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

Adharsh Rajasekar[1,*], *Raju Sekar*[2], *Eduardo Medina-Roldan*[3], *Jonathan Bridge*[4], *Charles K.S. Moy*[1], *Stephen Wilkinson*[5]

[1]Department of Civil Engineering, Xi'an Jiaotong-Liverpool University, Suzhou 215123, Jiangsu, China.

[2]Department of Biological Sciences, Xi'an Jiaotong- Liverpool University, Suzhou 215123, Jiangsu, China.

[3]Department of Environmental Science, Xi'an Jiaotong- Liverpool University, Suzhou 215123, Jiangsu, China.

[4]Department of the Natural and Built Environment, Sheffield Hallam University, Sheffield S1 1WB, United Kingdom.

[5]Department of Civil Engineering, University of Wolverhampton, Wolverhampton WV1 1LY, United Kingdom.

*Corresponding author:

Email: evolution.adharsh@gmail.com

Tel: +86 18261444592

Draft

ABSTRACT

The impact of contaminated leachate on groundwater from landfills is well known but specific effects on bacterial consortia are less well-studied. Bacterial communities in landfill and an urban site located in Suzhou, China were studied using Illumina high-throughput sequencing. A total number of 153944 good quality reads were produced and sequences assigned to 6388 operational taxonomic units (OTUs). Bacterial consortia consisted of up to 16 phyla including *Proteobacteria* (31.9 to 94.9% at landfill, 25.1 to 43.3% at urban sites), *Actinobacteria* (0 to 28.7% at landfill, 9.9 to 34.3% at urban sites), *Bacteroidetes* (1.4 to 25.6% at landfill, 5.6 to 7.8% at urban sites), *Chloroflexi* (0.4 to 26.5% at urban sites only) and unclassified bacteria. *Pseudomonas* was the dominant (67-93%) genus in landfill leachate. Arsenic concentrations in landfill raw leachate (RL) ($1.11x10^3$ µg/L) and fresh leachate (FL2) ($1.78x10^3$ µg/L), and mercury concentrations in RL (10.9 µg/L) and FL2 (7.37 µg/L) were higher than Chinese State Environmental Protection Administration (SEPA) standards for leachate in landfills. Shannon diversity index and Chao 1 richness estimate showed RL and FL2 lacked richness and diversity when compared with other samples. This is consistent with stresses imposed by elevated arsenic and mercury and has implications for ecological site remediation by bioremediation or natural attenuation.

Draft

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

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).

Draft

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).

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

Draft

sequencing was an effective technique to study the microbial community dynamics throughout while performing the dechlorination process.

In the present work, we investigated the potential for NGS to identify potential impacts on soil and groundwater bacterial communities due to heavy metal-rich landfill leachate in a conurbation in Suzhou, Jiangsu province, China. The objectives of this study were i) to characterize the composition of the bacterial communities of a selected landfill (leachate, soil and groundwater) and a non-landfill site in same conurbation, hereby referred to as "urban" (soil and groundwater); ii) to compare the unique and dominant bacterial taxa among the landfill and urban samples; and iii) to investigate and compare the bacterial diversity and heavy metal concentration of the soil and groundwater samples from a landfill and urban site. The study not only adds to the knowledge in respect of leachate impacts on subsurface consortia under urban areas, but assesses the potential of NGS for rapid monitoring of environmental impacts from landfills, and has implications for the design and implementation of biological remediation options such as natural attenuation or *in situ* microbially-induced carbonate precipitation.

MATERIALS AND METHODS

Sample locations

The selected landfill (located at 31°14'18.31"N 120°33'3.09"E) began operation in 1993 and receives about 1,500 tons/day of household wastes and industrial wastes from the Suzhou conurbation. A new landfill was constructed in 2006 on the surface of the older landfill (Rong et al., 2011). The urban site samples were collected from an area that was previously used for agriculture prior to reclamation for industrial development. The two sites are approximately 27

km from each other. The two sites are approximately 27 km from each other. Suzhou is situated on top of a 200 m deep sequence of Quaternary sediments. The depth of drift reduces to 0m directly to the West and South West of the City (Jiangsu Provincial Bureau of Geological and Mineral Exploration, 1984). At depth the bedrock is composed of Devonian quartzite and shales of the Wutong Formation, the sandstones shales and quartzites of the Maoshan Group and zones of Carboniferous limestone (the karstic features of which are known commercially as Taihu Stone, exposed at Dongting Mountain and in Linwu Cave) which forms the hills to the south and west of the city. This sequence is intruded by the Suzhou Granite which is exposed to the West of the city centre. The variable erosive bedrock surface, has been infilled by alluvial and lacustrine sediments of the lower flood plains of the Yangtze River. The subsurface materials vary from clays to silty sands (Shi et al., 2012). The structure of the quaternary strata below ground varies at the very large scale, due to the movement of the rivers and changes in the extent and location of the lakes with time. However, the extent of variation has been limited by the volume of materials being deposited within a geologically short period of time. Some of the silty/sandy subsurface zones are a result of reworking of loess by the Yangtze River. The silty sands have sufficient porosity to act as aquifer materials (Ma et al., 2011). Pumping works from these aquifers have caused the collapse of their porous structure resulting in approximately 1 m of settlement across the region increasing to 1.4m towards city centres, and reducing to 0m towards the locations of large permanent lakes (Shi et al., 2012). Details regarding Suzhou landfill construction and waste were briefly discussed by Rong et al. (2011).

The landfill sampling comprised of two leachates, soil from three different locations around the landfill (samples LS1, LS2 and LS3) and one groundwater from the landfill monitoring well

(samples BHGW) (Table 1). Leachate samples were either fresh (FL2, collected from an outlet pipe that runs beneath the landfill) or raw leachate (RL, sampled from a leachate pond). Soil samples were collected using a Spiral auger at 30cm depth. The first soil location was near the leachate pond; the second was close to agricultural land on the boundary of the site; and the third soil location was close to the groundwater monitoring borehole. The groundwater was collected at an approximate depth of 4 meters using a hand-held slow flow peristaltic pump. The samples were collected from well below the groundwater surface such that any residual floating matter would not be collected. Groundwater and leachate were collected in sterile high density polyethylene plastic bottles and soil samples were collected in a sterile plastic zip lock bags and transported to the laboratory under ambient temperature conditions, then stored in a cold room (4°C) prior to analysis.

Draft

To contrast the bacterial community from the landfill, soil (samples USS1 and USSur1) and groundwater (samples USGW) samples were collected from the urban site. Two samples from the two different locations in an urban area were selected for the soil sampling which were 200 meters apart. The groundwater borehole was chosen for the groundwater sampling. Ground water was collected at a depth of 4 meters. The first location of the soil sampling was located closer to the urban site groundwater and the second location of the soil sample was an isolated location.

Physicochemical analysis of soil and water samples

The following heavy metals were analysed for all samples: mercury (Hg), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn) and chromium (Cr). The heavy metals were analyzed at Tsingcheng Environment Company in Suzhou, China. Mercury and arsenic were analysed using Atomic Fluorescence Spectroscopy (AFS 2100, Haiguang Instruments Co. Ltd); zinc, lead and

copper were analysed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP 710, Agilent Technologies); cadmium was analysed using graphite furnace-Atomic Absorption Spectroscopy (240Z, Agilent technologies) and chromium was analysed using Flame-Atomic Absorption Spectroscopy (ICP 710, Agilent technologies). The pH of soil, groundwater and leachate samples was measured using a Suntex® TS 3000 pH/Temp portable probe in the Department of Environmental Science at XJTLU. The samples were stored at +4°C prior to analysis.

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

Preparation of samples for DNA extraction

One liter of groundwater was filtered on a 0.22 µm pore size polycarbonate membrane filter (Millipore, USA) using a vacuum pump. Samples were filtered and the filters were placed in sterile Petri dishes and stored at -20°C until they were used for DNA extraction. Due to the nature of the sample (high turbidity), 50 ml of leachate was centrifuged at 5000 rpm for 5 minutes and both the pellet and the supernatant were collected. The supernatant was filtered in a 0.22 µm membrane filter (Millipore, USA) and both pellet and membrane filter were used for DNA extraction. Soil samples were weighed (0.25 g) and used for DNA extraction.

DNA extraction

The genomic DNA from all the samples was extracted using a commercial DNA extraction Kit (MO BIO Power soil® DNA kit, USA) according to the manufacturer protocol. 50 µl of elution buffer was used to elute the DNA samples and these were frozen at −20 °C until further processing for bacterial community analysis. The DNA was quantified using Nanodrop (Thermo Scientific, Waltham, MA, USA) and examined by agarose gel electrophoresis (1% w/v).

Bacterial community analysis by next-generation sequencing

The bacterial diversity and community composition of soil, leachate and groundwater samples were studied by NGS using the Illumina MiseqPE250 platform. NGS was carried out at Shanghai Majorbio Pharmaceutical Technology Limited, China. 16S rRNA genes (V4 region) were amplified by PCR using 515F (5'barcoded GTGCCAGCMGCCGCGG3') and 806R (5'GGACTACHVGGGTWTCTAAT3') primer sets. PCR reactions contained in 20 µl: 4 µl of 5× FastPfu Buffer, 2 µl of 2.5 mM dNTPs, 0.8µl of forward and revers primers (5 µM), 0.4 µl of FastPfu polymerase, 10 ng of template DNA and DD water up to 20 µl. PCR conditions: a ABI GenAmp 9700 thermocycler was used. Initial denaturation 3 minutes at 95°C was followed by 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 for 10 min. The purified amplicons were pooled and sequenced on an Illumina MiSeq platform. Chimeric sequences were removed and the operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (Edgar, 2013). The phylogenetic affiliation of each 16S rRNA sequence was analysed by RDP classifier against the SILVA data base (Pruesse et al., 2007). The sequences were submitted to National Centre for Biotechnological Information (NCBI) Short Read Archive (SRA) database under the accession numbers SAMN06339740 to SAMN06339748.

Data analyses

The diversity within each sample (alpha diversity) was calculated by Shannon (H') and Simpson (D) diversity indices, abundance based coverage estimator (ACE) and Chao 1 richness estimator using MOTHUR (http://www.mothur.org). The diversity between samples were compared (beta diversity) by non-metric multidimensional scaling (NMDS) and cluster analysis by using QIIME. The relationship between the environmental parameters (pH and heavy metals) and bacterial

community was assessed by redundancy analysis (RDA) or canonical correspondence analysis (CCA) by using R language vegan package.

RESULTS

pH and heavy metals

Tables 2 and 3 show that the soil samples from the landfill and urban site were slightly acidic while landfill groundwater (BHGW), raw leachate (RL) and fresh leachate (FL2) sample were alkaline. To ensure accuracy in the results, two samples were collected for the landfill sites. The two readings labelled as $^{(1)}$ and $^{(2)}$ were taken from the same pool at slightly different location and interval. The Arsenic concentrations in RL and FL2 were 11.1-12.3 to 17.8-18.4 times higher than the Chinese SEPA guideline concentration value for landfill of 100 µg/L – Class V (Yang et al., 2008). Mercury concentrations were an order of magnitude higher in RL and FL2 samples and in the BHGW (landfill groundwater) than the Chinese SEPA guideline values (Yang et al., 2008). Heavy metal concentrations of the soil samples from the landfill were within the guideline range (Table 3). The As concentration of urban site soil 1 and 2 (USS1 and USSUR1) was at the threshold tolerance value of the guideline range. The heavy metal concentration of Hg in BHGW was found to be 340 times higher than USGW.

Bacterial diversity

Table 4 shows the number of reads obtained from the landfill samples varied from 13611 to 20464 and in urban site, it ranged from 14015 to 22643. The maximum reads obtained from LS3 and lowest from LS2 in the landfill environment. In urban site, USSUR1 had the lowest reads compared to other urban samples. OTU values ranged from 139 to 1018 for the landfill samples compared to 168 to 1167 in the urban site samples. FL2 had the lowest number and BHGW had the highest number of OTUs. In the urban site, USGW had the lowest OTU read compared to

USS1 which had the highest OTU read of 1224. The bacterial richness and diversity (Shannon *H'* index) of the urban soil samples (USS1 and USSUR1) were the highest of all the samples. Species diversity estimates obtained for the abundance-based coverage estimators (ACE) and the Chao1 index was higher in the urban site soil samples when compared to the landfill soil samples, despite As concentrations an order of magnitude higher in the urban site soil samples than in the landfill soil samples. Furthermore, the landfill groundwater (BHGW) had more bacterial diversity than the urban groundwater (USGW) by every metric despite the Hg concentration in BHGW being more than 340 times higher than USGW (Table 2).

Bacterial community structure

Figure 1 shows the bacterial community composition at phylum level in both landfill and urban site samples. Among all the phyla, only *Proteobacteria* and *Bacteroidetes* were found to be present in all the samples. The phylum *Proteobacteria* was dominant in all the samples from landfill site with their abundance ranging from 31.4% to 94.9% in the landfill samples. Across the urban site, their abundance ranged from 25.1% to 43.3% with USGW possessing a lower abundance compared to the USS1 and USSUR1. *Bacteroidetes* abundance ranged from 1.42% to 25.64% among the landfill samples with FL2 having the lowest abundance and LS2 the highest. In the urban site, samples they ranged from 5.69% to 7.86% in abundance with USGW having the higher presence of *Bacteroidetes*. Members of phylum *Actinobacteria* were found in all the samples except the leachate samples. The relative abundance of *Actinobacteria* ranged from 12.6 % to 28.6% and from 9.9% to 34.3% for the landfill site and urban site, respectively. USGW was again found to be higher for *Actinobacteria*. *Chlamydiae* was only found in USGW at 24.1%. *Firmicutes* and *Thermotogae* were only found in the RL sample with 6.4% and 8.2% abundance, respectively.

Draft

Figure 2 shows that at the order level, *Pseudomonadales* and *Sphingobacteriales* were present in all samples. *Pseudomonadales* were dominant in the landfill samples at RL (69.96 %), FL2 (92.97 %), LS2 (25.29 %) and LS3 (16.11 %). In LS2 and LS3, either *Xanthomonadales* (11.04% and 14.09%) or *Flavobacteriales* (20.88% and 10.55%) were the second or third dominant orders observed. However, in USGW samples, *Frankiales* (34.06%) and *Chlamydiales* (24.09%) were dominant and their abundance was either <1% or absent in other samples from both sites. *Sphingobacteriales* were found to be the second dominant order at 8.5% for BHGW and 7.81% for USGW. *Flavobacteriales* were present in higher percentages in LS2 (20.88%) and LS3 (10.55%) but their abundance were found to be less than <2% in other samples.

At genus level, the bacterial communities from the two sites were more diverse and unique. Figure 3a shows that *Pseudomonas* was the most dominant genus observed in FL2 and RL with a relative abundance of 92.9 and 69.9%, respectively. This genus was also dominant in LS2 and LS3 but their relative abundance was less (16-25%) as compared to leachate samples. *Sphingomonas* (6.5%) was found to be dominant in BHGW. In contrast the urban site samples (Figure 3b) show *Sporichthyaceae_unclassified* (34%) to be dominant followed by *Candidatus_Rhabdochlamydia* (24%) and *Sediminibacterium* (5.83%) in USGW sample. *Thiobacillus*, Anaerolineaceae_uncultured and Nitrosomonadaceae_uncultured were dominant in USS1 and USSUR1 samples.

Cluster analysis and NMDS was performed on the landfill and urban site samples (Fig. 4a, 4b, 5a, 5b). Fig 4a indicates a high level of similarity among the LS1, LS2 and LS3, BHGW, USS1

Draft

and USSUR1 samples. RL, FL2 and USGW are shown to be unique compared to the rest of the samples. Cluster analysis shown in Fig 5a and 5b support the results observed for RL, FL2 and USGW in Fig 4a. Fig 4b shows the least level of similarity observed among RL, FL2, LS1, LS2, LS3, USS1 and USSUR1 samples.

To study the relationship between environmental parameters and bacterial community composition, both multivariate redundancy analysis (RDA) and canonical correspondence analysis (CCA) were performed and compared since the length of the first axis gradient were between 3.0 and 4.0. Fig. 6 shows the RDA plot of the influence of As, Pb, Hg and pH on the soil samples from the different locations. The USS1 and USSUR1 samples were mainly correlated with the As and Pb content in the soil. The LS3 samples exhibited the reverse pattern and were correlated with the pH and Hg concentration in the soil. Canonical correspondence analysis (CCA) was performed to determine the possible linkages between the bacterial communities and environmental parameters by examining the leachate and groundwater samples. Canonical correspondence analysis (CCA) showed a negative correlation between As, pH, Hg and the bacterial community of the samples, indicating that they had the biggest impacts on the bacterial community of these samples (Fig. 7). Arsenic was the major factor that negatively correlated with bacterial communities from FL2 and RL samples. CCA identified both pH and heavy metals in the samples as a major environmental factor in affecting bacterial communities.

DISCUSSION

Comparison of pH and heavy metals between sites

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

https://mc06.manuscriptcentral.com/cjm-pubs

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-

Draft

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

Dominant phyla and genera in both sites

Leachate samples RL and FL2 had the least diverse phyla detection, in contrast to other landfill leachate studies (Song et al., 2015a, Wang et al., 2017). The high concentration of As and Hg in RL and FL2 could have inhibited the growth of other phyla, whereas *Pseudomonas* spp. have recently been identified as key members of arsenotrophic consortia in contaminated groundwater environments in Bangladesh (Sultana et al., 2017). The low diversity in leachate samples, compared with samples taken from within the landfill (e.g.,(Wang et al., 2017) may also be due to the concentration of landfill microbiota within surface-attached biofilms rather than in mobile planktonic forms (Costerton and Wilson, 2004). Landfill and urban site soil and groundwater samples shared most of the phyla except for *Chlamydiae*; which was only found in USGW. As far as we are aware, this is the first study to observe significant presence of *Chlamydiae* in urban groundwater microbial consortia; interestingly, given the high levels of lead and zinc in the urban soils, the phyla has previously been isolated in groundwater samples affected by lead-mine tailings (Zhang et al., 2008) .

Proteobacteria were most dominantly found in leachate samples from landfills (Song et al., 2015a, Song et al., 2015b) and aquifer sediments (Wan et al., 2012). It has been reported that members of *Proteobacteria* involved in the degradation of aromatic oils such as polycyclic aromatic hydrocarbons (Vukanti et al., 2009). These bacteria have been found to lose dominance in older leachate samples (Köchling et al., 2015) and they were detected at highly abundant levels in aged refuse from Shanghai landfills (Xie et al., 2012). *Actinobacteria* was found in the

soil and groundwater samples from both sites but not in the leachate samples. This was not expected as *Actinobacteria* has previously been found in leachate samples (Vukanti et al., 2009). The high arsenic and mercury concentrations of leachate could perhaps have restricted their growth. *Actinobacteria* are responsible for organic matter degradation contributing to carbon turnover (Song et al., 2015b). Since landfills receive waste ranging from households to industries, the amount of organic matter present in the soil could be a reason behind their presence in landfill soil compared to urban site soil. *Bacteroidetes* was observed in abundance at BHGW being twice as much as USGW. While LS2 & LS3 had three times the dominance as USS1 & USSUR1 which could possibly indicate early stages of organic matter degradation within the landfill samples as they commonly contain more soluble and easily degradable material (Schmidtova and Baldwin, 2011). *Bacteroidetes* tend to become more dominant than Proteobacteria as the waste in the landfill ages (Köchling et al., 2015). *Firmicutes* was only found to be dominant in the leachate samples which suggest that they are able to withstand and survive the toxic heavy metal concentrations found in the leachate. They have also been found in other toxic chemical environments such as sewers and drainage (Rodrigues et al., 2014).

Environmental factors may have fundamental impacts on the structure and function diversity of bacterial communities in landfill. Analysis from RDA showed that LS1, LS2, LS3 and BHGW were not influenced by pH and heavy metals, where USS1 and USur1 were shown to be lightly influenced by As and Pb. In this study, analysis from CCA has shown that higher concentrations of As and Hg influence the bacterial community of leachate. pH was also shown to significantly influence the bacterial community of leachate. The findings from this paper are consistent with previous results that show that heavy metals influence the bacterial community of landfill (Yao et al., 2017).

Draft

399

400 **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

413 **Acknowledgements**

414 This work was supported by grant no. PGRS-12-02-06 and RDF-13-01-06 awarded by Xi'an
415 Jiaotong-Liverpool University. Thanks to Departments of Biological Sciences and
416 Environmental Science at XJTLU for providing facilities for molecular microbiological and
417 chemical analyses, respectively. The authors express their gratitude to the editor and two
418 anonymous reviewers, whose comments contributed to the quality of the final manuscript.

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

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Draft

References

ADETUTU, E. M., GUNDRY, T. D., PATIL, S. S., GOLNESHIN, A., ADIGUN, J., BHASKARLA, V., ALEER, S., SHAHSAVARI, E., ROSS, E. & BALL, A. S. 2015. Exploiting the intrinsic microbial degradative potential for field-based in situ dechlorination of trichloroethene contaminated groundwater. *J Haz Mat,* 300**,** 48-57.

BROWN, B. L., WATSON, M., MINOT, S. S., RIVERA, M. C. & FRANKLIN, R. B. 2017. MinION™ nanopore sequencing of environmental metagenomes: a synthetic approach. *GigaScience,* 6**,** 1-10.

COSTERTON, W. J. & WILSON, M. 2004. Introducing Biofilm. *Biofilms,* 1**,** 1-4.

EDGAR, R. C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods,* 10**,** 996-1001.

EL-SALAM, M. M. A. & ABU-ZUID, G. I. 2015. Impact of landfill leachate on the groundwater quality: A case study in Egypt. *J Adv res,* 6**,** 579-586.

HUANG, K., GUO, J., LIN, K. F., ZHOU, X. Y., WANG, J. X., ZHOU, P., XU, F. & ZHANG, M. L. 2013. Distribution and temporal trend of polybrominated diphenyl ethers in one Shanghai municipal landfill, China. *Environ Sci Pollut Res Int,* 20**,** 5299-5308.

HUANG, L.-N., CHEN, Y.-Q., ZHOU, H., LUO, S., LAN, C.-Y. & QU, L.-H. 2003. Characterization of methanogenic Archaea in the leachate of a closed municipal solid waste land¢ll. *FEMS microbiol ecol,* 46**,** 171-177.

JIANGSU PROVINCIAL BUREAU OF GEOLOGICAL AND MINERAL EXPLORATION. 1984. *Geological map of Jiangsu Province and Shanghai Municipality, PRC*.

KÖCHLING, T., SANZ, J. L., GAVAZZA, S. & FLORENCIO, L. 2015. Analysis of microbial community structure and composition in leachates from a young landfill by 454 pyrosequencing. *Appl Microbiol and Biotechnol,* 99**,** 5657-5668.

LI, Y., LI, J. & DENG, C. 2014. Occurrence, characteristics and leakage of polybrominated diphenyl ethers in leachate from municipal solid waste landfills in China. *Environ Pollut,* 184**,** 94-100.

MA, F. S., YANG, Y. S., YUAN, R. M., YAO, B. K. & GUO, J. 2011. Effect of regional land subsidence on engineering structures: a case study of the 6 km long Su-tong Yantze River Bridge. *Bulletin of Engineering Geology and the Environment,* 70**,** 449-459.

MECKENSTOCK, R. U., ELSNER, M., GRIEBLER, C., LUEDERS, T., STUMPP, C., AAMAND, J., AGATHOS, S. N., ALBRECHTSEN, H.-J., BASTIAENS, L., BJERG, P. L., BOON, N., DEJONGHE, W., HUANG, W. E., SCHMIDT, S. I., SMOLDERS, E., SØRENSEN, S. R., SPRINGAEL, D. & BREUKELEN, B. M. V. 2015. Biodegradation: Updating the Concepts of Control for Microbial Cleanup in Contaminated Aquifers. *Environ sci technol,* 49**,** 7073-7081.

MOR, S., RAVINDRA, K., DAHIYA, R. P. & CHANDRA, A. 2006. Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site. *Environ Monit Assess,* 118**,** 435-456.

MULLER, A. K., WESTERGAARD, K., CHRISTENSEN, S. & SORENSON, S. J. 2001. The effect of long-term mercury pollution on the soil microbial community. *FEMS Microbiol Ecol,* 36**,** 11-19.

PRUESSE, E., QUAST, C., KNITTEL, K., FUCHS, B. M., LUDWIG, W., PEPLIES, J. & GLOCKNER, F. O. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research,* 35**,** 7188-7196.

RODRIGUES, V. D., TORRES, T. T. & OTTOBONI, L. M. 2014. Bacterial diversity assessment in soil of an active Brazilian copper mine using high-throughput sequencing of 16S rDNA amplicons. *Anton Van Leeuw,* 106**,** 879-90.

ROLING, W. F. M., VAN BREUKELEN, B. M., BRASTER, M., LIN, B. & VAN VERSEVELD, H. W. 2001. Relationships between Microbial Community Structure and Hydrochemistry in a Landfill Leachate-Polluted Aquifer. *Appl Environ Microbiol,* 67**,** 4619-4629.

RONG, F., ZHAOGUI, G. & TUGEN, F. 2011. Analysis of Stability and Control in Landfill Sites Expansion. *Proc Eng,* 24**,** 667-671.

SANDAA, R.-A., TORSVIK, V., ENGER, O., DAAE, F. L., CASTBERG, T. & HAHN, D. 1999. Analysis of bacterial communities in heavy metal contaminated soils at diferrent levels of resolution. *FEMS Microbiol Ecol,* 30**,** 237-251.

SCHMIDTOVA, J. & BALDWIN, S. A. 2011. Correlation of bacterial communities supported by different organic materials with sulfate reduction in metal-rich landfill leachate. *Water res,* 45**,** 1115-1128.

SHI, X., FANG, R., WU, J., XU, H., SUN, Y. & YU, J. 2012. Sustainable development and utilization of groundwater resources considering land subsidence in Suzhou, China. *Eng geol,* 124**,** 77-89.

SONG, L., WANG, Y., TANG, W. & LE, Y. 2015a. Bacterial community diversity in municipal waste landfill sites. *Appl Microbiol Biotechnol,* 99**,** 7745-7756.

SONG, L., WANG, Y., ZHAO, H. & LONG, D. T. 2015b. Composition of bacterial and archaeal communities during landfill refuse decomposition processes. *Microbiol res,* 181**,** 105-111.

STALEY, B. F., SAIKALY, P. E., DE LOS REYES, F. L., 3RD & BARLAZ, M. A. 2011. Critical evaluation of solid waste sample processing for DNA-based microbial community analysis. *Biodegradation,* 22**,** 189-204.

SULTANA, M., MOU, T. J., SANYAL, S. K., DIBA, F., MAHMUD, Z. H., PARVEZ, A. K. & HOSSAIN, M. A. 2017. Investigation of Arsenotrophic Microbiome in Arsenic-Affected Bangladesh Groundwater. *Ground Water,* 55**,** 736-746.

VISHNIVETSKAYA, T. A., MOSHER, J. J., PALUMBO, A. V., YANG, Z. K., PODAR, M., BROWN, S. D., BROOKS, S. C., GU, B., SOUTHWORTH, G. R., DRAKE, M. M., BRANDT, C. C. & ELIAS, D. A. 2011. Mercury and Other Heavy Metals Influence Bacterial Community Structure in Contaminated Tennessee Streams. *Appl Environ Microbiol,* 77**,** 302-311.

VUKANTI, R., CRISSMAN, M., LEFF, L. G. & LEFF, A. A. 2009. Bacterial communities of tyre monofill sites: growth on tyre shreds and leachate. *J Appl Microbiol,* 106**,** 1957-1966.

WAN, R., ZHANG, S. & XIE, S. 2012. Microbial community changes in aquifer sediment microcosm for anaerobic anthracene biodegradation under methanogenic condition. *J Environ sci,* 24**,** 1498-1503.

WANG, J., LIN, Z., LIN, K., WANG, C., ZHANG, W., CUI, C., LIN, J., DONG, Q. & HUANG, C. 2011. Polybrominated diphenyl ethers in water, sediment, soil, and biological samples from different industrial areas in Zhejiang, China. *J Haz Mat,* 197**,** 211-219.

WANG, X., CAO, A., ZHAO, G., ZHOU, C. & XU, R. 2017. Microbial community structure and diversity in a municipal solid waste landfil. *Waste Manage,* 66**,** 79-87.

WIJESEKARA, S. S. R. M. D. H. R., SIRIWARDANA, A. R., KAWAMOTO, K., SILVA, N. D., MAYAKADUWA, S. S., BASNAYAKE, B. F. A. & VITHANAGE, M. 2014. Fate and transport of pollutants through a municipal solid waste landfill leachate in Sri Lanka. *Environ Earth Sci,* 72**,** 1707-1719.

XIE, B., XIONG, S., LIANG, S., HU, C., ZHANG, X. & LU, J. 2012. Performance and bacterial compositions of aged refuse reactors treating mature landfill leachate. *Biores Technol,* 103**,** 71-77.

YANG, K., ZHOU, X.-N., YAN, W.-A., HANG, D.-R. & STEINMANN, P. 2008. Landfills in Jiangsu province, China, and potential threats for public health: Leachate appraisal and spatial analysis using geographic information system and remote sensing. *Waste Manage,* 28**,** 2750-2757.

YAO, X.-F., ZHANG, J.-M., TIAN, L. & GUO, J.-H. 2017. The effect of heavy metal contamination on the bacterial community structure at Jiaozhou Bay, China. *Braz J Microbiol,* 48**,** 71-78.

ZHANG, H.-B., SHI, W., YANG, M.-X., SHA, T. & ZHAO, Z.-W. 2008. Bacterial Diversity at Different Depths in Lead-Zinc Mine Tailings as Revealed by 16S rRNA Gene Libraries. *J Microbiol,* 45**,** 479-484.

Draft

ZHANG, Q. Q., TIAN, B. H., ZHANG, X., GHULAM, A., FANG, C. R. & HE, R. 2013a. Investigation on characteristics of leachate and concentrated leachate in three landfill leachate treatment plants. *Waste Manage,* 33**,** 2277-2286.

ZHANG, Q. Q., TIAN, B. H., ZHANG, X., GHULAM, A., FANG, C. R. & HE, R. 2013b. Investigation on characteristics of leachate and concentrated leachate in three landfill leachate treatment plants. *Waste Manag,* 33**,** 2277-86.

ZOU, J., DAI, W., GONG, S. & MA, Z. 2014. Analysis of Spatial Variations and Sources of Heavy Metals in Farmland Soils of Beijing Suburbs. *PLOS one,* 10**,** 13.

Draft

Table captions:

Table 1. Collection and description for landfill samples.

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

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

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

Draft

Figure captions:

Fig 1. Phylum level bacterial community composition observed in the samples collected from landfill site (a) and an urban site (b). FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3 = landfill soil; BHGW = landfill ground water; USGW = urban site ground water; USS1 and USSUR1 = urban site soil samples.

Fig 2. Bacterial community composition and cluster analysis at order level in samples collected from landfill site and an urban site. FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3 = landfill soil locations; BHGW = landfill ground water; USGW = urban site ground water; USS1 and USSUR1 = urban site soil samples.

Fig 3. Genus level bacterial community composition observed in the samples collected from landfill site (a) and an urban site (b). FL2=fresh leachate; RL= raw leachate; LS1, LS2 and LS3 = landfill soil; BHGW= landfill ground water; USGW= urban site ground water; USS1 and USSUR1= urban site soil samples.

Fig 4. Cluster analysis based on order level bacterial abundance. (a) LEA, USO, LSO; (b) GW, LEA, LSO. FL2=fresh leachate; RL= raw leachate; LS1, LS2 and LS3 = landfill soil; BHGW= landfill ground water; USGW= urban site ground water; USS1 and USSUR1= urban site soil samples; GW=combination of groundwater from both sites.

Fig 5. Non-metric multidimensional scaling (NMDS) analysis of sequences. (a) LF and US; (b) LEA, LSO, USO. FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3 = landfill soil locations; BHGW = landfill ground water; LF = combination of all landfill samples; USGW = urban site ground water; USS1 and USSUR1 = urban site soil samples; US = combination of all urban sites.

Fig 6. Redundancy analysis (RDA) of soil bacterial communities in landfill and urban site soil samples. RDA1 explained 89.2 %, and RDA2 explained 7.65 % of the total variance. LS1, LS2 and LS3 = landfill soil locations USS1 and USSUR1 = urban site soil samples , respectively

Draft

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

Draft

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	$1.11x10^3$	ND	ND	ND	ND	0.508
RL[2]	7.9	11.42	$1.23x10^3$	ND	ND	ND	ND	0.581
FL2[1]	8.12	7.37	$1.78x10^3$	ND	0.107	0.027	ND	0.586
FL2[2]	8.3	8.20	$1.84x10^3$	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

Draft

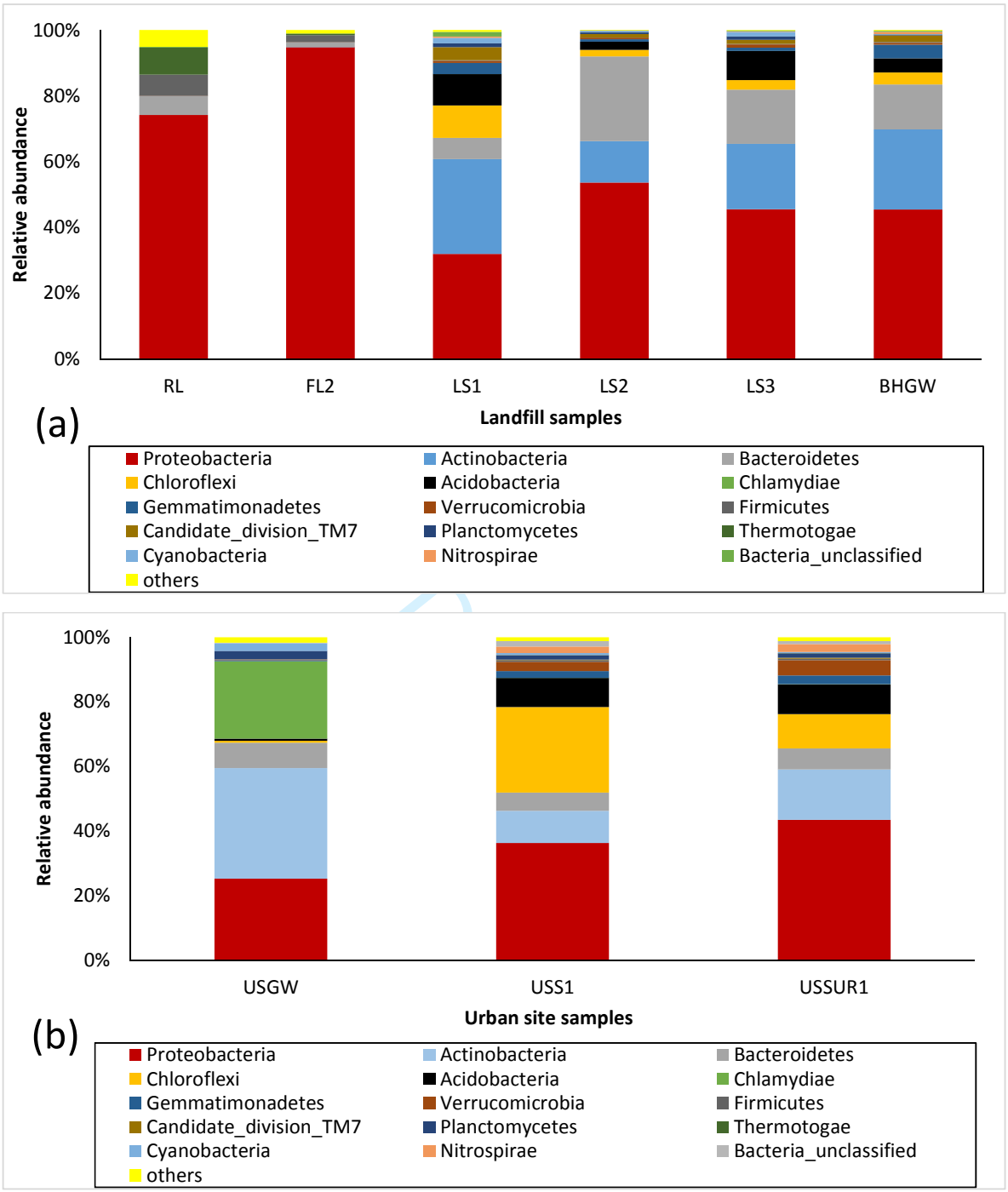

100%
80%
60%
40%
20%
0%
Relative abundance
RL
FL2
LS1
LS2
LS3
BHGW
Landfill samples
(a)
Proteobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Acidobacteria
Chlamydiae
Gemmatimonadetes
Verrucomicrobia
Firmicutes
Candidate_division_TM7
Planctomycetes
Thermotogae
Cyanobacteria
Nitrospirae
Bacteria_unclassified
others
100%
80%
60%
40%
20%
0%
Relative abundance
USGW
USS1
USSUR1
Urban site samples
(b)
Proteobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Acidobacteria
Chlamydiae
Gemmatimonadetes
Verrucomicrobia
Firmicutes
Candidate_division_TM7
Planctomycetes
Thermotogae
Cyanobacteria
Nitrospirae
Bacteria_unclassified
others

Fig 1.

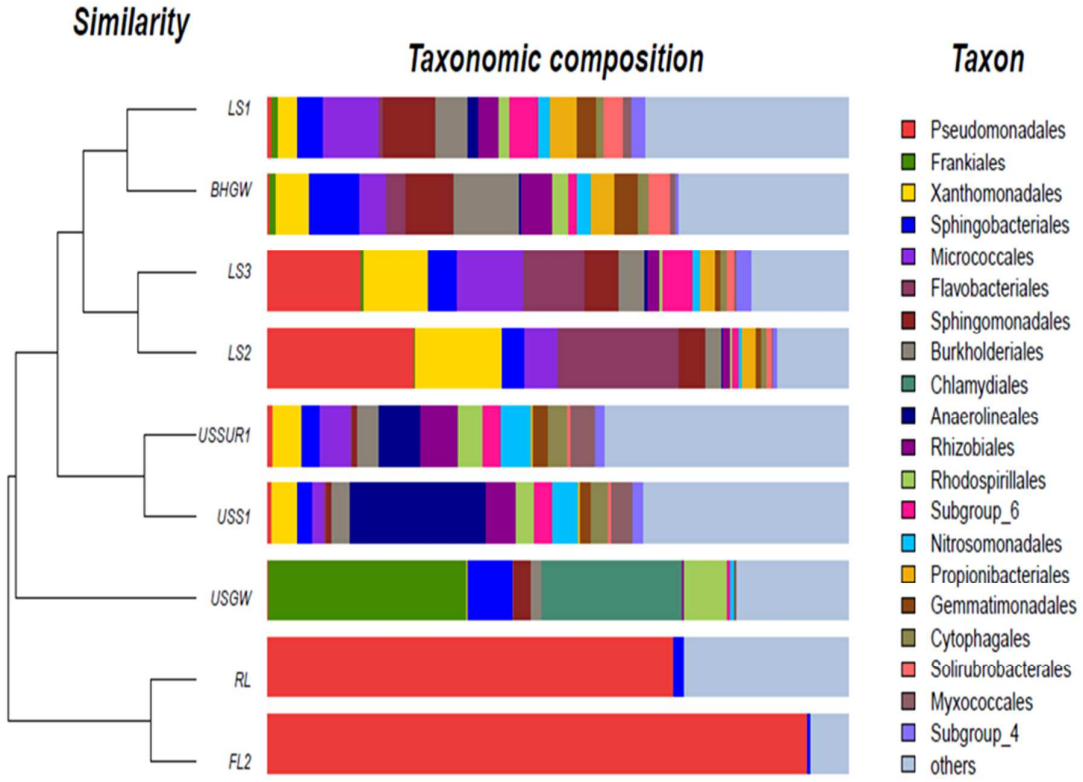

Similarity
Taxonomic composition
Taxon
LS1
BHGW
LS3
LS2
USSUR1
USS1
USGW
RL
FL2
Pseudomonadales
Frankiales
Xanthomonadales
Sphingobacteriales
Micrococcales
Flavobacteriales
Sphingomonadales
Burkholderiales
Chlamydiales
Anaerolineales
Rhizobiales
Rhodospirillales
Subgroup_6
Nitrosomonadales
Propionibacteriales
Gemmatimonadales
Cytophagales
Solirubrobacterales
Myxococcales
Subgroup_4
others

Fig 2.

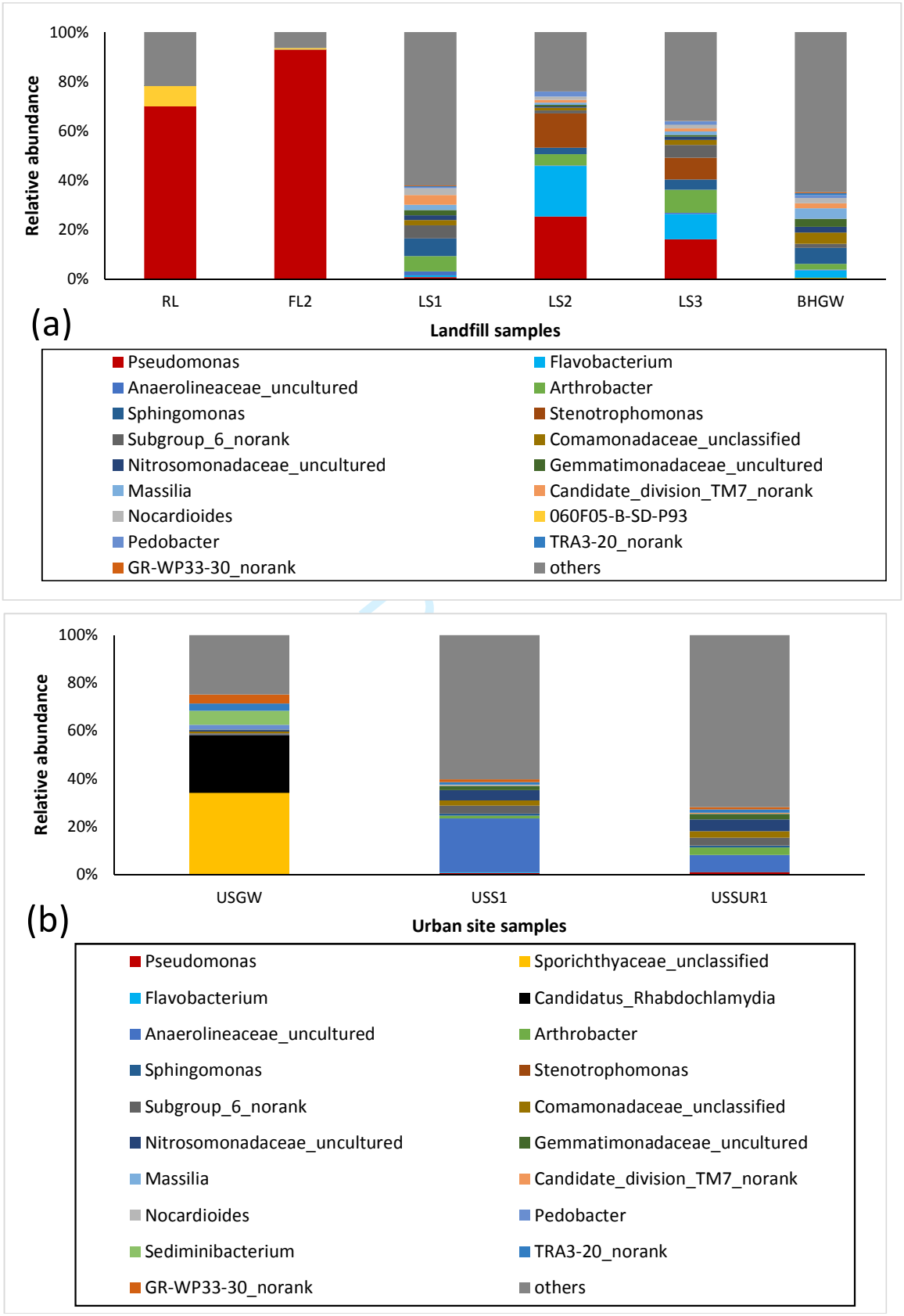
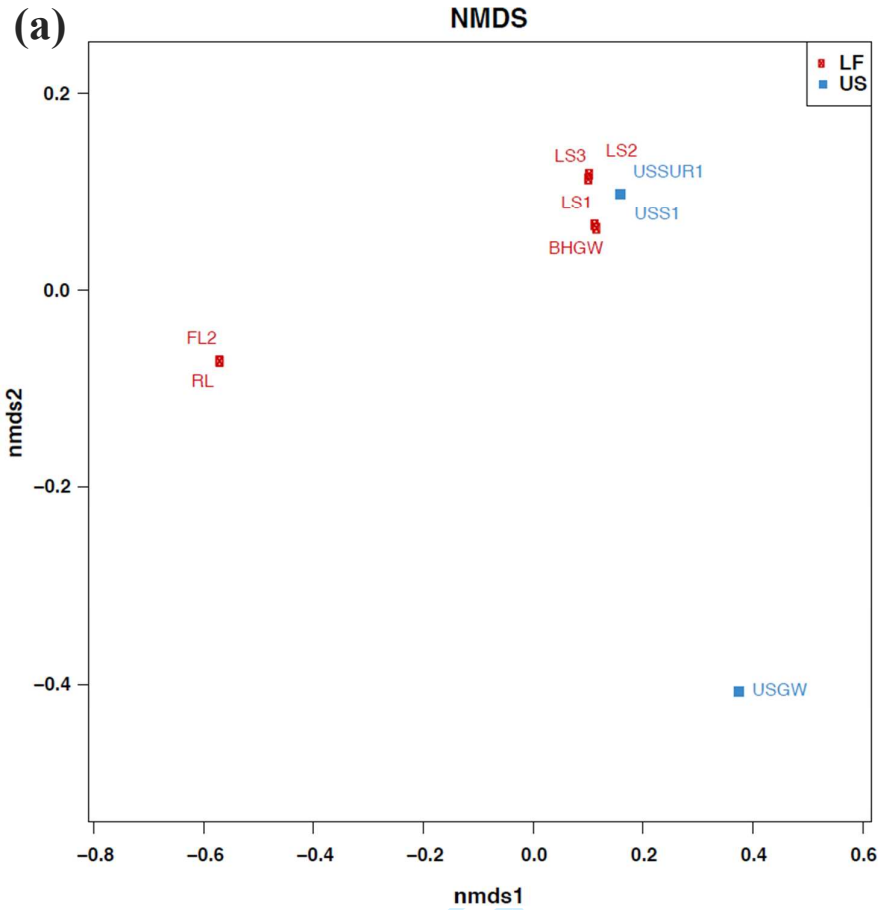

100%
80%
60%
40%
20%
0%
Relative abundance
RL
FL2
LS1
LS2
LS3
BHGW
(a)
Landfill samples
Pseudomonas
Flavobacterium
Anaerolineaceae_uncultured
Arthrobacter
Sphingomonas
Stenotrophomonas
Subgroup_6_norank
Comamonadaceae_unclassified
Nitrosomonadaceae_uncultured
Gemmatimonadaceae_uncultured
Massilia
Candidate_division_TM7_norank
Nocardioides
060F05-B-SD-P93
Pedobacter
TRA3-20_norank
GR-WP33-30_norank
others
100%
80%
60%
40%
20%
0%
Relative abundance
USGW
USS1
USSUR1
(b)
Urban site samples
Pseudomonas
Sporichthyaceae_unclassified
Flavobacterium
Candidatus_Rhabdochlamydia
Anaerolineaceae_uncultured
Arthrobacter
Sphingomonas
Stenotrophomonas
Subgroup_6_norank
Comamonadaceae_unclassified
Nitrosomonadaceae_uncultured
Gemmatimonadaceae_uncultured
Massilia
Candidate_division_TM7_norank
Nocardioides
Pedobacter
Sediminibacterium
TRA3-20_norank
GR-WP33-30_norank
others

Fig 3.


(a)
NMDS
LF
US
LS3
LS2
USSUR1
LS1
USS1
BHGW
FL2
RL
USGW
nmds2
nmds1
0.2
0.0
−0.2
−0.4
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6

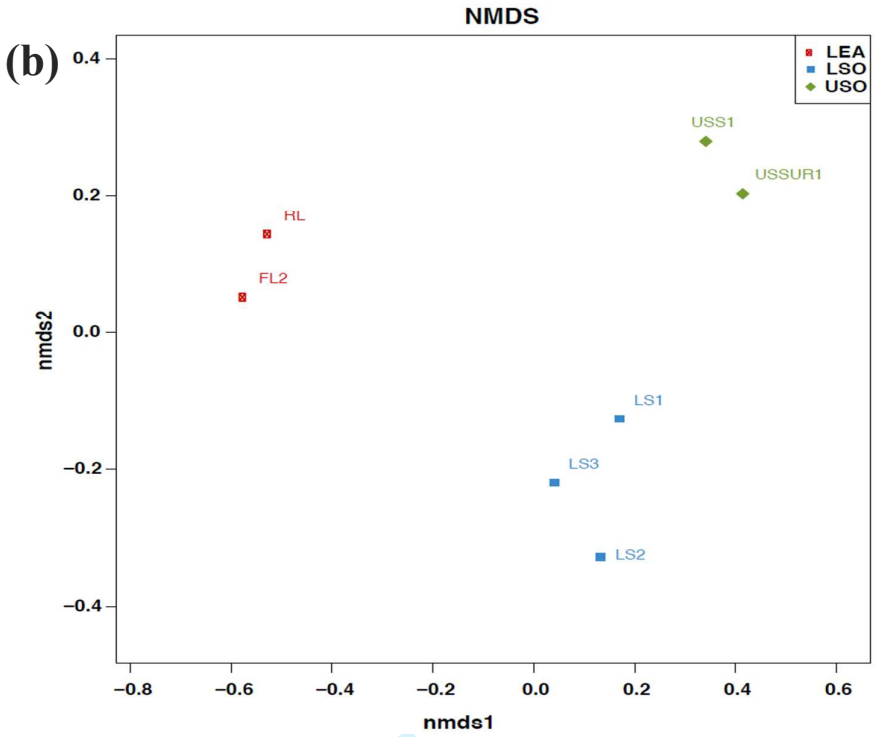

(b)
NMDS
LEA
LSO
USO
USS1
USSUR1
HL
FL2
LS1
LS3
LS2
nmds2
nmds1
0.4
0.2
0.0
−0.2
−0.4
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6

Fig 4.

Draft

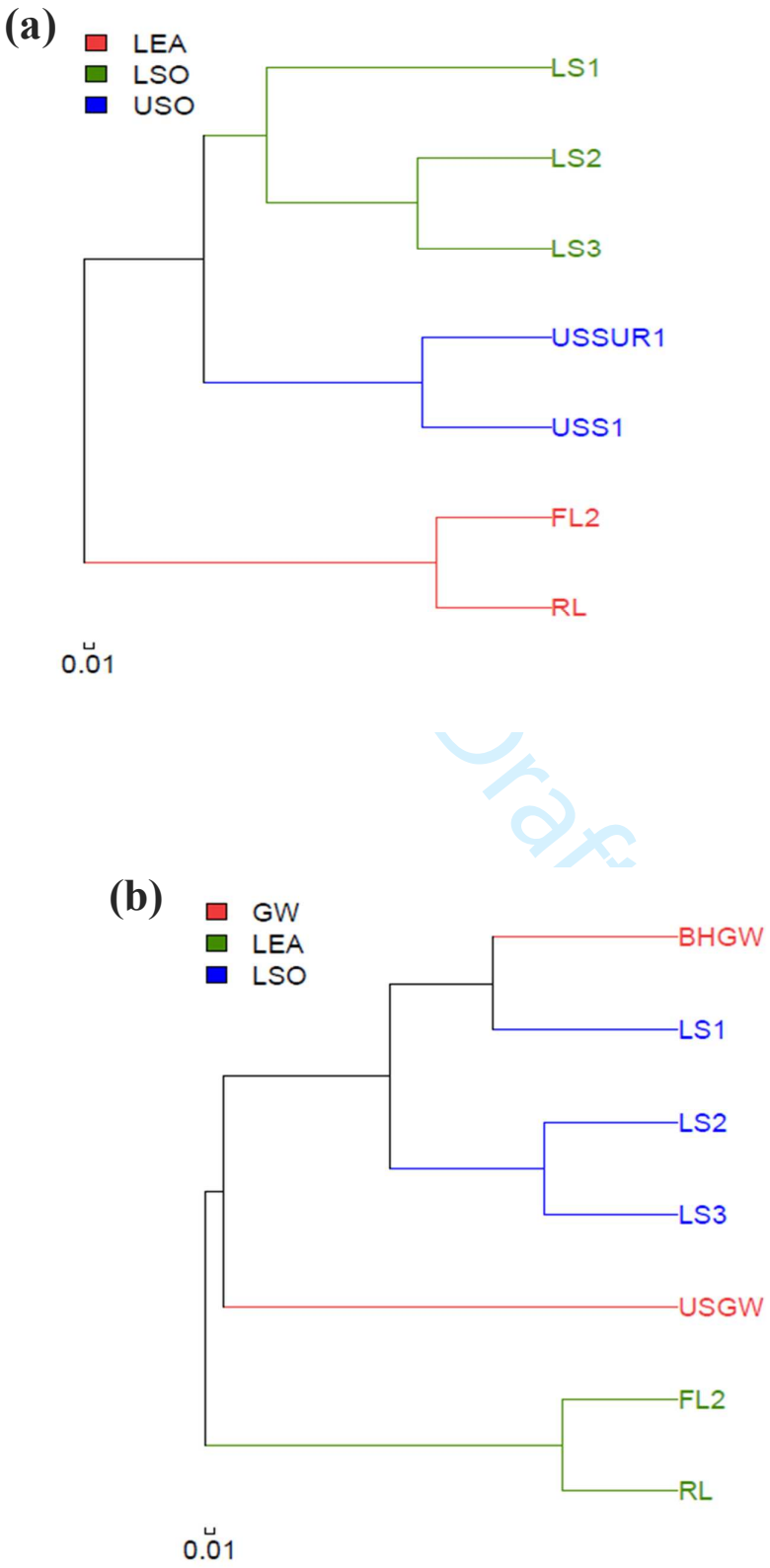

(a)
LEA
LSO
USO
LS1
LS2
LS3
USSUR1
USS1
FL2
RL
0.01
(b)
GW
LEA
LSO
BHGW
LS1
LS2
LS3
USGW
FL2
RL
0.01

Fig 5.

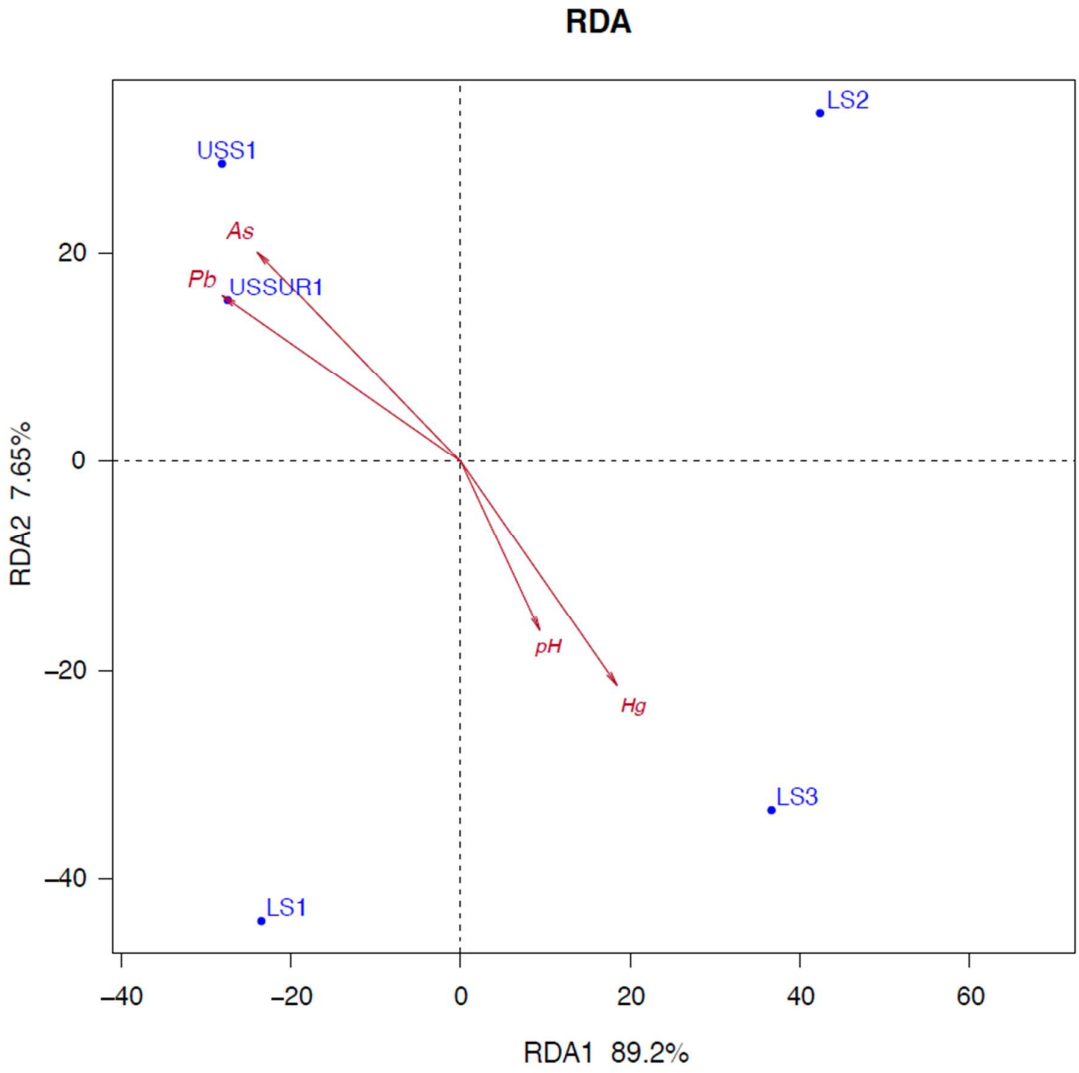

RDA
LS2
USS1
As
Pb
USSUR1
pH
Hg
LS3
LS1
20
0
−20
−40
RDA2 7.65%
−40
−20
0
20
40
60
RDA1 89.2%

Fig 6.

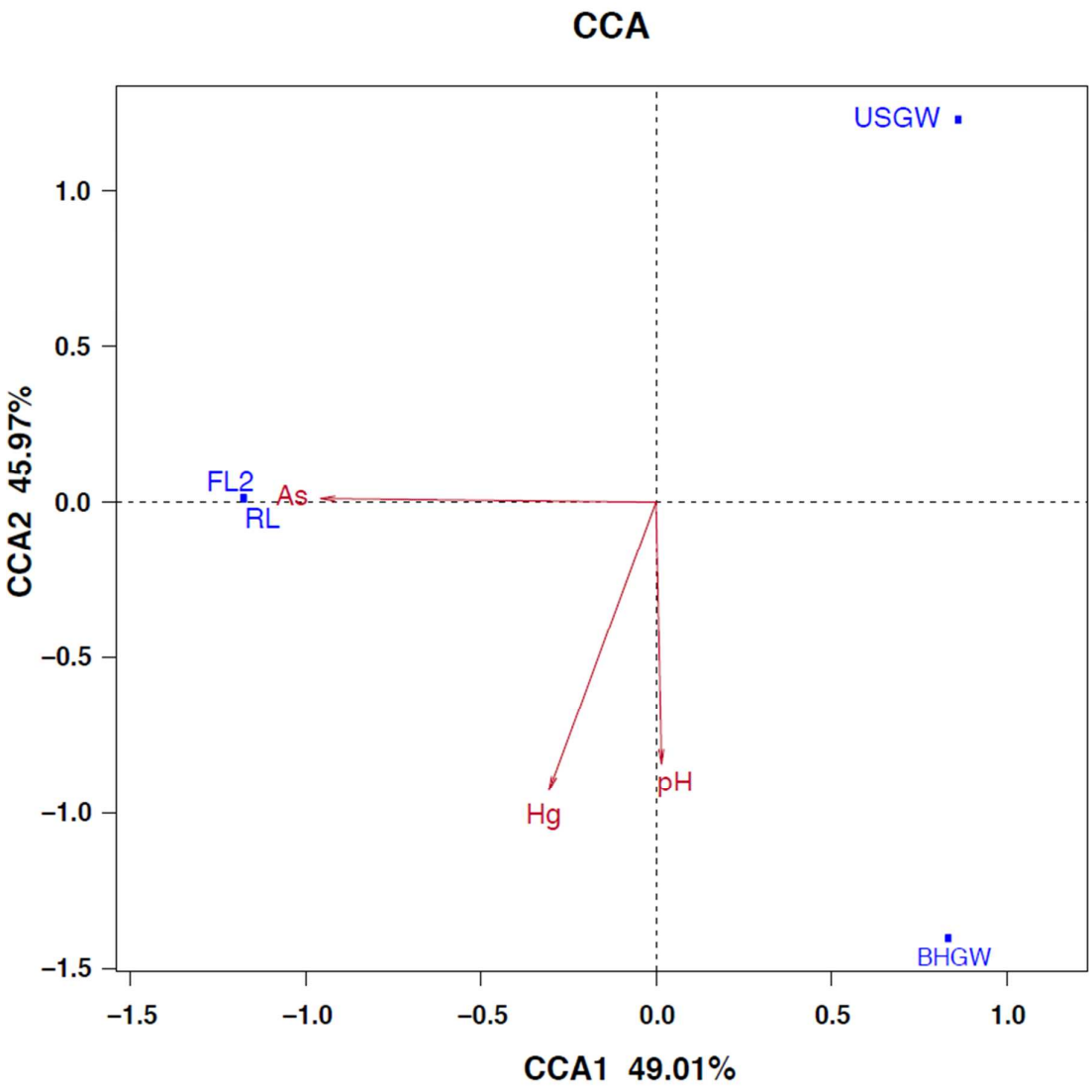

CCA
USGW
FL2
RL
As
Hg
pH
BHGW
1.0
0.5
0.0
−0.5
−1.0
−1.5
CCA2 45.97%
−1.5
−1.0
−0.5
0.0
0.5
1.0
CCA1 49.01%

Fig 7.