



# Ecological effects of soil properties and metal concentrations on the composition and diversity of microbial communities associated with land use patterns in an electronic waste recycling region

Wencheng Wu<sup>a,b</sup>, Changxun Dong<sup>c</sup>, Jiahui Wu<sup>b</sup>, Xiaowen Liu<sup>b</sup>, Yingxin Wu<sup>b</sup>, Xianbin Chen<sup>b</sup>, Shixiao Yu<sup>a,\*</sup>

<sup>a</sup> School of Life Science/State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, PR China

<sup>b</sup> South China Institute of Environmental Sciences, Ministry of Environmental Protection, Guangzhou 510655, PR China

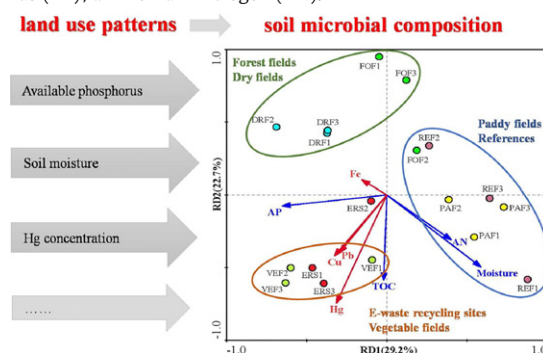
<sup>c</sup> College of Sciences, Nanjing Agricultural University, Nanjing 210095, PR China

## HIGHLIGHTS

- E-waste recycling activities affected soil microbial communities in fields.
- Microbial community composition was strongly influenced by land use patterns.
- Microbial assemblages were primarily influenced by available P, moisture and mercury.
- Mercury concentration was the key factor regulating microbial community diversity.
- *Nitrososphaera*, *Solibacter*, and *Nitrospira* dominated in e-waste recycling sites.

## GRAPHICAL ABSTRACT

Redundancy analyses of microbial data and a subset of eight environmental variables. Paddy field (PAF); vegetable field (VEF); dry field (DRF); forest field (FOF); e-waste recycling site (ERS); reference site (REF); copper (Cu); mercury (Hg); lead (Pb); iron (Fe); moisture content (moisture); total organic carbon (TOC); available phosphorus (AP); ammonium nitrogen (AN).



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## ABSTRACT

Soil microbes play vital roles in ecosystem functions, and soil microbial communities may be strongly structured by land use patterns associated with electronic waste (e-waste) recycling activities, which can increase the heavy metal concentration in soils. In this study, a suite of soils from five land use types (paddy field, vegetable field, dry field, forest field, and e-waste recycling site) were collected in Longtang Town, Guangdong Province, South China. Soil physicochemical properties and heavy metal concentrations were measured, and the indigenous microbial assemblages were profiled using 16S rRNA high-throughput sequencing and clone library analyses. The results showed that mercury concentration was positively correlated with both Faith's PD and Chao1 estimates, suggesting that the soil microbial alpha diversity was predominantly regulated by mercury. In addition, redundancy analysis indicated that available phosphorus, soil moisture, and mercury were the three major drivers affecting the microbial assemblages. Overall, the microbial composition was determined primarily by land use patterns, and this study provides a novel insight on the composition and diversity of microbial communities in soils associated with e-waste recycling activities.

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\* Corresponding author.

E-mail address: [lssyx@mail.sysu.edu.cn](mailto:lssyx@mail.sysu.edu.cn) (S. Yu).

## 1. Introduction

Landscapes dominated by human activities, including agriculture, industry, and forestry can fundamentally alter soil quality and nutrient cycling (e.g., C, N, and P), which consequently structure the soil microbial communities (Guo et al., 2016; Mganga et al., 2016). Microbial communities participate in specific soil biochemical reactions, and these communities play important roles in the regulation of soil nutrients and ecosystem processes. Thus, microbial communities can also have a strong influence on soil quality and plant biomass production (Hernández et al., 2016; Tischer et al., 2015).

Microbial community composition and diversity are increasingly studied because they are sufficiently sensitive to provide integrative information regarding environmental change, and numerous studies have attempted to elucidate the factors that influence microbes in various habitats. For instance, on the basis of the investigations of natural forest, shrub land, young pasture, old pasture, re-established pasture, and abandoned pasture sites, Tischer et al. (2015) reported that the structure of soil microbial communities was well adapted to environmental changes along land use sequences. In addition, soil pH, carbon content, and C:N ratio were considered the predominant environmental drivers. Moreover, one study in metal-contaminated forests nearby a Pb/Zn industrial region revealed that the structure and diversity of the soil microbial community depended mainly on soil pH (Chodak et al., 2013). Similarly, Jiang et al. (2016) studied the soil bacterial communities within the rhizosphere of a Cu-tolerant plant and found that soil pH, rather than Cu concentration, was the most important environmental factor affecting the bacterial community. Comparatively, the findings of another study conducted in the grassland of a Pb/Zn mining region revealed that high nutrient availability and plant species richness could apparently counteract the toxic effects of metal contamination, which subsequently proved beneficial for soil microbes (Stefanowicz et al., 2012). These studies identified distinct environmental drivers that influence microbes, suggesting that soil microbial communities might be well adapted to local environmental variation.

In recent decades, with the rapid development of the electronics industry and information technology, electronic waste (e-waste) has become an emerging environmental issue worldwide, and most e-waste has been transported to developing countries. Even worse, informal e-waste recycling processes, such as manual disassembling, open incineration, and acid dipping are extensively employed, and these activities have been identified as important sources of environmental contaminants (e.g., heavy metals) in e-waste recycling regions (Song and Li, 2014), owing to the common use of various heavy metals in electronic products (Robinson, 2009). In this regard, Luo et al. (2011) found that the concentrations of Cd, Cu, Pb, and Zn in the soils of former incineration sites reached 17, 11,140, 4500, and 3690 mg kg<sup>-1</sup>, respectively. In addition, Wu et al. (2015) also found that the surface soils of former burning and acid-leaching sites suffered from severe contamination by multiple metals. Nevertheless, the responses of microbial communities to metal contamination and the properties of soils under different land use patterns have not been well explored. In particular, given the uncontrolled and informal nature of e-waste recycling, the resulting contamination might be remarkably different than those caused by industrial or mining activities. Therefore, a deeper understanding of the changes in microbial community structure in relation to land use would greatly benefit land management and restoration in e-waste recycling regions.

Here, an in-depth and comprehensive investigation of the microbial assemblages in soils from area under different land use in a notorious e-waste recycling region was conducted. In particular, the study aimed to elucidate the composition and diversity of soil microbial communities in the e-waste recycling region and to examine how microbial assemblages are shaped by physicochemical properties and heavy metals in the soils associated with different land use patterns.

## 2. Materials and methods

### 2.1. Study area

Longtang, a town located in the city of Qingyuan, Guangdong Province, South China (Fig. 1), has been a booming e-waste recycling aggregative region since the 1990s. It has a typical subtropical monsoon climate with annual temperature and rainfall of 22.6 °C and 1700 mm, respectively. The prevailing winds move from the southeast to the northeast throughout the year. The air, water, and soil of the region have been heavily contaminated by multiple metals because of crude e-waste recycling activities in a large number of simple household e-waste recycling workshops (Leung et al., 2008; Wu et al., 2015).

### 2.2. Sample collection

Soil samples were collected in May 2016. Representative soil samples were collected at depths of 0–20 cm from three e-waste recycling sites (ERS1, ERS2, and ERS3), three paddy fields (PAF1, PAF2, and PAF3), three vegetable fields (VEF1, VEF2, and VEF3), three dry fields (DRF1, DRF2, and DRF3) and three forest fields (FOF1, FOF2, and FOF3), all of which represent the most typical land use types in the studied region. The VEF are cultivated with various vegetables (e.g., lettuce, Chinese cabbage, and celery), and the ERS have been developed from VEF since the 1990s. Both the VEF and ERS are located along village edges. The DRF are planted with xerophilous crops, and the sites are close to FOF, which are dominated by pine, eucalyptus, and shrubs. Both DRF and FOF have relatively higher elevations and do not require irrigation, comparing to other fields. On the other hand, the PAF are frequently irrigated and cultivated with rice, particularly representing the major agricultural fields in the studied region and attracting much attention owing to the considerable heavy metal contamination in soils and rice grains (Fu et al., 2013). Thus, three reference soil samples were also collected from paddy fields (REF1, REF2, and REF3) that were located 10 km upstream and upwind from the study area. These sites were unlikely to be affected by e-waste recycling activities. The coordinates of the sampling location were determined using a portable GPS unit (Table S1). Each sample consisted of five subsamples collected randomly in a 100 m<sup>2</sup> area. All samples were collected using a bamboo spade, transported to the laboratory in cooler boxes, and then stored at 4 °C prior to subsequent analyses.

### 2.3. Analyses of soil properties and metal concentrations

Moisture content was determined by weighing subsamples before and after oven-drying at 105 °C until a constant weight was reached. The samples were air-dried and ground prior to passage through 10-mesh (i.e., 2 mm) and 100-mesh (i.e., 0.147 mm) sieves in preparation for the determination of the physicochemical properties and the concentrations of heavy metal. The pH and electrical conductivity (EC) were measured using a pH meter and EC meter (model 744, Metrohm, Switzerland), respectively, in 1:2.5 (w/v) H<sub>2</sub>O suspensions after 1 h of shaking. Total organic carbon (TOC) was measured using a TOC-L analyzer (Shimadzu, Japan). Total nitrogen (TN) was determined using the Kjeldahl method (AOAC, 1990), and ammonium nitrogen (AN) was colorimetrically determined using a 752 N spectrophotometer (LengGuang Tech, China). Total phosphorus (TP) and available phosphorus (AP, NaHCO<sub>3</sub> extractable fraction) were determined using the Olsen method (Olsen et al., 1954), and available potassium (AK) was analyzed using the ammonium acetate method (Haby et al., 1990). Total potassium (TK) and metals concentrations (including those of Fe, Mn, Cr, Cu, Ni, Pb, and Zn) were determined using inductively coupled plasma-optical emission spectrophotometry (710, Agilent, USA) after acid digestion (HCl, HNO<sub>3</sub>, HF, and HClO<sub>4</sub>). The Cd concentration was measured using graphite furnace-atomic absorption spectrometry (PinAAcle 900 T, Perkin Elmer, USA) due to the low concentrations in

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