they were used for DNA extraction. Due to the

175   nature of the sample (high turbidity), 50 ml of leachate was centrifuged at 5000 rpm for 5

176   minutes and both the pellet and the supernatant were collected. The supernatant was filtered in a

177   0.22 μm membrane filter (Millipore, USA) and both pellet and membrane filter were used for

178   DNA extraction. Soil samples were weighed (0.25 g) and used for DNA extraction.

179

180   ***DNA extraction***

181   The genomic DNA from all the samples was extracted using a commercial DNA extraction Kit

182   (MO BIO Power soil® DNA kit, USA) according to the manufacturer protocol. 50 μl of elution

183   buffer was used to elute the DNA samples and these were frozen at −20 °C until further

184   processing for bacterial community analysis. The DNA was quantified using Nanodrop (Thermo

185   Scientific, Waltham, MA, USA) and examined by agarose gel electrophoresis (1% w/v).

186

187 **Bacterial community analysis by next-generation sequencing**

188 The bacterial diversity and community composition of soil, leachate and groundwater samples

189 were studied by NGS using the Illumina MiseqPE250 platform. NGS was carried out at

190 Shanghai Majorbio Pharmaceutical Technology Limited, China. 16S rRNA genes (V4 region)

191 were amplified by PCR using 515F (5'barcoded GTGCCAGCMGCCGCGG3') and 806R

192 (5'GGACTACHVGGGTWTCTAAT3') primer sets. PCR reactions contained in 20 µl: 4 µl of

193 5× FastPfu Buffer, 2 µl of 2.5 mM dNTPs, 0.8µl of forward and revers primers (5 µM), 0.4 µl of

194 FastPfu polymerase, 10 ng of template DNA and DD water up to 20 µl. PCR conditions: a ABI

195 GenAmp 9700 thermocycler was used. Initial denaturation 3 minutes at 95°C was followed by

196 28 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C; final extension was carried out at 72°C

197 for 10 min. The purified amplicons were pooled and sequenced on an Illumina MiSeq platform.

198 Chimeric sequences were removed and the operational taxonomic units (OTUs) were clustered

199 with 97% similarity cutoff using UPARSE (Edgar, 2013). The phylogenetic affiliation of each

200 16S rRNA sequence was analysed by RDP classifier against the SILVA data base (Pruesse et al.,

201 2007). The sequences were submitted to National Centre for Biotechnological Information

202 (NCBI) Short Read Archive (SRA) database under the accession numbers SAMN06339740 to

203 SAMN06339748.

204 **Data analyses**

205 The diversity within each sample (alpha diversity) was calculated by Shannon (H') and Simpson

206 (D) diversity indices, abundance based coverage estimator (ACE) and Chao 1 richness estimator

207 using MOTHUR (http://www.mothur.org). The diversity between samples were compared (beta

208 diversity) by non-metric multidimensional scaling (NMDS) and cluster analysis by using QIIME.

209 The relationship between the environmental parameters (pH and heavy metals) and bacterial

210 community was assessed by redundancy analysis (RDA) or canonical correspondence analysis

211 (CCA) by using R language vegan package.

212

213 **RESULTS**

214 **pH and heavy metals**

215 Tables 2 and 3 show that the soil samples from the landfill and urban site were slightly acidic

216 while landfill groundwater (BHGW), raw leachate (RL) and fresh leachate (FL2) sample were

217 alkaline. To ensure accuracy in the results, two samples were collected for the landfill sites. The

218 two readings labelled as [1] and [2] were taken from the same pool at slightly different location

219 and interval. The Arsenic concentrations in RL and FL2 were 11.1-12.3 to 17.8-18.4 times

220 higher than the Chinese SEPA guideline concentration value for landfill of 100 μg/L – Class V

221 (Yang et al., 2008). Mercury concentrations were an order of magnitude higher in RL and FL2

222 samples and in the BHGW (landfill groundwater) than the Chinese SEPA guideline values (Yang

223 et al., 2008). Heavy metal concentrations of the soil samples from the landfill were within the

224 guideline range (Table 3). The As concentration of urban site soil 1 and 2 (USS1 and USSUR1)

225 was at the threshold tolerance value of the guideline range. The heavy metal concentration of Hg

226 in BHGW was found to be 340 times higher than USGW.

227

228 **Bacterial diversity**

229 Table 4 shows the number of reads obtained from the landfill samples varied from 13611 to

230 20464 and in urban site, it ranged from 14015 to 22643. The maximum reads obtained from LS3

231 and lowest from LS2 in the landfill environment. In urban site, USSUR1 had the lowest reads

232 compared to other urban samples. OTU values ranged from 139 to 1018 for the landfill samples

233 compared to 168 to 1167 in the urban site samples. FL2 had the lowest number and BHGW had

234 the highest number of OTUs. In the urban site, USGW had the lowest OTU read compared to

10

235   USS1 which had the highest OTU read of 1224. The bacterial richness and diversity (Shannon

236   *H'* index) of the urban soil samples (USS1 and USSUR1) were the highest of all the samples.

237   Species diversity estimates obtained for the abundance-based coverage estimators (ACE) and the

238   Chao1 index was higher in the urban site soil samples when compared to the landfill soil

239   samples, despite As concentrations an order of magnitude higher in the urban site soil samples

240   than in the landfill soil samples. Furthermore, the landfill groundwater (BHGW) had more

241   bacterial diversity than the urban groundwater (USGW) by every metric despite the Hg

242   concentration in BHGW being more than 340 times higher than USGW (Table 2).

243

244   **Bacterial community structure**

245   Figure 1 shows the bacterial community composition at phylum level in both landfill and urban

246   site samples. Among all the phyla, only *Proteobacteria* and *Bacteroidetes* were found to be

247   present in all the samples. The phylum *Proteobacteria* was dominant in all the samples from

248   landfill site with their abundance ranging from 31.4% to 94.9% in the landfill samples. Across

249   the urban site, their abundance ranged from 25.1% to 43.3% with USGW possessing a lower

250   abundance compared to the USS1 and USSUR1. *Bacteroidetes* abundance ranged from 1.42% to

251   25.64% among the landfill samples with FL2 having the lowest abundance and LS2 the highest.

252   In the urban site, samples they ranged from 5.69% to 7.86% in abundance with USGW having

253   the higher presence of *Bacteroidetes*. Members of phylum *Actinobacteria* were found in all the

254   samples except the leachate samples. The relative abundance of *Actinobacteria* ranged from

255   12.6 % to 28.6% and from 9.9% to 34.3% for the landfill site and urban site, respectively.

256   USGW was again found to be higher for *Actinobacteria*. *Chlamydiae* was only found in USGW

257   at 24.1%. *Firmicutes* and *Thermotogae* were only found in the RL sample with 6.4% and 8.2%

258   abundance, respectively.

11

259

260   Figure 2 shows that at the order level, *Pseudomonadales* and *Sphingobacteriales* were present in

261   all samples. *Pseudomonadales* were dominant in the landfill samples at RL (69.96 %), FL2

262   (92.97 %), LS2 (25.29 %) and LS3 (16.11 %). In LS2 and LS3, either *Xanthomonadales*

263   (11.04% and 14.09%) or *Flavobacteriales* (20.88% and 10.55%) were the second or third

264   dominant orders observed. However, in USGW samples, *Frankiales* (34.06%) and *Chlamydiales*

265   (24.09%) were dominant and their abundance was either <1% or absent in other samples from

266   both sites. *Sphingobacteriales* were found to be the second dominant order at 8.5% for BHGW

267   and 7.81% for USGW. *Flavobacteriales* were present in higher percentages in LS2 (20.88%) and

268   LS3 (10.55%) but their abundance were found to be less than <2% in other samples.

269

270   At genus level, the bacterial communities from the two sites were more diverse and unique.

271   Figure 3a shows that *Pseudomonas* was the most dominant genus observed in FL2 and RL with a

272   relative abundance of 92.9 and 69.9%, respectively. This genus was also dominant in LS2 and

273   LS3 but their relative abundance was less (16-25%) as compared to leachate samples.

274   *Sphingomonas* (6.5%) was found to be dominant in BHGW. In contrast the urban site samples

275   (Figure 3b) show *Sporichthyaceae_unclassified* (34%) to be dominant followed by

276   *Candidatus_Rhabdochlamydia* (24%) and *Sediminibacterium* (5.83%) in USGW sample.

277   *Thiobacillus*, Anaerolineaceae_uncultured and Nitrosomonadaceae_uncultured were dominant in

278   USS1 and USSUR1 samples.

279

280   Cluster analysis and NMDS was performed on the landfill and urban site samples (Fig. 4a, 4b,

281   5a, 5b). Fig 4a indicates a high level of similarity among the LS1, LS2 and LS3, BHGW, USS1

12

282   and USSUR1 samples. RL, FL2 and USGW are shown to be unique compared to the rest of the

283   samples. Cluster analysis shown in Fig 5a and 5b support the results observed for RL, FL2 and

284   USGW in Fig 4a. Fig 4b shows the least level of similarity observed among RL, FL2, LS1, LS2,

285   LS3, USS1 and USSUR1 samples.

286

287   To study the relationship between environmental parameters and bacterial community

288   composition, both multivariate redundancy analysis (RDA) and canonical correspondence

289   analysis (CCA) were performed and compared since the length of the first axis gradient were

290   between 3.0 and 4.0. Fig. 6 shows the RDA plot of the influence of As, Pb, Hg and pH on the

291   soil samples from the different locations. The USS1 and USSUR1 samples were mainly

292   correlated with the As and Pb content in the soil. The LS3 samples exhibited the reverse pattern

293   and were correlated with the pH and Hg concentration in the soil. Canonical correspondence

294   analysis (CCA) was performed to determine the possible linkages between the bacterial

295   communities and environmental parameters by examining the leachate and groundwater samples.

296   Canonical correspondence analysis (CCA) showed a negative correlation between As, pH, Hg

297   and the bacterial community of the samples, indicating that they had the biggest impacts on the

298   bacterial community of these samples (Fig. 7). Arsenic was the major factor that negatively

299   correlated with bacterial communities from FL2 and RL samples. CCA identified both pH and

300   heavy metals in the samples as a major environmental factor in affecting bacterial communities.

301

302   **DISCUSSION**

303   **Comparison of pH and heavy metals between sites**

304   The pH of leachate samples RL and FL2 were 7.78 and 8.12, respectively (Table 2). This range

305   of pH has been reported in other landfill leachate studies conducted in China (Song et al., 2015a,

306   Song et al., 2015b, Li et al., 2014). Since this landfill has an onsite incinerator, the alkaline pH

307   could be attributed to the disposal of ash in the landfill. The pH of BHGW and urban site

308   groundwater (USGW) was also alkaline at 8.2 and 7.75, respectively (Table 2). The pH values of

309   landfill and urban site soil were between 6.6 and 7.1 which indicate that the samples are slightly

310   more acidic in nature than the natural groundwater (Table 3). The pH values of the soil are not

311   surprising given the sites were previously used as agricultural lands (Zou et al., 2014) and the

312   regional presence of limestone formations (Jiangsu Provincial Bureau of Geological and Mineral

313   Exploration, 1984).

314

315   The heavy metal concentrations for As and Hg were above the guidelines range in both leachate

316   samples (Table 2). These hazardous ranges of As and Hg could be due to the solid waste

317   decomposition (mostly from waste water and MSW) and indicates the age of the landfill (more

318   than 10 years old) (Zhang et al., 2013b, Huang et al., 2013, Huang et al., 2003). The Hg level in

319   BHGW was 340 times higher when compared with USGW, indicating a possible percolation of

320   mercury from the landfill leachate to landfill groundwater. Very low concentrations in LS1, LS2

321   & LS3 indicating Hg-bearing leachate and groundwater are not interacting with the soils. On this

322   chemical evidence, it might be concluded that at this site, the near surface environment around

323   the landfill remains relatively uncontaminated and leachate was not percolating directly to the

324   groundwater below the water table (Roling et al., 2001) (Wang et al., 2011).

325

326   The concentration ofAs in RL & FL2 was very high in comparison to other landfills in Jiangsu

327   province which was between 0.03 to 0.113 mg/L. (Yang et al., 2008). Given that both sites were

328   agricultural land prior to rapid urbanisation in the late 20th century, agri-chemical residues

329   within the soil at USS1 & USSUR1 could explain the elevated arsenic levels (Zou et al., 2014).

330   The remaining heavy metals were analyzed from both sites and are typical of soils in urban

331   contexts subject to uncontrolled disposal of consumer and industrial chemicals, road runoff and

332   deposition of airborne pollutants (Mor et al., 2006). (Wijesekara et al., 2014). This context of

333   high background contamination presents the key challenge for both chemical and

334   microbiological investigation of leachate impacts.

335

336   **Analysis of bacterial community structure in landfill**

337   *Comparison OTU and community composition among samples*

338   Figs. 4 and 5 shows OTU based NMDS and cluster analysis plots which demonstrate the level of

339   similarity among the samples from both sites. When aggregated together, similarity between

340   landfill soil samples (LSO) and urban site soil samples (USO) was high when compared against

341   the similarity between groundwater samples from both sites (Fig. 4a). Landfill groundwater

342   (BHGW) consortia were also closely similar with the soil samples. The reason behind the low

343   similarity between the groundwater samples could be due to the poor diversity and richness of

344   the urban groundwater (USGW) (Table 3). It is also clear that the bacterial communities in the

345   raw and fresh leachate were markedly distinct from any of the soil or groundwater communities;

346   this is evident at both genus and order level (Figs. 2 and 3). On the basis of bacterial community

347   analysis, the dramatic differences between leachate and environmental samples offer the

348   potential for fingerprinting the presence of leachate contamination through identification of

349   leachate-specific DNA in environmental samples. Although such detailed mapping was not

350   possible in this study, we note that all three landfill soil samples contained *Pseudomonas*, in

351   common with the leachate samples, which was not present in soils or groundwater from non-

15

352   landfill locations. This may indicate surface or in-soil transport of leachates not evident from the

353   heavy metals analysis.

354

355   ***Dominant phyla and genera in both sites***

356   Leachate samples RL and FL2 had the least diverse phyla detection, in contrast to other landfill

357   leachate studies (Song et al., 2015a, Wang et al., 2017). The high concentration of As and Hg in

358   RL and FL2 could have inhibited the growth of other phyla, whereas *Pseudomonas* spp. have

359   recently been identified as key members of arsenotrophic consortia in contaminated groundwater

360   environments in Bangladesh (Sultana et al., 2017). The low diversity in leachate samples,

361   compared with samples taken from within the landfill (e.g.,(Wang et al., 2017) may also be due

362   to the concentration of landfill microbiota within surface-attached biofilms rather than in mobile

363   planktonic forms (Costerton and Wilson, 2004). Landfill and urban site soil and groundwater

364   samples shared most of the phyla except for *Chlamydiae*; which was only found in USGW. As

365   far as we are aware, this is the first study to observe significant presence of *Chlamydiae* in urban

366   groundwater microbial consortia; interestingly, given the high levels of lead and zinc in the

367   urban soils, the phyla has previously been isolated in groundwater samples affected by lead-mine

368   tailings (Zhang et al., 2008) .

369

370   *Proteobacteria* were most dominantly found in leachate samples from landfills (Song et al.,

371   2015a, Song et al., 2015b) and aquifer sediments (Wan et al., 2012). It has been reported that

372   members of *Proteobacteria* involved in the degradation of aromatic oils such as polycyclic

373   aromatic hydrocarbons (Vukanti et al., 2009). These bacteria have been found to lose dominance

374   in older leachate samples (Köchling et al., 2015) and they were detected at highly abundant

375   levels in aged refuse from Shanghai landfills (Xie et al., 2012). *Actinobacteria* was found in the

16

376   soil and groundwater samples from both sites but not in the leachate samples. This was not

377   expected as *Actinobacteria* has previously been found in leachate samples (Vukanti et al., 2009).

378   The high arsenic and mercury concentrations of leachate could perhaps have restricted their

379   growth. *Actinobacteria* are responsible for organic matter degradation contributing to carbon

380   turnover (Song et al., 2015b). Since landfills receive waste ranging from households to

381   industries, the amount of organic matter present in the soil could be a reason behind their

382   presence in landfill soil compared to urban site soil. *Bacteroidetes* was observed in abundance at

383   BHGW being twice as much as USGW. While LS2 & LS3 had three times the dominance as

384   USS1 & USSUR1 which could possibly indicate early stages of organic matter degradation

385   within the landfill samples as they commonly contain more soluble and easily degradable

386   material (Schmidtova and Baldwin, 2011). *Bacteroidetes* tend to become more dominant than

387   Proteobacteria as the waste in the landfill ages (Köchling et al., 2015). *Firmicutes* was only

388   found to be dominant in the leachate samples which suggest that they are able to withstand and

389   survive the toxic heavy metal concentrations found in the leachate. They have also been found in

390   other toxic chemical environments such as sewers and drainage (Rodrigues et al., 2014).

391   Environmental factors may have fundamental impacts on the structure and function diversity of

392   bacterial communities in landfill. Analysis from RDA showed that LS1, LS2, LS3 and BHGW

393   were not influenced by pH and heavy metals, where USS1 and USur1 were shown to be lightly

394   influenced by As and Pb. In this study, analysis from CCA has shown that higher concentrations

395   of As and Hg influence the bacterial community of leachate. pH was also shown to significantly

396   influence the bacterial community of leachate. The findings from this paper are consistent with

397   previous results that show that heavy metals influence the bacterial community of landfill (Yao

398   et al., 2017).

17

399

Potential of NGS for fingerprinting leachate interactions with soil and groundwater

401 In this study, Illumina MiSeq technique was used to investigate the bacterial community in

402 samples collected from landfill and urban sites. Bacterial richness and abundance were found to

403 vary significantly among the landfill and urban site samples. Further bacterial analysis revealed

404 lack of diversity in leachate samples when compared with soil and groundwater samples. OTU

405 data from NGS could be used in mapping the interactions between the samples at a site. In our

406 study, OTU data helped in understanding the similarity among the samples from both sites. More

407 studies are now being published using MiSeq methodology since it offers high-resolution

408 microbial community data which helps us in understanding the influence of external factors such

409 as heavy metals towards soil and groundwater microbial consortia. Further study needs to be

410 conducted to understand the long term effects of leachate interactions with soil and groundwater

411 in a landfill to observe the changes in microbial community.

412

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419

420 **Conflict of Interest**

421 The authors mentioned in this paper have no conflict of interest regarding the paper's content

422 and submission.

423

18

424   **Ethical approval**

425   This article does not contain any studies with human participants or animals performed by any of

426   the authors.

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532

533 **Table captions:**

534 **Table 1.** Collection and description for landfill samples.

535 **Table 2.** pH and heavy metal composition in landfill leachate (RL & FL2) and ground water
536 samples (BHGW) and urban site groundwater sample (USGW) respectively; [1] represents the
537 first reading and [2] represents the second reading. ND = Not detected

538 **Table 3.** pH and heavy metal composition of samples obtained from landfill (LS1, LS2 & LS3)
539 and urban site (USS1 & USSUR1) soil respectively; [1] represents the first reading and [2]
540 represents the second reading. ND = Not detected

541 **Table 4.** Bacterial diversity based on 16S rRNA gene retrieved by NGS from a landfill and an
542 urban site. ACE = Abundance based coverage estimators

543   **Figure captions:**

544   **Fig 1.** Phylum level bacterial community composition observed in the samples collected from

545   landfill site (a) and an urban site (b). FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3

546   = landfill soil; BHGW = landfill ground water; USGW = urban site ground water; USS1 and

547   USSUR1 = urban site soil samples.

548

549   **Fig 2.** Bacterial community composition and cluster analysis at order level in samples collected

550   from landfill site and an urban site. FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3

551   = landfill soil locations; BHGW = landfill ground water; USGW = urban site ground water;

552   USS1 and USSUR1 = urban site soil samples.

553

554   **Fig 3.** Genus level bacterial community composition observed in the samples collected from

555   landfill site (a) and an urban site (b). FL2=fresh leachate; RL= raw leachate; LS1, LS2 and LS3

556   = landfill soil; BHGW= landfill ground water; USGW= urban site ground water; USS1 and

557   USSUR1= urban site soil samples.

558

559   **Fig 4**. Cluster analysis based on order level bacterial abundance. (a) LEA, USO, LSO; (b) GW,

560   LEA, LSO. FL2=fresh leachate; RL= raw leachate; LS1, LS2 and LS3 = landfill soil; BHGW=

561   landfill ground water; USGW= urban site ground water; USS1 and USSUR1= urban site soil

562   samples; GW=combination of groundwater from both sites.

563

564   **Fig 5.** Non-metric multidimensional scaling (NMDS) analysis of sequences. (a) LF and US; (b)

565   LEA, LSO, USO. FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3 = landfill soil

566   locations; BHGW = landfill ground water; LF = combination of all landfill samples; USGW =

567   urban site ground water; USS1 and USSUR1 = urban site soil samples; US = combination of all

568   urban sites.

569

570   **Fig 6.** Redundancy analysis (RDA) of soil bacterial communities in landfill and urban site soil

571   samples. RDA1 explained 89.2 %, and RDA2 explained 7.65 % of the total variance. LS1, LS2

572   and LS3 = landfill soil locations USS1 and USSUR1 = urban site soil samples , respectively

573 **Fig 7.** Canonical correspondence analysis (CCA) of bacterial communities in RL, FL2, BHGW

574 and USGW. CCA1 explained 49.01 %, and CCA2 explained 45.97 % of the total variance. FL2

575 = fresh leachate; RL = raw leachate; BHGW = landfill ground water; USGW = urban site ground

576 water, respectively.

577

Table 1

Samples acronyms	Sample name	Reason for collection
RL	Raw Leachate	Due to its long term storage in the landfill that might influence variation in the microbial diversity.
FL2	Fresh Leachate	Provides an in depth understanding on the microbial diversity when compared with raw leachate
LS1	Landfill soil location 1	Closer to the landfill which might provide data on any leakage from leachate.
LS2	Landfill soil location 2	Closer to the agricultural land; data can be used to compare with landfill soil location 1.
LS3	Landfill soil location 3	Closer to the groundwater monitoring borehole; data can be used to compare the permeability of the landfill.
BHGW	Landfill groundwater monitoring borehole	Only functioning borehole used to check the contamination levels of the groundwater.
USGW	Urban site groundwater	Accessible borehole close to the soil locations.
USS1	Urban site soil sample 1	Location of the soil sampling was located closer to the urban site groundwater. It was collected from the surface.
USSur1	Urban site soil sample 2	Isolated soil location 500 m away from USS1 and USGW. It was collected 30 cm depth.

Table 2

	pH	Mercury (µg/L)	Arsenic (µg/L)	Cadmium (µg/L)	Copper (µg/L)	Lead (µg/L)	Zinc (µg/L)	Chromium (µg/L)
RL[1]	7.78	10.9	1.11×10^3	ND	ND	ND	ND	0.508
RL[2]	7.9	11.42	1.23×10^3	ND	ND	ND	ND	0.581
FL2[1]	8.12	7.37	1.78×10^3	ND	0.107	0.027	ND	0.586
FL2[2]	8.3	8.20	1.84×10^3	ND	ND	ND	ND	0.541
BHGW[1]	8.2	12.7	ND	ND	0.048	ND	0.186	0.015
BHGW[2]	8.25	5.59	ND	ND	ND	ND	0.062	0.011
USGW	7.75	0.037	ND	ND	ND	0.078	0.030	ND

Table 3

	pH	Mercury (mg/kg)	Arsenic (mg/kg)	Cadmium (mg/kg)	Copper (mg/kg)	Lead (mg/kg)	Zinc (mg/kg)	Chromium (mg/kg)
LS1[1]	6.71	0.175	0.766	ND	69.5	10.3	49.1	62.3
LS1[2]	6.87	0.152	0.854	ND	75.3	10.1	81.4	67.3
LS2[1]	6.63	0.150	0.937	ND	79.3	5.72	55.7	70.4
LS2[2]	6.42	0.184	0.726	ND	77.2	7.90	71.2	71.5
LS3[1]	7.1	0.146	0.998	ND	79.5	6.91	76.9	73.8
LS3[2]	6.95	0.143	0.907	ND	71.8	8.73	64.8	68.2
USS1	6.82	0.075	11.3	0.169	5.8	27.6	64.9	43.45
USSUR1	6.74	0.058	9.28	0.137	7.57	26.3	63.6	50.2

Table 4

Sample ID	Number of Reads	Number of OTUs	ACE index	Chao 1 richness estimate	Shannon diversity index (H')	Simpson diversity index (D)	Coverage
					0.97		
RL	15386	154	159	164	2.06	0.3716	0.999
FL2	15746	139	174	163	0.98	0.6584	0.997
LS1	15313	996	1109	1103	5.77	0.0089	0.989
LS2	13611	647	892	862	3.43	0.125	0.983
LS3	20464	875	1080	1112	4.49	0.0516	0.989
BHGW	20141	1018	1201	1259	5.6	0.0093	0.988
USGW	22643	168	189	190	2.65	0.177	0.999
USS1	16625	1224	1331	1332	6.1	0.0056	0.989
USSUR1	14015	1167	1322	1328	5.94	0.0079	0.983

Fig 1.

Fig 2.

Fig 3.

(a)

(b)

Fig 4.

(a)

Legend:
- ■ LEA
- ■ LSO
- ■ USO

LS1
LS2
LS3
USSUR1
USS1
FL2
RL

0.01

(b)

Legend:
- ■ GW
- ■ LEA
- ■ LSO

BHGW
LS1
LS2
LS3
USGW
FL2
RL

0.01

Fig 5.

RDA

Fig 6.

Fig 7.