The influence of e-waste recycling on the molecular ecological network of soil microbial communities in Pakistan and China☆

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ABSTRACT

Primitive electronic waste (e-waste) recycling releases large amounts of organic pollutants and heavy metals into the environment. As crucial moderators of geochemical cycling processes and pollutant remediation, soil microbes may be affected by these contaminants. We collected soil samples heavily contaminated by e-waste recycling in China and Pakistan, and analyzed the indigenous microbial communities. The results of this work revealed that the microbial community composition and diversity, at both whole and core community levels, were affected significantly by polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and heavy metals (e.g., Cu, Zn, and Pb). The geographical distance showed limited impacts on microbial communities compared with geochemical factors. The constructed ecological network of soil microbial communities illustrated microbial co-occurrence, competition and antagonism across soils, revealing the response of microbes to soil properties and pollutants. Two of the three main modules constructed with core operational taxonomic units (OTUs) were sensitive to nutrition (total organic carbon and total nitrogen) and pollutants. Five key OTUs assigned to *Acidobacteria*, *Proteobacteria*, and *Nitrospirae* in ecological network were identified. This is the first study to report the effects of e-waste pollutants on soil microbial network, providing a deeper understanding of the ecological influence of crude e-waste recycling activities on soil ecological functions.

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1. Introduction

The crude recycling technology applied to electronic waste (e-waste) releases very high amounts of persistent organic pollutants (POPs) and heavy metals into environment, such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). (Huang et al., 2014; Luo et al., 2015; Tang et al., 2014; Wang et al., 2015). Given their increasing potential threat to human health and ecosystem, e-waste pollution has caused considerable concern (Chen et al., 2015a; Robinson, 2009; Zhao et al., 2015).

Microbes, which are crucial to the functioning of almost all

ecosystems, are sensitive to environmental change, due to their rapid growth and active metabolism (Cantarel et al., 2012). Several studies have reported that environmental contaminants (organic pollutants and heavy metals) can inhibit microbial enzyme activity, damage microbial metabolic ability, weaken the resistance of the soil microbial community to subsequent disturbance, and decrease microbial community diversity (Chen et al., 2014; Correa et al., 2010; Guo et al., 2012; Johnston and Leff, 2015; Li et al., 2016; Liu et al., 2011; Petric et al., 2011; Sullivan et al., 2013). These pollutants may also alter microbial community structure by significantly promoting species with remarkable adaptability or biodegradability, and reducing the abundance of other species with normal ecological functions (Liu et al., 2011; Nogales et al., 1999). Since microbial community diversity plays an important role in environmental stress adaption and functional stabilization of ecosystem (Jones and Lennon, 2010), the loss of microbial community diversity caused by environmental contaminants can inhibit various

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functions of ecosystem involving in nutrient cycling. Thus, the alteration of microbial community composition and diversity at e-waste recycling sites might reflect the environmental toxicity of pollutants, suggesting that soil microbial community may be a possible indicator of soil environmental quality (Zhang et al., 2012b). Recently, the effects of organic pollutants and heavy metals at e-waste recycling sites on soil microbial patterns are growing concerns. Laboratory experiments have been performed to investigate the ecotoxicological effects of e-waste pollution on microbes. PBDEs and Cu are reported to have a cocktail of effects on the enzymatic activities of urease, catalase, and saccharase (Zhang et al., 2012a). PBDEs and Pb can decrease microbial biomass and repress microbial basal respiration (Chen et al., 2015b). Deca-bromodiphenyl ether and tetrabromobisphenol A have also been found to have antagonistic toxic effects on microbes within 14 days of release, followed by synergistic toxic effects as time passes (Zhang et al., 2015). However, laboratory studies cannot fully approximate real world scenarios, and these results do not perfectly represent *in situ* conditions. Addressing this challenge, Liu et al. conducted a comprehensive survey of the microbial communities in e-waste contaminated soils in 2014 (Liu et al., 2015). They found that environmental variables could explain approximately 70% of the variation observed in microbial communities, where moisture content, decabromodiphenyl ether, and Cu were identified as the most important factors. All of the studied soils were located within a small area in the town of Guiyu, South China, and it is still unknown whether similar impacts of pollutants occur over long distances. Furthermore, at a large scale, microbial distribution patterns in e-waste contaminated soils may be revealed. The "Everything is Everywhere" (EisE) hypothesis is widely used to explain the phenomenon whereby microorganisms can diffuse easily at a global scale and that the environment influences their abundance via natural selection (Martiny et al., 2006). However, the EisE hypothesis has been challenged by molecular evidence indicating that geographical barriers may restrict microbial distribution patterns (Taylor et al., 2006). This debate remains unresolved. In the present study, we investigated the microbial community and attempted to distinguish whether the EisE hypothesis can better explain community variation at e-waste recycling sites.

Ecological networks show the co-occurrence, competition, and antagonism among different microbial populations within microbial communities (Zhou et al., 2011). One approach, developed by Zhou et al. (2011), originated from a new random matrix theory and successfully characterized ecological networks in microbial communities based on high-throughput metagenomic sequencing. In this model, co-occurrence, such as commensalism or a mutualistic relationship, is represented by a positive correlation, whereas a negative correlation may suggest the presence of competition and antagonism (Chow et al., 2014). Mantel tests detect relationships between microbial network interactions and soil properties (Zhou et al., 2011). Therefore, constructing and analyzing the microbial network can provide a deep understanding of microbial communities at e-waste recycling sites, particularly the interrelationships between different species, and identify the influence of organic pollutants and heavy metals. To date, no studies have been performed to determine the interactions among microbes in e-waste recycling soils *in situ*.

We collected samples from e-waste contaminated soils over a long distance between China and Pakistan, two major e-waste recycling countries. The 454 high-throughput sequencing was applied to target the gene encoding for 16S rRNA and determine the composition and diversity of soil microbial community. The present study aims to: (1) illustrate the soil microbial community in e-waste contaminated soils from wide region; (2) identify the key factors affecting microbial community structure; and (3) explore

the microbial network and its impact factors. Our results provided a deep view of microbial communities in e-waste contaminated soils over long distances and uncovered the patterns in the microbial communities that had been shaped by e-waste organic pollutants and heavy metals. These findings provide important insights into the impacts on microbial community structure, diversity, and co-occurrence patterns of e-waste recycling activities.

2. Materials and methods

2.1. Sample collection

Soil samples were collected from five sites in China and three sites in Pakistan in June 2012, where e-waste recycling activities are intense and all the sampling locations are near the e-waste recycling sites (Fig. S1). The detailed information of each sampling site was: C1 (Shijiao, 23°56′ N, 113°29′ E) and C2 (Longtang, 23°33′ N, 113°40′ E) in Qingyuan, C3 (Guiyu, 23°19′ N, 116°20′ E) in Shantou, C4 (Fengjiang, 28°32′ N, 121°23′ E) and C5 (Xinqiao, 28°32′ N, 121°25′ E) in Taizhou, P1 (Karachi, 24°55′ N, 67°30′ E), P2 (Multan, 30°14′ N, 71°29′ E) and P3 (Lahore, 31°32′ N, 74°20′ E). From each site, the soil samples were collected in triplicates at a depth of 0–10 cm, and transported to the laboratory with ice packs. The soil textural classification at each site is shown in Table S1.

2.2. Soil properties and pollutant analysis

Total organic carbon (TOC) and total nitrogen (TN) were determined as previously described (Hedges and Stern, 1984) with modifications. Briefly, 2 g of freeze-dried soils were blended and treated twice with 25 mL of 1 M HCl, followed by washing with ultrapure water to a final pH of 6–7. After drying and homogenizing, TOC and TN were analyzed using an elemental analyzer (vario EL cube, Elementar Analysensysteme GmbH). The instrument was calibrated using the standard provided by manufacturers before testing and every ten samples. Each sample was measured thrice to ensure the data accuracy (standard error less than 0.3%). The soil dissolved organic carbon (DOC) was extracted into a 100 mL centrifuge bottle with 10 g of soil and 50 mL of ultrapure water. After shaking for 30 min and centrifuging at 5000 rpm for 10 min, the supernatants were filtered through 0.45 μL polycarbonate filter membrane. Then, DOC was measured using a TOC-VCPH analyzer. The standard curve was constructed using a range of stock solutions prepared with potassium hydrogen phthalate with concentrations of 0, 20, 50, 100, 200, and 800 mg/L. Soil pH was measured in a suspension with soil/0.01 M $CaCl_2$ solution (1:5, w/v) using a pH meter.

PAHs, PCBs and PBDEs were extracted by dichloromethane (DCM) in a Soxhlet apparatus for 48 h spiking with relevant recovery standards (Table S2). During the extraction, activated copper was used to remove sulfur. After solvent exchange to hexane, the extracts were concentrated to approximately 0.5 mL. The PCB and PBDE extracts were purified in a multilayer column containing (from bottom to top) neutral alumina (3% deactivated), neutral silica gel (3% deactivated), 50% (w/w) sulfuric acid-silica gel, and anhydrous Na_2SO_4, via elution with 20 mL hexane/DCM (1:1, v/v). PAH extracts were cleaned using the same multilayer column without sulfuric acid-silica gel. The internal standards were then added to the corresponding extracts, following the evaporation process using N_2 to concentrate the extracts to approximately 50 μL. A total of 15 PAHs (using the DB-5MS column, 30 m × 0.25 mm × 0.25 μm), 32 PCBs (using the CP-Sil 8 CB column, 0.25 mm × 0.25 μm), 8 PBDEs (using the DB5-MS column, 30 m × 0.25 mm × 0.25 μm and CP-Sil 13 CB column) (Table S3) were detected using gas chromatography mass spectrometry (GC-

MS: Agilent 7890). For PAHs, the temperature of the injector was 280 °C. The GC oven temperature was set at 60 °C for 1 min, then 4 °C/min to 290 °C for 10 min, 4 °C/min to 300 °C, and held for 5 min. For PCBs, the injector temperature was 250 °C. The oven temperature was 150 °C for 3 min, rose to 290 °C at a rate of 4 °C/min and kept for 10 min, then held for 5 min. For PBDEs, the injector temperature was 290 °C. The oven temperature was 110 °C for 5 min, rose to 200 °C at a rate of 15 °C/min and kept for 3 min, rose to 295 °C at a rate of 5 °C/min and held for 22 min, then kept at 295 °C for 10 min. All samples were carried by high purity helium with a flow rate of 1.83 mL/min. The recovery rate ranging from 80% to 120% was acceptable. A standard sample was tested every ten samples, and the standard error was less than 15%.

The concentrations of heavy metals (Cu, Pb, and Zn) in the soils were determined by flame-atomic absorption spectrometry (AAS; novAA 400, Analytik Jena AG) after homogenization and strong acid digestion (concentrated HNO_3 and $HClO_4$, 4:1, v/v) of about 200 mg soil for 32 h. The standard was measured every ten samples and the recovery rate ranged from 95% to 105%.

2.3. DNA extraction, 16S rRNA amplification, and sequencing

DNA was extracted in triplicate from 0.5 g of soil using a PowerSoil DNA Isolation Kit according to the manufacturer's instructions. The combined DNA solutions were stored at −20 °C for further analysis after measuring the concentration/quality via a NanoDrop 2000 Spectrophotometer.

The universal primer pair (341F: 5′-CCTACGGGNGGCWGCAG-3′, 802R: 5′-TACNVGGGTATCTAATCC-3′) targeting the V3–V4 hypervariable region was used to amplify 16S rRNA. Polymerase chain reaction (PCR) mixtures (50 μL) contained 50–100 ng (1 μL) of DNA template, 25 μL of Taq premix buffer (TaKaRa), 100 nM of each primer (1 μL), and 22 μL of H_2O. The 16S rRNA was amplified in triplicate with the following process: 94 °C for 5 min; 28 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s and a final extension at 72 °C for 5 min. The negative control was used to test the contamination, replacing DNA template by H_2O. The amplification products without contamination were tested by agarose gel (1.5%) and purified using the MicroElute Cycle-Pure Kit (Omega Bio-Tek), following the manufacturer's instructions. Products from the same sample were combined and quantified as above, and finally sent to the Beijing Genomics Institute (BGI) for sequencing, based on the 454 platform.

2.4. Processing of pyrosequencing data

The original data have been submitted to NCBI (accession number: SRP111893). The data were analyzed using Mothur (Schloss et al., 2009) and QIIME (Caporaso et al., 2010). Reads with a low quality (quality score < 25, length < 250, ambiguous base > 0) were removed and the singleton sequences were discarded. Operational taxonomic units (OTUs) with 97% similarity were picked out, and the representative sequence set was chosen. Chimeric sequences were then identified and discarded (Edgar et al., 2011). The OTUs were then normalized according to the sample with minimum number of sequence for further analysis. The phylotype information was identified according to the Greengenes 13.5 database using "assign_taxonomy.py".

The relative abundance of each taxon was estimated by comparing the number of sequences classified as the specific taxon to the total number of sequences in the individual sample. OTUs occurring in more than five samples were picked up as core OTUs. The α-diversity indices (Chao1, Shannon, and ACE) and β-diversity weighted UniFrac phylogenetic distance between samples (Lozupone and Knight, 2005) were measured using the QIIME

script.

2.5. Network construction

The construction of the co-occurrence ecological network was performed using the online Molecular Ecological Network Analyses (MENA) pipeline (http://ieg2.ou.edu/MENA) (Deng et al., 2012). The selected core OTUs were analyzed during network construction. The relationship between OTUs was tested via Pearson correlation analysis and the threshold was determined by a random matrix theory (RMT)-based approach. The network was then visualized via Cytoscape. The relationships between the network and soil properties were measured by a Mantel test (Zhou et al., 2010).

2.6. Statistical analysis

Statistical analysis was performed using SPSS and R. The relationships between soil properties were determined by a Spearman's correlation analysis (two-tailed). The influence of soil properties on bacterial α-diversity was detected using a Spearman's analysis (two-tailed). To determine the major soil properties affecting microbial communities (whole microbial communities and core OTUs), the "Bioenv" function in the R "vegan" package was used with default parameters. Then, redundancy analysis (RDA) and canonical correspondence analysis (CCA) were performed to explain relationships between microbial communities and the selected soil properties using the "vegan" package. A Mantel test was used to determine the influence of geographic distance and soil properties on microbial communities and β-diversity with a two-tailed test and 999 permutations.

3. Results

3.1. Microbial community profiles

After filtering the low quality reads (score < 25), a total of 84,403 reads were generated from 454 sequencing and further analyzed in a downstream investigation. Total of 19,407 OTUs was identified based on 97% similarity. The number of OTUs and sequences varied across soils, ranging from 1,370 to 4,871 and from 7,124 to 15,396, respectively (Table S4).

At the phylum level, 45 phyla were assigned to bacteria. More than 97.6% of the sequences were classified with abundances larger than 0.1% (Fig. 1, Table S5A and S5B). Less than 2.41% of the total sequences were classified as rare phyla (<0.1%). Six predominant phyla (>5%), *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, and *Planctomycetes*, accounted for 84.7% (on average) of the total sequences. Of the total OTUs, 183 OTUs occurred in more than five sites were selected as core OTUs (Fig. S2). The OTU with the highest abundance (ID: 1051517) was classified as *Bacillus*. The taxonomic compositions of the whole and core community at phyla, class, order, family and genus level were shown in SI-II.

Bacterial diversity was calculated based on OTU level. The indices of Chao1, Shannon, and Ace, ranging from 3,420 to 11,800, 7.55–11.7, and 3,660–12,700, were used to characterize alpha diversity (Table S6). Beta diversity (weighted Unifrac) containing phylogenetic information is presented in Table S7 and Fig. 2A. The P3 microbial community was similar to those in soils from China, but not to those in Pakistan sites (P1 and P2).

3.2. Effects of soil properties and pollutants on microbial composition and diversity

Soil properties and pollutant content are summarized in Table 1. Both soil properties and contamination levels varied over a wide
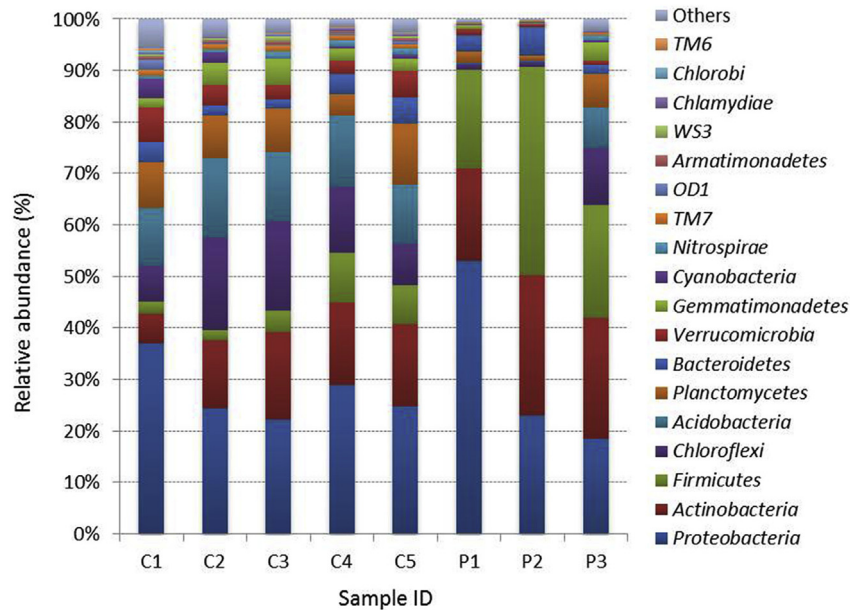
Fig. 1. Relative abundances (%) of dominant lineages (phylum level) across different soil samples. The other phyla are composed of 27 rare ones and the unclassified sequences affiliated to unknown phylum. Soils C1 to C5 were collected from China, and P1 to P3 were from Pakistan.

range. For example, pH ranged from 4.46 to 12.6, and PAHs ranged from 307 to 2,940 ng/g. PCBs (23—8,780 ng/g) and Cu (151—3,130 mg/kg) showed the greatest amount of variation. Generally, samples from the same country had similar soil properties and pollutant content (Fig. 2B). Soils separated by long distances (P3 in Pakistan and all soils from China) had similar soil properties (Fig. 2B).

To identify the influence of soil properties on microbial communities, two subsets having the maximum correlation with whole community and core OTUs were selected: (1) pH, TN, TOC, PAHs, Cu, and Zn (whole community level); (2) pH, TN, DOC, PAHs, Cu, and Pb (core OTU level). Variables of both subsets were significantly related to microbial composition at the community and core OTU levels, respectively (Mantel test, $p < 0.05$). The relationship between microbial composition and a subset of variables was detected using RDA. At the whole community level, the six variables explained 93.6% of the microbial community composition variation (Fig. S3A), and the contributions of soil properties and pollutants to community variation were 21.5% and 15.7% respectively. The co-effects explained 56.4% of community variation (Fig. S3A). Zinc was the most important factor, explaining 5.5% of community variation, followed by PAHs (4.8%) and pH (4.8%). At the core OTUs

level, soil properties and pollutants explained 36.6% and 39.3% of total variation, respectively (Fig. S3B). All subset variables could explain 90% of the variation (Fig. S3B). PAHs (12.7%), Cu (12.5%), and Pb (12.0%) were the most relevant factors explaining core OTUs variation. The contributions of all individual variables are shown in Table S8.

PBDEs were the only pollutants significantly negatively related to alpha diversity: Chao1 (Spearman, $r = -0.81$, $p < 0.05$) and Ace (Spearman, $r = -0.81$, $p < 0.05$). No relationship between alpha diversity and other pollutants was detected. In addition, geographic distance, soil properties and pollutants did not affect the microbial composition and Unifrac distance (beta-diversity) (Mantel test, $p > 0.05$).

3.3. Network structure and its association with soil properties

Topological properties, commonly used to describe the complex patterns of bacterial interrelationships, are listed in Table 2. Here, we considered four empirical network indices as: avgK (Average connectivity level of OTUs), avgCC (Average clustering coefficient describing how an OTU is connected with its neighbors in the average level), GD (Average geodesic distance. Smaller GD indicates
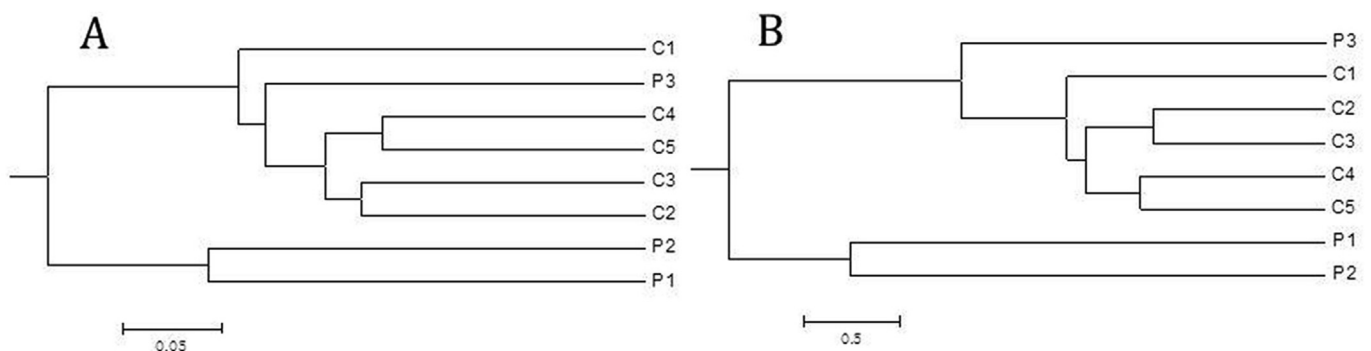

Fig. 2. Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering based on phylogenetic distance of microbial communities (A) and Euclidean distance of soil characteristics (B). Soils C1 to C5 were collected from China, and P1 to P3 were from Pakistan.

Table 1
Properties of soil samples taken from China (C1 to C5) and Pakistan (P1 to P3) (Mean ± SD, n = 3).

Sample	pH	TN (%)	TOC(%)	DOC(mg/kg)	PAHs(ng/g)	PCBs(ng/g)	PBDEs (ng/g)	Cu(mg/kg)	Pb(mg/kg)	Zn (mg/kg)
C1	5.03 ± 0.02	0.29 ± 0.017	3.60 ± 0.20	201 ± 8.9	308 ± 5.0	23 ± 1.0	85 ± 3.61	156 ± 14.0	81 ± 4.6	117 ± 21.7
C2	4.46 ± 0.07	0.17 ± 0.020	2.08 ± 0.03	141 ± 13.1	469 ± 32.9	101 ± 5.3	708 ± 14.4	178 ± 12.0	52 ± 8.2	104 ± 16.1
C3	7.38 ± 0.13	0.09 ± 0.012	0.94 ± 0.85	75.4 ± 3.8	307 ± 12.1	38 ± 2.0	1080 ± 133.0	187 ± 15.6	165 ± 14.8	503 ± 16.6
C4	5.69 ± 0.11	0.16 ± 0.017	1.95 ± 0.04	80.3 ± 3.7	680 ± 10.8	310 ± 8.5	225 ± 26.1	371 ± 7.6	482 ± 9.17	2110 ± 93.7
C5	4.75 ± 0.38	0.10 ± 0.026	1.12 ± 0.01	48.3 ± 2.4	1010 ± 34.8	30 ± 2.7	13 ± 1.0	151 ± 16.6	122 ± 13.2	562 ± 50.8
P1	7.20 ± 0.06	0.27 ± 0.020	8.11 ± 0.09	2110 ± 104.0	2350 ± 57.7	8780 ± 147.0	699 ± 31.5	3080 ± 45.5	1670 ± 88.4	3030 ± 96.2
P2	12.6 ± 0.05	0.33 ± 0.013	12.9 ± 0.18	3090 ± 127.0	2940 ± 60.0	473 ± 16.4	1010 ± 23.1	3000 ± 195.0	290 ± 15.0	1130 ± 158.0
P3	7.25 ± 0.10	0.10 ± 0.010	2.30 ± 0.29	256 ± 27.2	2180 ± 146.0	6630 ± 91.9	49 ± 11.4	3130 ± 59.6	558 ± 57.3	895 ± 21.1

Table 2
Topological properties of the ecological network of microbial communities and their associated random networks.

Empirical Network							Random Networks		
St	Network size	Link	avgK	GD	avgCC	Modularity	GD±SD	avgCC±SD	Modularity±SD
0.780	156	440	5.64	3.77	0.391	0.615	2.83 ± 0.116	0.094 ± 0.011	0.356 ± 0.009

avgK stands for average connectivity; avgCC signifies average clustering coefficient; GD indicates average geodesic distance, St represents threshold value.

the closer OTUs in the network), and modularity (measuring the strength of division of a network into modules). The network indices are much higher than the random network indices in the present study (Table 2), suggesting the network possesses typical small-world, which indicated this network can be highly clustered and the network nodes are closely related with each other (Watts and Strogatz, 1998). Of the 15 modules detected, three most dominant modules (each containing more than 30 nodes) possess 118 nodes and 75.6% of the total network size. The OTUs within the same module are closely connected and have limited relationship with those from other modules. The three dominant modules were constructed by ten phyla, and *Acidobacteria*, *Actinobacteria*, and *Proteobacteria* were the three dominant phyla (Fig. 4). Five phyla, including one unique phylum, *Cyanobacteria*, were detected in Module 2. All *Gemmatimonadetes* were in Module 1 and *Verrucomicrobia* only occurred in Module 3 (Fig. 4).

The relationship between bacterial network interactions and soil properties was evaluated using Mantel test. The square of the correlation between the signal intensity of individual OTU and soil properties was calculated to determine the role of soil variables in network modules (Horvath and Dong, 2008; Zhou et al., 2011). Module 1 was sensitive to pollutants, and its inner interaction was significantly affected by PAHs, PCBs, and Cu ($p < 0.05$) (Table 3). Module 3 was closely correlated with TOC and TN. The interactions of OTUs in Module 2 were independent of soil properties.

To identify the key OTUs, the *Z-P* plot is exhibited in Fig. 5. The x-axis, *Pi*, reflects the degree of the connections between OTUs from different modules. The y-axis, *Zi*, describes the connections between OTUs from the same module. The five module hubs ($Zi > 2.5$, $Pi \leq 0.62$) play an important role in bacterial interaction, assigned to *Acidobacteria*, *Proteobacteria*, and *Nitrospirae*. Their taxonomic information is presented in Table 4. No network hubs ($Zi > 2.5$, $Pi > 0.62$) or connectors ($Zi \leq 2.5$, $Pi > 0.62$) OTUs were observed in the present study.
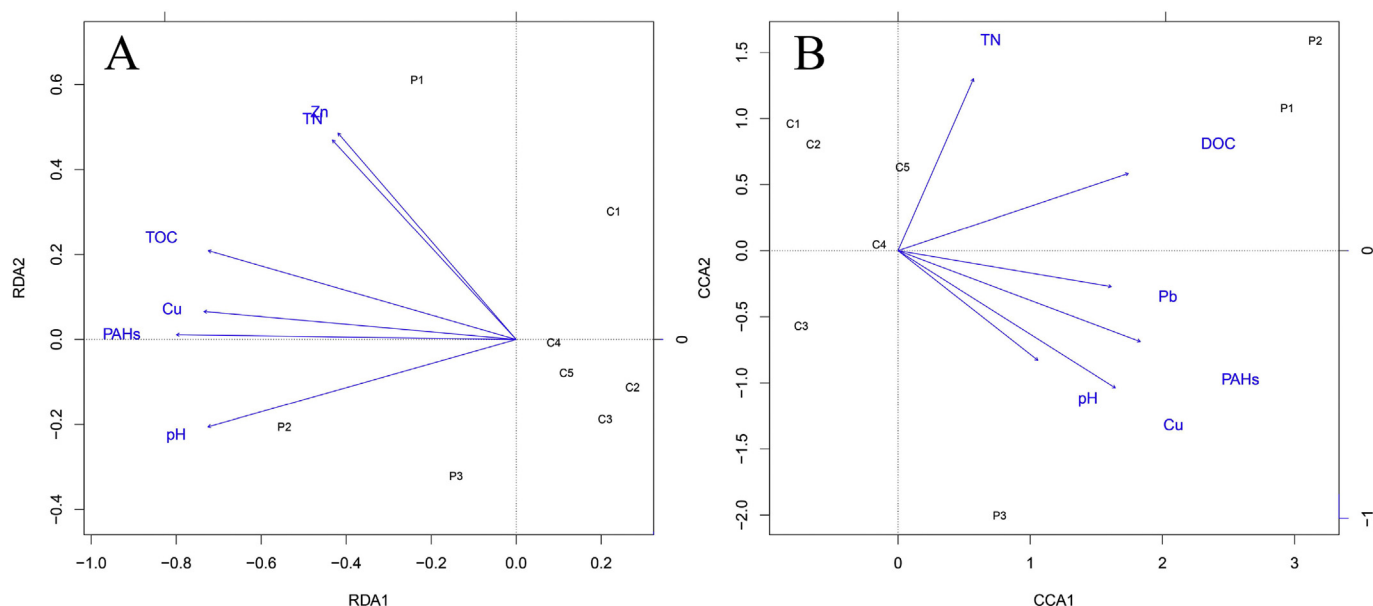

Fig. 3. (A) RDA plot of the relationship between bacterial community structure at the OTU level from different sampling sites (C1 to C5 from China and P1 to P3 from Pakistan) and six subset variables (pH, TN, TOC, PAHs, Cu and Zn). (B) CCA plot of the correlation between core OTUs and six subset variables (pH, TN, DOC, PAHs, Cu and Pb).
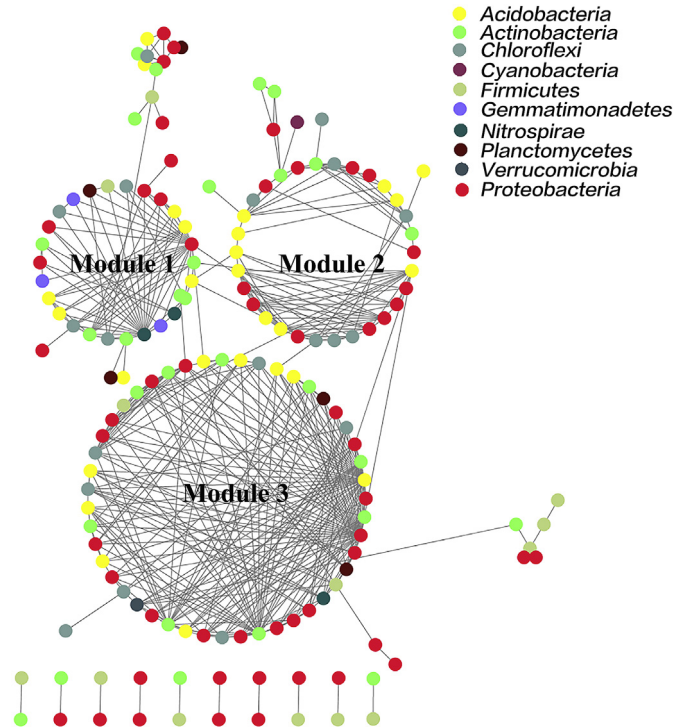
Fig. 4. The co-occurrence ecological network constructed by core OTUs. Each node represents an OTU, and the connectivity between two OTUs is indicated by the edges. Colors of the nodes signify different phylum. A total number of 15 modules are detected in ecological network. The three dominant modules (Module 1, 2, and 3) include more than 30 OTUs and are constructed by ten phyla, possessing 75.6% of the total network size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

In this study, 45 bacterial phyla were identified, which was remarkably different from a previous study of the Qingyuan e-waste recycling site, in which 29 bacterial phyla were detected using the Miseq platform (Liu et al., 2015), and indicating higher diversity than previously detected. However, most of the novel bacteria were classified into rare phyla with small abundance. Among the core OTUs, which represented the main microbes in e-
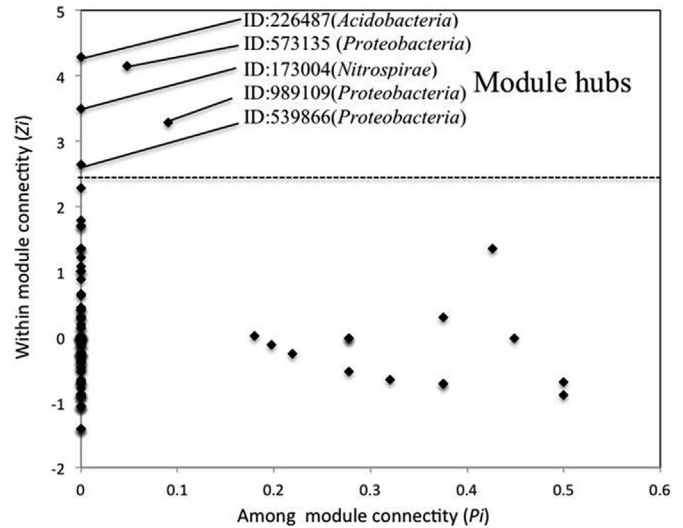

Fig. 5. Z-P plot exhibiting the distribution of OTUs based on their topological roles. Each point represents an OTU. The location of each OTU is determined according to the within-module connectivity (Zi) and among-module connectivity (Pi). The module hub is defined according to Zi and Pi values ($Zi > 2.5$, $Pi \leq 0.62$). The five identified module hubs were marked with ID numbers.

waste soils (Fig. S2), the OTU representing *Bacillus* was the most abundant and the results indicate its high capacity to adapt to the contaminants. *Bacillus* can produce highly dormant endospores in response to environmental stress (Earl et al., 2008), and it is capable of resisting heavy metals and degrading organic pollutants (Mulligan et al., 2001; Zeng et al., 2016).

Numerous environmental variables are capable of affecting microbial community. Compared with soil physical and chemical properties, the seasonal temporal variation was reported to be negligible by previous studies (Fierer and Jackson, 2006; Krave et al., 2002), and it was therefore ignored in the present study since all the samples were collected at a fixed time. To detect the main variables in soils affecting microbial community structure, "Bioenv" was performed and several influencing soil properties were selected. The respective patterns were due to the influence of different soil properties and pollutants on the microbial community at each level. At the whole community level, the co-effects of soil properties (e.g., pH and nutrition) and pollutants explained

Table 3
The relationship between soil properties (pH, TN, TOC, DOC), pollutants (PAHs, PCBs, PBDEs, Cu, Pb, Zn) and three dominant modules revealed by Mantel test.

	pH	TN	TOC	DOC	PAHs	PCBs	PBDEs	Cu	Pb	Zn
Module 1	−0.06	−0.40	−0.70*	−0.73	−0.93**	−0.77*	−0.18	−0.99**	−0.63	−0.47
Module 2	−0.48	−0.48	−0.45	−0.38	−0.34	−0.041	0.17	−0.31	−0.16	−0.34
Module 3	−0.37	−0.79*	−0.72*	−0.68	−0.59	−0.36	0.05	−0.54	−0.37	−0.20

*represents $p < 0.05$; ** represents $p < 0.01$.

Table 4
Five module hubs and their taxonomic information.

	Phylum	Class	Order	Family	Genus
ID: 226487	*Acidobacteria*	*Acidobacteria-6*	*iii1-15*	NA	NA
ID: 573135	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Bradyrhizobiaceae*	NA
ID: 173004	*Nitrospirae*	*Nitrospira*	*Nitrospirales*	*Nitrospiraceae*	*Nitrospira*
ID: 989109	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	*Kaistobacter*
ID: 539866	*Proteobacteria*	*Gammaproteobacteria*	*Thiotrichales*	*Piscirickettsiaceae*	NA

NA represents the taxonomic information cannot be identified. The IDs are in accordance with Greengenes database.

most of the microbial community structure (Fig. S3A). Relationships between soil properties and pollutants have been reported in many previous studies. The pH and organic matter can alter the mobility of heavy metals (Calugaru et al., 2016; Pakzad et al., 2016), which may affect the microbial community significantly. Soil organic carbon was closely linked to the availability of organic pollutants including PAHs, PCBs, and PBDEs (Calugaru et al., 2016; Jiang et al., 2016), further influencing microbial access to these pollutants and their biological toxicity. It is therefore foreseeable that soil properties have co-effects with various types of contaminants. Although as a necessary micronutrient for cell metabolism, high concentration of Zn can damage microbial activities, alter microbial communities, decrease microbial diversity, and inhibit enzyme activities (Chapman et al., 2013; Gomez-Balderas et al., 2014; Lessard et al., 2014). Zn is therefore identified as the most important factor affecting microbial community at these e-waste polluted sites (Fig. 3A, Table S8).

Although pH showed a certain influence on microbial community in the present study, its impact was not as significant as previous studies showing pH is one of the most important factors in shaping soil microbial community (Dumbrell et al., 2010; Mukherjee et al., 2014). The intense organic contamination at e-waste polluted sites might explain this phenomenon. The existence of PAHs not only possesses toxicity on soil microbes, which changes microbial community composition, but also enriches the bacterial species capable of metabolizing PAHs to alter the microbial community structure (Jones et al., 2011; Vila et al., 2010; Zhang et al., 2010). The coupled toxicity and metabolic effects are not negligible in the present study, since PAHs concentration varied from 307 to 2,940 ng/g. Meanwhile, the resilience of microbial community composition might be the reason explaining the limited pH influence. Several studies have proved the possibility that microbial community is resilient and could return to its predisturbance composition even if it is sensitive to environment (Allison and Martiny, 2008; Shade et al., 2012). The resilient process needs time. The PAHs contamination is a short-term effect at these e-waste polluted sites, whereas the soil pH values might be stable for long-time. Therefore, microbial community in present study is more sensitive to PAHs than pH. Our results suggest that historical factors should be considered when we investigated the microbial community affected by environmental factors on different time scale.

Although soil properties and pollutants affected both the whole and core microbial communities in this study, high co-effects were only identified for whole communities, not core OTUs. This may be explained by the dominance of the microbes represented by core OTUs in the soil community, which are possibly better adapted to e-waste contamination. However, the mechanism remains unclear and there is a lack of previous studies to support or counter this speculation. PAHs and Cu were determined to be the most important factors influencing core microbial communities, and are both tightly related to microbial communities (Fig. 3). The effects of PAHs on microbial communities (Muckian et al., 2009) have been observed previously as the alteration of microbial community structure and diversity (Sawulski et al., 2014). In e-waste contaminated soils, the role of PAHs was dependent on their concentration and availability. Heavy PAH contamination significantly reduces the diversity of bacterial communities, whereas slight contamination may increase microbial diversity in e-waste contaminated soils (Zhang et al., 2010). Cu, as the second most powerful factor, commonly represents one of the most important toxic substances in e-waste contaminated soils (Wong et al., 2007) and shifts microbial communities; a similar result was shown by Liu (Liu et al., 2015). PBDEs, a typical group of organic contaminants, are frequently detected in soils around e-waste recycling sites (Tang et al., 2010) and can produce reactive oxygen species to selectively inhibit the growth of specific lineages (Liu et al., 2011). We found that the negative relationship between PBDEs and alpha diversity supported this conclusion.

We found a more highly significant divergence in microbial communities in samples at close distances than those at remote distances (Fig. 2, Table S7). Some studies have shown that spatial distance is a powerful driver of microbial community structure (Green et al., 2004; Griffiths et al., 2011; Martiny et al., 2011), challenging the EisE hypothesis "Everything is everywhere, but environment selects". Our results are consistent with the EisE hypothesis. For instance, the soil samples from sites at remote distances (P3 in Pakistan and all soils in China) were close in terms of phylogenetics (Fig. 2A). Such similar taxa at different e-waste recycling sites were hardly explained by geographical distance. Additionally, microbial communities in soils with similar characteristics (Fig. 2B), in spite of a geographical distance, were located at closer phylogenetics distances (Fig. 2A), suggesting that environmental conditions play a more important role in microbial community structure. Although our findings are supported by some previous studies agreeing with EisE hypothesis (Van der Gucht et al., 2007), the relatively small sample size in the present study questions the outcome, and future work is needed for a larger sample size for more reliable and accurate conclusions.

We examined the topological roles of core OTUs, which describe the co-occurrence among bacterial populations, to explore the cooperation of the microbial community in e-waste soils. Up to now, there is still no study focusing on microbial network in e-waste contamination soils. This network can give us the information what microbes are physically and/or functionally associated in a microbial community (Zhou et al., 2011). In our study, most of the OTUs are classified into three main modules. The OTUs within the same module are closely connected and have limited relationship with those from other modules. In other words, the modules stand for the complex web among the microbial community, behaving important for microbial community structure stabilization which benefits the ecological functions such as nutrition cycling, pollutant degradation, etc. (Faust and Raes, 2012; Hansen et al., 2007). The co-occurrence levels of OTUs in the three main modules are: (1) sensitive to pollutants, (2) sensitive to nutrition, and (3) independent of soil properties (Table 3). Module 1, sensitive to pollutants, is significantly related to PAHs, PCBs and Cu. The increase in these three pollutants reduces the connections between the OTUs clustered in Module 1, suggesting that a high concentration of these pollutants could reduce the cooperation of microbes. The significance level follows the order of Cu > PAHs > PCBs (Table 3), based on the *p* values. TOC is also negatively correlated to OTU connection in Module 1. First, TOC affects the availability of metals and organic chemicals, and then reduces their toxicity on microbes (Calugaru et al., 2016; Jiang et al., 2016; Song et al., 2004). Second, we speculate that microbes need to cooperate to a greater extent to resist stress (Yin et al., 2015) and high levels of TOC ease nutrition stress, which leads to a weak connection. *Gemmatimonadetes* are found only in Module 1, suggesting that this phylum may be tightly related to pollutants. Compared with Module 1, Module 3 is more sensitive to nutrition content. Both TOC and TN affect microbial connection in this module. All OTUs belonging to *Verrucomicrobia* are detected in Module 3, possibly due to its close correlation to nutrition. For Module 2, no soil variables alter the interaction strength of microbes and fewer phyla are detected, indicating their high adaptability to e-waste-contaminated soils. It is predicted that some key factors not detected in this study, such as temperature, vegetation, oxygen level, and moisture content, may also significantly affect microbial communities (Fierer et al., 2003; Hofmann et al., 2016).

The module hubs and connectors of the network may be key phyla playing crucial roles in microbial communities (Montoya et al., 2006). From the Z-P plot, no connectors were detected in the present study (Fig. 5), possibly due to the fact that heavy contamination in e-waste-contaminated soils splits the connection among different modules. Five module hubs were identified through the Z-P plot (Fig. 5, Table 4). Iii1-15 affiliating with Acidobacteria and Piscirickettsiaceae are uncultured and no information about their function has been reported to date. Bradyrhizobiaceae and Nitrospira are related to nitrogen metabolism (Feng et al., 2016; Vila-Costa et al., 2014), indicating the strong link and interaction between the nitrogen cycle and microbes in e-waste contaminated soils. Kaistobacter (Sphingomonadaceae) exhibits a powerful ability to degrade pollutants, such as the synthetic organochloride hexachlorocyclohexane and PCBs (Hu et al., 2015; Pearce et al., 2015), suggesting its importance in microbial community network structure.

In this study, we make an in-depth discussion on microbial community profiles in e-waste contaminated soils, based on ecological network, to determine whether influencing factors vary over a long distance. Our results indicate that microbial communities at e-waste recycling sites are following the EisE hypothesis. Factors influencing microbial communities (microbial abundance, diversity, and interrelationships) include soil properties (TOC, TN, and pH), organic pollutants (PAHs, PCBs, and PBDEs) and heavy metals (Cu, Zn, and Pb). The limited roles of soil pH are explained by the intensive levels of organic, heavy metal contamination and microbial community resilience. Additionally, we firstly reported the roles of pollutants and nutrition on the microbial ecological network in e-waste polluted soils, helping us understand key factors affecting microbial co-occurrence patterns, which may be important for various functions of microbial community (Faust and Raes, 2012). Due to the limitation of small sample size in this study, future work should cover more sites and larger sample size to disclose more universal and reliable fundamentals of microbial community behavior at e-waste polluted sites.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.08.003.

References

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. U. S. A. 105, 11512–11519.

Calugaru, I.L., Neculita, C.M., Genty, T., Bussiere, B., Potvin, R., 2016. Performance of thermally activated dolomite for the treatment of Ni and Zn in contaminated neutral drainage. J. Hazard. Mater. 310, 48–55.

Cantarel, A.A.M., Bloor, J.M.G., Pommier, T., Guillaumaud, N., Moirot, C., Soussana, J.F., Poly, F., 2012. Four years of experimental climate change modifies the microbial drivers of N2O fluxes in an upland grassland ecosystem. Glob. Change Biol. 18, 2520–2531.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Chapman, E.E.V., Dave, G., Murimboh, J.D., 2013. A review of metal (Pb and Zn) sensitive and pH tolerant bioassay organisms for risk screening of metal-contaminated acidic soils. Environ. Pollut. 179, 326–342.

Chen, C., Yang, K., Yu, C.N., Qin, Z.H., Huang, R.L., Tang, X.J., Shen, C.F., Shi, H.X., 2015a. Polychlorinated biphenyls attenuation in soil from E-waste recycling area under flooded and dryland conditions. Clean-Soil Air Water 43, 584–591.

Chen, J.H., He, F., Zhang, X.H., Sun, X., Zheng, J.F., Zheng, J.W., 2014. Heavy metal pollution decreases microbial abundance, diversity and activity within particle-size fractions of a paddy soil. Fems Microbiol. Ecol. 87, 164–181.

Chen, L., Zhang, W., Zhang, R., Lin, K., He, L., Wu, L., 2015b. The bioavailability and adverse impacts of lead and decabromodiphenyl ether on soil microbial activities. Environ. Sci. Pollut. Res. Int. 22, 12141–12149.

Chow, C.E.T., Kim, D.Y., Sachdeva, R., Caron, D.A., Fuhrman, J.A., 2014. Top-down controls on bacterial community structure: microbial network analysis of bacteria, T4-like viruses and protists. Isme J. 8, 816–829.

Correa, P.A., Lin, L.S., Just, C.L., Hu, D.F., Hornbuckle, K.C., Schnoor, J.L., Van Aken, B., 2010. The effects of individual PCB congeners on the soil bacterial community structure and the abundance of biphenyl dioxygenase genes. Environ. Int. 36, 901–906.

Deng, Y., Jiang, Y.H., Yang, Y.F., He, Z.L., Luo, F., Zhou, J.Z., 2012. Molecular ecological network analyses. Bmc Bioinforma. 13, 113.

Dumbrell, A.J., Nelson, M., Helgason, T., Dytham, C., Fitter, A.H., 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. Isme J. 4, 337–345.

Earl, A.M., Losick, R., Kolter, R., 2008. Ecology and genomics of Bacillus subtilis. Trends Microbiol. 16, 269–275.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.

Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10, 538–550.

Feng, G.F., Sun, W., Zhang, F.L., Karthik, L., Li, Z.Y., 2016. Inhabitancy of active Nitrosopumilus-like ammonia-oxidizing archaea and Nitrospira nitrite-oxidizing bacteria in the sponge Theonella swinhoei. Sci. Rep. 6, 24966.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 103, 626–631.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Influence of drying-rewetting frequency on soil bacterial community structure. Microb. Ecol. 45, 63–71.

Gomez-Balderas, C.D.C., Cochet, N., Bert, V., Tarnaud, E., Sarde, C.O., 2014. 16S rDNA analysis of bacterial communities associated with the hyper accumulator Arabidopsis halleri grown on a Zn and Cd polluted soil. Eur. J. Soil Biol. 60, 16–23.

Green, J.L., Holmes, A.J., Westoby, M., Oliver, I., Briscoe, D., Dangerfield, M., Gillings, M., Beattie, A.J., 2004. Spatial scaling of microbial eukaryote diversity. Nature 432, 747–750.

Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial biogeography of British soils. Environ. Microbiol. 13, 1642–1654.

Guo, X.L., Gu, J., Gao, H., Qin, Q.J., Chen, Z.X., Shao, L., Chen, L., Li, H.L., Zhang, W.J., Chen, S.N., Liu, J., 2012. Effects of Cu on metabolisms and enzyme activities of microbial communities in the process of composting. Bioresour. Technol. 108, 140–148.

Hansen, S.K., Rainey, P.B., Haagensen, J.A.J., Molin, S., 2007. Evolution of species interactions in a biofilm community. Nature 445, 533–536.

Hedges, J.I., Stern, J.H., 1984. Carbon and nitrogen determinations of carbonate-containing solids. Limnol. Oceanogr. 29, 657–663.

Hofmann, K., Lamprecht, A., Pauli, H., Illmer, P., 2016. Distribution of prokaryotic abundance and microbial nutrient cycling across a high-alpine altitudinal gradient in the austrian central alps is affected by vegetation, temperature, and soil nutrients. Microb. Ecol. 72, 704–716.

Horvath, S., Dong, J., 2008. Geometric interpretation of gene coexpression network analysis. Plos Comput. Biol. 4, e1000117.

Hu, J.X., Qian, M.R., Zhang, Q., Cui, J.L., Yu, C.N., Su, X.M., Shen, C.F., Hashmi, M.Z., Shi, J.Y., 2015. Sphingobium fuliginis HC3: a novel and robust isolated biphenyl- and polychlorinated biphenyls-degrading bacterium without dead-end intermediates accumulation. Plos One 10, e0122740.

Huang, D.Y., Liu, C.P., Li, F.B., Liu, T.X., Liu, C.S., Tao, L., Wang, Y., 2014. Profiles, sources, and transport of polycyclic aromatic hydrocarbons in soils affected by electronic waste recycling in Longtang, south China. Environ. Monit. Assess. 186, 3351–3364.

Jiang, Y.F., Yves, U.J., Sun, H., Hu, X.F., Zhan, H.Y., Wu, Y.Q., 2016. Distribution, compositional pattern and sources of polycyclic aromatic hydrocarbons in urban soils of an industrial city, Lanzhou, China. Ecotoxicol. Environ. Saf. 126, 154–162.

Johnston, G.P., Leff, L.G., 2015. Bacterial community composition and biogeochemical heterogeneity in PAH-contaminated riverbank sediments. J. Soils Sediments 15, 225–239.

Jones, M.D., Crandell, D.W., Singleton, D.R., Aitken, M.D., 2011. Stable-isotope probing of the polycyclic aromatic hydrocarbon-degrading bacterial guild in a contaminated soil. Environ. Microbiol. 13, 2623–2632.

Jones, S.E., Lennon, J.T., 2010. Dormancy contributes to the maintenance of microbial diversity. Proc. Natl. Acad. Sci. U. S. A. 107, 5881–5886.

Krave, A.S., Lin, B., Braster, M., Laverman, A.M., van Straalen, N.M., Roling, W.F.M., van Verseveld, H.W., 2002. Stratification and seasonal stability of diverse bacterial communities in a Pinus merkusii (pine) forest soil in central Java, Indonesia. Environ. Microbiol. 4, 361–373.

Lessard, I., Sauve, S., Deschenes, L., 2014. Toxicity response of a new enzyme-based functional diversity methodology for Zn-contaminated field-collected soils. Soil Biol. Biochem. 71, 87–94.

Li, J., Wang, J.T., Hu, H.W., Ma, Y.B., Zhang, L.M., He, J.Z., 2016. Copper pollution

decreases the resistance of soil microbial community to subsequent dry-rewetting disturbance. J. Environ. Sci. (China) 39, 155–164.

Liu, J., He, X.X., Lin, X.R., Chen, W.C., Zhou, Q.X., Shu, W.S., Huang, L.N., 2015. Ecological effects of combined pollution associated with E-waste recycling on the composition and diversity of soil microbial communities. Environ. Sci. Technol. 49, 6438–6447.

Liu, L., Zhu, W., Xiao, L., Yang, L.Y., 2011. Effect of decabromodiphenyl ether (BDE 209) and dibromodiphenyl ether (BDE 15) on soil microbial activity and bacterial community composition. J. Hazard. Mater. 186, 883–890.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235.

Luo, C.L., Wang, S.R., Wang, Y., Yang, R.X., Zhang, G., Shen, Z.G., 2015. Effects of EDDS and plant-growth-promoting bacteria on plant uptake of trace metals and PCBs from e-waste-contaminated soil. J. Hazard. Mater. 286, 379–385.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Ovreas, L., Reysenbach, A.L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. Nat. Rev. Microbiol. 4, 102–112.

Martiny, J.B.H., Eisen, J.A., Penn, K., Allison, S.D., Horner-Devine, M.C., 2011. Drivers of bacterial beta-diversity depend on spatial scale. Proc. Natl. Acad. Sci. U. S. A. 108, 7850–7854.

Montoya, J.M., Pimm, S.L., Sole, R.V., 2006. Ecological networks and their fragility. Nature 442, 259–264.

Muckian, L.M., Grant, R.J., Clipson, N.J.W., Doyle, E.M., 2009. Bacterial community dynamics during bioremediation of phenanthrene- and fluoranthene-amended soil. Int. Biodeterior. Biodegrad. 63, 52–56.

Mukherjee, S., Juottonen, H., Siivonen, P., Quesada, C.L., Tuomi, P., Pulkkinen, P., Yrjala, K., 2014. Spatial patterns of microbial diversity and activity in an aged creosote-contaminated site. Isme J. 8, 2131–2142.

Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Heavy metal removal from sediments by biosurfactants. J. Hazard. Mater. 85, 111–125.

Nogales, B., Moore, E.R.B., Abraham, W.R., Timmis, K.N., 1999. Identification of the metabolically active members of a bacterial community in a polychlorinated biphenyl polluted moorland soil. Environ. Microbiol. 1, 199–212.

Pakzad, H.R., Pasandi, M., Yeganeh, S., Alizadeh Ketek Lahijani, H.A., 2016. Assessment of heavy metal enrichment in the offshore fine-grained sediments of the Caspian Sea. Environ. Monit. Assess. 188, 303.

Pearce, S.L., Oakeshott, J.G., Pandey, G., 2015. Insights into ongoing evolution of the hexachlorocyclohexane catabolic pathway from comparative genomics of ten sphingomonadaceae strains. G3-Genes Genomes Genet. 5, 1081–1094.

Petric, I., Bru, D., Udikovic-Kolic, N., Hrsak, D., Philippot, L., Martin-Laurent, F., 2011. Evidence for shifts in the structure and abundance of the microbial community in a long-term PCB-contaminated soil under bioremediation. J. Hazard. Mater. 195, 254–260.

Robinson, B.H., 2009. E-waste: an assessment of global production and environmental impacts. Sci. Total Environ. 408, 183–191.

Sawulski, P., Clipson, N., Doyle, E., 2014. Effects of polycyclic aromatic hydrocarbons on microbial community structure and PAH ring hydroxylating dioxygenase gene abundance in soil. Biodegradation 25, 835–847.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

Shade, A., Read, J.S., Youngblut, N.D., Fierer, N., Knight, R., Kratz, T.K., Lottig, N.R., Roden, E.E., Stanley, E.H., Stombaugh, J., Whitaker, R.J., Wu, C.H., McMahon, K.D., 2012. Lake microbial communities are resilient after a whole-ecosystem disturbance. Isme J. 6, 2153–2167.

Song, J., Zhao, F.J., Luo, Y.M., McGrath, S.P., Zhang, H., 2004. Copper uptake by Elsholtzia splendens and Silene vulgaris and assessment of copper phytoavailability in contaminated soils. Environ. Pollut. 128, 307–315.

Sullivan, T.S., McBride, M.B., Thies, J.E., 2013. Soil bacterial and archaeal community

composition reflects high spatial heterogeneity of pH, bioavailable Zn, and Cu in a metalliferous peat soil. Soil Biol. Biochem. 66, 102–109.

Tang, X.J., Shen, C.F., Shi, D.Z., Cheema, S.A., Khan, M.I., Zhang, C.K., Chen, Y.X., 2010. Heavy metal and persistent organic compound contamination in soil from Wenling: an emerging e-waste recycling city in Taizhou area, China. J. Hazard. Mater. 173, 653–660.

Tang, X.J., Zeng, B., Hashmi, M.Z., Long, D.Y., Yu, B.B., Ullah, N., Shen, C.F., Chen, Y.X., 2014. PBDEs and PCDD/Fs in surface soil taken from the Taizhou e-waste recycling area, China. Chem. Ecol. 30, 245–251.

Taylor, J.W., Turner, E., Townsend, J.P., Dettman, J.R., Jacobson, D., 2006. Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. Phil. Trans. R. Soc. B-Biol. Sci. 361, 1947–1963.

Van der Gucht, K., Cottenie, K., Muylaert, K., Vloemans, N., Cousin, S., Declerck, S., Jeppesen, E., Conde-Porcuna, J.M., Schwenk, K., Zwart, G., Degans, H., Vyverman, W., De Meester, L., 2007. The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. Proc. Natl. Acad. Sci. U. S. A. 104, 20404–20409.

Vila-Costa, M., Bartrons, M., Catalan, J., Casamayor, E.O., 2014. Nitrogen-cycling genes in epilithic biofilms of oligotrophic high-altitude lakes (central pyrenees, Spain). Microb. Ecol. 68, 60–69.

Vila, J., Nieto, J.M., Mertens, J., Springael, D., Grifoll, M., 2010. Microbial community structure of a heavy fuel oil-degrading marine consortium: linking microbial dynamics with polycyclic aromatic hydrocarbon utilization. Fems Microbiol. Ecol. 73, 349–362.

Wang, S.R., Wang, Y., Lei, W.R., Sun, Y.T., Wang, Y.J., Luo, C.L., Zhang, G., 2015. Simultaneous enhanced removal of Cu, PCBs, and PBDEs by corn from e-waste-contaminated soil using the biodegradable chelant EDDS. Environ. Sci. Pollut. Res. 22, 18203–18210.

Watts, D.J., Strogatz, S.H., 1998. Collective dynamics of 'small-world' networks. Nature 393, 440–442.

Wong, M.H., Wu, S.C., Deng, W.J., Yu, X.Z., Luo, Q., Leung, A.O.W., Wong, C.S.C., Luksemburg, W.J., Wong, A.S., 2007. Export of toxic chemicals - a review of the case of uncontrolled electronic-waste recycling. Environ. Pollut. 149, 131–140.

Yin, H.Q., Niu, J.J., Ren, Y.H., Cong, J., Zhang, X.X., Fan, F.L., Xiao, Y.H., Zhang, X., Deng, J., Xie, M., He, Z.L., Zhou, J.Z., Liang, Y.L., Liu, X.D., 2015. An integrated insight into the response of sedimentary microbial communities to heavy metal contamination. Sci. Rep. 5, 14266.

Zeng, J., Zhu, Q.H., Wu, Y.C., Lin, X.G., 2016. Oxidation of polycyclic aromatic hydrocarbons using Bacillus subtilis CotA with high laccase activity and copper independence. Chemosphere 148, 1–7.

Zhang, W., Chen, L., An, S., Liu, K., Lin, K.F., Fu, R.B., 2015. Effects of the joint exposure of decabromodiphenyl ether and tetrabromobisphenol A on soil bacterial community structure. Environ. Sci. Pollut. Res. 22, 1054–1065.

Zhang, W., Wang, H., Zhang, R., Yu, X.Z., Qian, P.Y., Wong, M.H., 2010. Bacterial communities in PAH contaminated soils at an electronic-waste processing center in China. Ecotoxicology 19, 96–104.

Zhang, W., Zhang, M., An, S., Lin, K., Li, H., Cui, C., Fu, R., Zhu, J., 2012a. The combined effect of decabromodiphenyl ether (BDE-209) and copper (Cu) on soil enzyme activities and microbial community structure. Environ. Toxicol. Pharmacol. 34, 358–369.

Zhang, W., Zhang, M., An, S., Lin, K.F., Li, H., Cui, C.Z., Fu, R.B., Zhu, J., 2012b. The combined effect of decabromodiphenyl ether (BDE-209) and copper (Cu) on soil enzyme activities and microbial community structure. Environ. Toxicol. Pharmacol. 34, 358–369.

Zhao, W.T., Ding, L., Gu, X.W., Luo, J., Liu, Y.L., Guo, L., Shi, Y., Huang, T., Cheng, S.G., 2015. Levels and ecological risk assessment of metals in soils from a typical e-waste recycling region in southeast China. Ecotoxicology 24, 1947–1960.

Zhou, J.Z., Deng, Y., Luo, F., He, Z.L., Tu, Q.C., Zhi, X.Y., 2010. Functional molecular ecological networks. Mbio 1 (4) e00169–10.

Zhou, J.Z., Deng, Y., Luo, F., He, Z.L., Yang, Y.F., 2011. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO2. Mbio 2 (4) e00122–11.