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Soil Microbiomes Reversely Respond to Heavy Metals and Polycyclic Aromatic Hydrocarbons in Highly Contaminated Industrial Sites

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Abstract:	Industrial sites from e-waste dismantling plants and coking plants are characterized by severe pollution with heavy metals (HMs) and/or polycyclic aromatic hydrocarbons (PAHs) in soil. Mixed contaminations (HMs+PAHs) complicate land reclamation and influence the microbial diversity and function of soil microbiomes. In this study, we determined HMs and PAHs contaminations of an e-waste dismantling plant and a coking plant, and evaluated the influences of HMs and PAHs on soil microbiomes. The

	microbiomes (diversity and abundance) of all sites were determined using high-throughput sequencing of 16S rRNA genes, and canonical correlation analysis was conducted to investigate the relations between soil microbiomes and contaminants. The e-waste dismantling plant was polluted mainly with HMs (such as 2,379.07±227.46 mg/kg of Mn) and slightly with PAHs (10.36±0.74 mg/kg), whereas the coking plant was contaminated severely with PAHs (12,558.06±611.19 mg/kg) and moderately with HMs (such as 672.61±7.13 mg/kg of Mn). The abundances of bacterial taxa such as MND1, Bryobacter and Galiella were positively related to the concentration of HMs, whereas the abundance of genera such as Sulfuritalea , Pseudomonas and Sphingobium were positively related to the concentration of PAHs. To our knowledge, we found for the first time that HMs and PAHs in the severely contaminated industrial sites reversely changed the soil microbiomes. This study contributes to the understanding of the combined effect of HMs and PAHs on soil microbiomes.
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Dear Editors:

With the agreement from all listed authors, I submit a manuscript entitled "*Soil Microbiomes Reversely Respond to Heavy Metals and Polycyclic Aromatic Hydrocarbons in Highly Contaminated Industrial Sites*"(authors: Zhen-Ni Yang, Ze-Shen Liu, Ke-Huan Wang, Zong-Lin Liang, Rashidin Abdugheni, Ye Huang, Run-Hua Wang, Hong-Lin Ma, Xiao-Kang Wang, Mei-Ling Yang, Bing-Ge Zhang, De-Feng Li, Cheng-Ying Jiang, Philippe F.-X. Corvini and Shuang-Jiang Liu) for your consideration as an article in *Environmental Science and Ecotechnology*. We confirm that the manuscript has not been published previously by any of the authors and is not under consideration for publication in another journal at the time of submission.

We believe that this article is of great interest to the readership of *Environmental Science and Ecotechnology*. Soil contaminations from industries have attracted serious concerns. Electronic waste (e-waste) dismantling and coking are important contamination sources of heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs). The mixed contaminations (HMs+PAHs) affected the diversity and function of the soil microbiomes. Intensive understanding of how soil microbiomes interact with contaminants is pivotal to develop more effective processes for bioremediation.

In the study, we aimed to determine the contaminations of HMs and PAHs in an e-waste dismantling plant and a coking plant, and investigate the effect of the mixed contamination on the soil microbiomes. We found that the e-waste dismantling plant site was featured by heavy contamination of HMs but minor PAHs, whereas the coking plant site was featured by serious contamination of PAHs and moderate HMs. The abundance of bacterial genera such as *Sulfuritalea* increased in soil contaminated severely with PAHs; however, other bacterial genera such as *Bryobacter* increased in soil polluted with HMs. To our knowledge, we found for the first time that HMs and PAHs in the severely contaminated industrial sites reversely changed the soil microbiomes. This study promotes the understanding of the mixed effect of HMs and PAHs on soil microbiomes.

We believe that the present results will be of immediate interest to scientists who have interest in soil microbiomes and mixed contaminations of HMs and PAHs. For these reasons, we believe that this manuscript warrants publication in Article form in *Environmental Science and Ecotechnology*.

Thank you very much for your kind consideration. We are looking forward to hearing from you soon.

Yours sincerely,

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Soil Microbiomes Reversely Respond to Heavy Metals and Polycyclic Aromatic Hydrocarbons in Highly Contaminated Industrial Sites

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Abstract: Industrial sites from e-waste dismantling plants and coking plants are characterized by severe pollution with heavy metals (HMs) and/or polycyclic aromatic hydrocarbons (PAHs) in soil. Mixed contaminations (HMs+PAHs) complicate land reclamation and influence the microbial diversity and function of soil microbiomes. In this study, we determined HMs and PAHs contaminations of an e-waste dismantling plant and a coking plant, and evaluated the influences of HMs and PAHs on soil microbiomes. The microbiomes (diversity and abundance) of all sites were determined using high-throughput sequencing of 16S rRNA genes, and canonical correlation analysis was conducted to investigate the relations between soil microbiomes and contaminants. The e-waste dismantling plant was polluted mainly with HMs (such as 2,379.07±227.46 mg/kg of Mn) and slightly with PAHs (10.36±0.74 mg/kg), whereas the coking plant was contaminated severely with PAHs (12,558.06±611.19 mg/kg) and moderately with HMs (such as 672.61±7.13 mg/kg of Mn). The abundances of bacterial taxa such as MND1, *Bryobacter* and *Galiella* were positively related to the concentration of HMs, whereas the abundance of genera such as *Sulfuritalea*, *Pseudomonas* and *Sphingobium* were positively related to the concentration of PAHs. To our knowledge, we found for the first time that HMs and PAHs in the severely contaminated industrial sites reversely changed the soil microbiomes. This study contributes to the understanding of the combined effect of HMs and PAHs on soil microbiomes.

Keywords: soil microbiomes; e-waste; coking plant; heavy metal; polycyclic aromatic hydrocarbons

1. Introduction

Soil contaminations resulting from industrial activities have raised serious concerns (Park *et al.* 2021, Zhao *et al.* 2021). Currently, industrial processes such as electronic waste (e-waste) dismantling, metal smelting and coking are important sources of contamination with heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) (Guo *et al.* 2021, Kong *et al.* 2021). Previous studies demonstrated that mixed contaminations with HMs and PAHs have different influences on the diversity and community structure of the soil microbiomes compared to single contamination (Gauthier *et al.* 2014). Intensive understanding of how soil microbiomes responding to mixed pollution is essential to develop more effective bioremediation processes (Lee *et al.* 2018).

HMs widely occur in soil (Li *et al.* 2021b) and human activities such as industrial processes might lead to high concentration of HMs in soil (Zhou *et al.* 2016). HMs such as Cd, Cr and Pb are toxic to organisms even at low concentration (Rehman *et al.* 2018, Seleiman *et al.* 2020, Usman *et al.* 2013). Studies have reported that soil microbiomes respond and adapt to HMs, resulting in microbial metabolic activity, population diversity and abundance changes (Beattie *et al.* 2018, Lin *et al.* 2019b, Zhao *et al.* 2020). For example, some species of *Achromobacter* and *Bacillus* were able to eliminate the toxicity of some HMs (*e.g.*, Cr, Mn and As) via adsorption, oxidation and reduction mechanisms (Bachate *et al.* 2013, Yin *et al.* 2019). PAHs are also relevant soil pollutants (Marsh *et al.* 2005) and are released into the environment

as a consequence of industrial processes and human activities, *e.g.*, incomplete combustion and pyrolysis of organic substances, transportation and waste incineration (Song *et al.* 2021, Wheatley and Sadhra 2004, Zhang and Wang 2011). PAHs cause great concerns due to their high toxicity and low bioavailability (Tomczyk *et al.* 2020). Besides, PAHs represent a threat to human health due to their teratogenic, carcinogenic, and mutagenic properties (Wu *et al.* 2021). PAHs, especially those with more than four benzene rings, are recalcitrant to degradation in the environment, owing to their high hydrophobicity and their low bioavailability (Gupta *et al.* 2019). Soil contamination with PAHs resulted in the decrease of soil microbial diversity and abundance and metabolic function. The abundances of genera such as *Rhizobacter*, *Sphingobium*, *Mycobacterium*, *Bacillus* and *Pseudarthrobacter* increased in PAHs-contaminated soil (Li *et al.* 2019).

Many contaminated sites are affected with both HMs and PAHs, and mixed contamination has caused increasing concerns (Liu *et al.* 2017). The effects of mixed contamination with HMs and PAHs on microbes and environments are complex and difficult to understand (Haarstad *et al.* 2012). Studies demonstrated that HMs affect PAH degradation (Guo *et al.* 2010, Zhang *et al.* 2011) and conversely, that PAHs altered the transport of HMs (Cao *et al.* 2008, Gorria *et al.* 2006). In addition, the effect of mixed contamination is species-and-dose-dependent. For example, the microbial adsorption and degradation of PAHs were dependent on both the bacterial species and concentration of HMs (Chen *et al.* 2016, Yin *et al.* 2020). The adsorption

of phenanthrene on cyanobacteria cells decreased with the increase of Cu(II) concentration below 0.04 mM, but enhanced with increasing Cu(II) concentration in the range from 0.04 mM to 0.2 mM (Tao *et al.* 2013). Bourceret *et al*. (Bourceret *et al.* 2016) found that *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the dominant phyla in soil contaminated with HMs and PAHs. Gran-Scheuch *et al*. (Gran-Scheuch *et al.* 2020) reported that the population of PAHs degraders or metal resistant bacteria increased in the soil of King George Island (Antarctica) where HMs and PAHs were detected. Although attention has been paid to mixed contamination with HMs and PAHs (Lin *et al.* 2019a, Picariello *et al.* 2020), the understanding of how microbiomes respond and adapt to mixed contamination is still limited (Liu *et al.* 2017).

In this study, we aimed to determine the level of contamination with HMs and PAHs in an e-waste dismantling plant and a coking plant and to investigate the effect of this mixed contamination on the soil microbiomes. We found that the e-waste dismantling plant was polluted mainly with HMs and slightly with PAHs, whereas the coking plant was contaminated severely with PAHs and moderately with HMs. The abundance of bacterial taxa such as MND1, *Bryobacter* and *Galiella* increased with the increasing concentration of HMs, whereas that of *Sulfuritalea*, *Pseudomonas* and *Sphingobium* increased with the increasing concentration of PAHs. To our knowledge, we report for the first time that HMs and PAHs in severely contaminated industrial sites reversely changed the soil microbiomes.

2. Materials and Methods

2.1. Soil sampling

An initial investigation of the level of soil contamination with HMs and PAHs was carried out at an e-waste dismantling plant and a coking plant. The two plants were located 48.8 km far from each other and showed similar climatic features. Based on the degree of contamination with HMs and PAHs, five samples (annotated samples A-E) were selected for further investigation (Table 1).

Table 1. Information of sampling sites.

Samples	Sampling sites	Characteristics[1]	Depth (cm)
A	Paddy field (control)	HMs (I) and PAHs (I)	0–30
B	E-waste dismantling plant	HMs (V) and PAHs (II)	0–30
C	Coking plant	HMs (III) and PAHs (III)	0–30
D	Coking plant	HMs (IV) and PAHs (IV)	0–30
E	Coking plant	HMs (II) and PAHs (V)	0–30

[1] Roman numbers from 'I' (low) to 'V' (high) refer to the contamination levels of HMs and PAHs according to their total concentration. The concentration of HMs and PAHs in detail were given in Figure 1.

Sample A collected from the paddy field near the e-waste dismantling plant served as the negative control in this study. Sample B was obtained from the e-waste dismantling plant, whereas samples C-E were from the coking plant. In the coking plant, sample C was collected from the field where coal and iron were stacked, sample D was taken near the coking process, and sample E was significantly polluted with tar oil. The five samples were classified in different level categories, *i.e.*, Level 'I' to Level 'V' (Table 1) on the basis of the concentration of HMs and PAHs. Sampling

was conducted on December 12th, 2020. Five soil subsamples from each site were collected and mixed to obtain one composite soil sample. After drying and sieving (0.15 mm), triplicate soil samples of 100 g for each sampling site were used for soil characterization.

2.2. Chemical analysis

Chemical reagents including hydrochloric acid, nitric acid, acetonitrile, acetone and *n*-hexane of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Decafluorobiphenyl and dichloromethane were from Shanghai Eon Chemical Technology Co., Ltd (China) and Shanghai Macklin Regent Co., Ltd (China), respectively. Acetonitrile of HPLC grade was from Concord Technology (Tianjin) Co., Ltd (China). Standard solution of PAHs was from TMstandard Co., Ltd (China).

2.2.1. Analyses of HMs

Concentration of HMs were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES; Optima 5300 DV, PerkinElmer, USA) after acidic digestion (da Silva *et al.* 2016). Triplicate soil samples (around 1.0 g each) in 7.0 mL of aqua regia (HCl:HNO_3, 3:1, v/v) were digested at 185°C for 40 min using microwave (Topwave, analytikjena, Germany). After digestion, the acid solution kept faint boiling on a hot plate in the fuming cupboard until 2~3 mL of volume left. The residual acid solution was dissolved in ultrapure Milli-Q water to a final volume of 50 mL. The concentration of 15 elements (*i.e.*, Mn, As, Cr, Pb, Cu, Zn, Ni, Ba, Sr, V, La,

Li, Co, Mo and Sc) in the solution was measured using ICP-OES, and the final concentration in the soil samples was calculated for each element and the total concentration (∑HMs) was also determined.

2.2.2. Analyses of PAHs

PAHs in the soil were extracted using the Soxhlet method and analyzed by means of high-performance liquid chromatography (HPLC; 1260 Infinity, Agilent Technologies, USA) (Wu *et al.* 2018). Triplicate soil samples of 10.0 g each were extracted with 100 mL of acetone and *n*-hexane (1:1, v/v) using a Soxhlet extractor and spiked with 200 ng of decafluorobiphenyl. The extract was then filtered through fiberglass meshes and concentrated and cleaned up using a rotary evaporator and Si SPE Cartridge (CNW, ANPEL Laboratory Technologies (Shanghai) Inc., China), respectively. A mixture of *n*-hexane and dichloromethane (1:1, v/v) was used to dissolve the PAHs. The extract was concentrated by a rotary evaporator and dissolved in acetonitrile to a final volume of 5.0 mL. The concentration of PAHs in the concentrated acetonitrile solution was analyzed using a HPLC with Eclipse plus C18 column (Agilent, USA) and acetonitrile-water as mobile phase according to the gradient (6:4, acetonitrile/water, from the start to 18 min; only acetonitrile from 18 to 28.5 min; finally, 6:4, acetonitrile/water, from 28.5 min to the end). The PAHs analyzed were naphthalene (NAP), acenaphthylene (ACY), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF),

benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DhA), benzo[ghi]perylene (BgP) and indeno[1,2,3-cd]pyrene (IcP). The concentration of the 15 PAHs in the soil samples was calculated for each PAH and the total concentration (∑PAHs) was also calculated.

2.3. Characteristics of microbial communities

The soil DNA was extracted from triplicate samples using DNeasy® PowerSoil® Kit (QIAGEN, Germany). The primer set 515F (5‘-GTGCCAGCMGCCGCGG-3’)/907R (5’-CCGTCAATTCMTTTRAGTTT-3’) was used to amplify the V4–V5 region of bacterial 16S ribosomal RNA (16S rRNA) genes (Shan *et al.* 2015). The PCR products were sequenced by Shanghai Majorbio Bio-pharm Technology Co., Ltd (China) using Illumina MiSeq Platform (San Diego, USA). Microbial diversity and composition were analyzed using QIIME2 (v2020.2). Shannon index was calculated using software MOTHUR (v1.30). One-way analysis of variance (ANOVA) was used to establish statistical differences between the groups with $p < 0.05$. Beta diversity on the level of amplicon sequence variants (ASV) was evaluated by principal co-ordinates analysis (PcoA). Linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe) analysis was performed to screen different bacterial species. Canonical correlation analysis (CCA) was conducted by means of the software R (v.3.3.1) using Vegan package to determine the correlation between the soil microbiomes diversity and the level of PAHs/HMs.

3. Results and Discussion

3.1. The e-waste dismantling plant and the coking plant sites are featured with HMs and PAHs

Soil samples from the e-waste dismantling plant and the coking plant were screened according to their contamination with HMs and PAHs. Five soil samples were finally selected to investigate the effect of the co-occurrence of HMs and PAHs on the soil microbiomes, *i.e.* sample A from the paddy field as the negative control, sample B from the e-waste dismantling plant and samples C-D from the coking plant. The concentration of HMs and PAHs in these samples is displayed in Figure 1. Sample A showed the lowest ∑HMs (1,609.56±65.85 mg/kg) and the lowest ∑PAHs (4.40±0.84 mg/kg). Mn (679.6±37.29 mg/kg), Zn (307.7±21.4 mg/kg) and Ba (229.62±25.95 mg/kg) were the major HMs (75.61%), and FLO consisting of 3 rings (3.00±0.68 mg/kg) was the major PAH (68.18%). Sample B from the e-waste dismantling plant had ∑HMs of 16,780.08±1,278.91 mg/kg, which was ten times higher than that in sample A and also the highest among all the soil samples. Metals such as Cu, Pb, Zn, Ni and As were widely used in electronic products and their total concentration in sample B accounted for 75.92% of ∑HMs (Yang *et al.* 2020a, Yang *et al.* 2020b). ∑PAHs in sample B was twice that of sample A. All the fifteen PAHs were detected, and FLO (2.06±0.11 mg/kg) and BbF (1.65±0.15 mg/kg) were the major PAHs (35.82%).

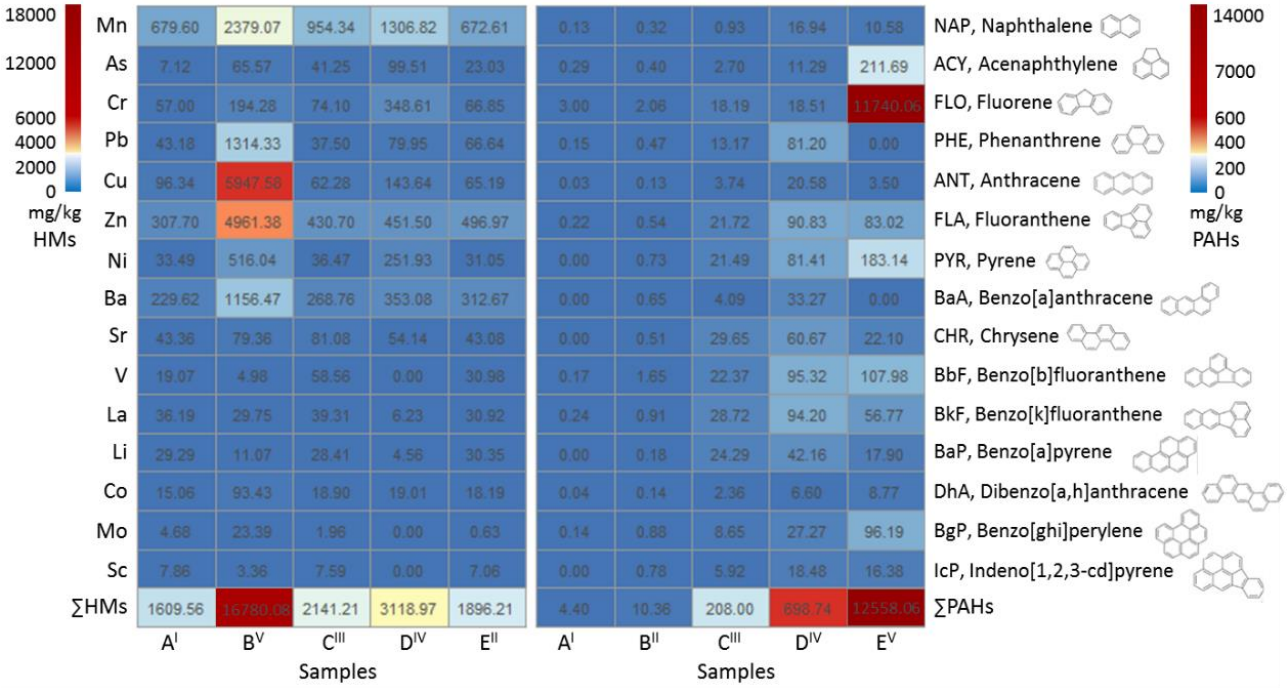

Figure 1. Analyses of HMs (left) and PAHs (right) in the soil samples. Number in each cell indicates the mean concentration of the corresponding pollutant. Sample A (negative control) was collected from the paddy field, sample B was from the e-waste dismantling plant, and samples C-E were from the coking plant. Roman numbers in superscript refer to the contamination levels based on ∑HMs or ∑PAHs, *i.e.*, 'I' (low) to 'V' (high).

In the coking plant, ∑HMs in samples C-E were 1.17 to 1.94 times of that in sample A. In samples C-E, Mn showed the highest concentration (672.61 to 1,306.82 mg/kg). The concentration of As (23.03 to 99.51 mg/kg) was the most heterogeneously distributed in samples C-E compared to sample A, followed by Zn, Cr and Ni. The high concentration of Mn, As, Zn, Cr and Ni in soil may be caused by the storage and combustion of coal (Zajusz-Zubek *et al.* 2017). The ∑PAHs in samples C-E ranged from 208.00 to 12,558.06 mg/kg, which were 47.24 to 2,852.29 times higher than that of sample A. In samples C and D, the concentration of PAHs

with more than 4 rings (*e.g.*, PYR of 21.49 to 81.41 mg/kg, CHR of 29.65 to 60.67 mg/kg, and BbF of 22.37 to 95.32 mg/kg) were higher than that with 3 rings and less (*e.g.*, NAP of 0.93 to 16.94 mg/kg and ACY of 2.70 to 11.29 mg/kg). Sample E showed the highest ∑PAHs (12,558.06±611.19 mg/kg) among the five samples, and 3-ring FLO of 11,740.06±620.1 mg/kg (93.49%) was the main PAH while the concentration of the other PAH congeners (*i.e.*, ACY, PYR and BbF) was below 211.68 mg/kg. The high ∑PAHs probably resulted from coking activity and the very high concentration of FLO could be originated from tar oil, the main product of the coking activity (Vasilieva *et al.* 2012).

Previous studies showed that e-waste dismantling plants have generally high concentration of HMs but low concentration of PAHs, and coking plant usually have high concentration of PAHs but low concentration of HMs (Rachwal *et al.* 2015, Sun *et al.* 2014, Tang *et al.* 2010). This study showed similar features and the concentration of HMs and PAHs were much higher than those reported in the literature. The high contaminations with HMs and PAHs might help in understanding the response of soil microbiomes to mixed contamination with HMs and PAHs.

3.2. The α- and β-diversities of the microbiomes at the e-waste dismantling and the coking sites

After quality control, 831,803 sequences of 16S rRNA genes were obtained. The measurement of the sequences was sufficient according to the rarefaction curves (Figure S1). These sequences were clustered into 28,746 ASV (amplicon sequence

variants) and assigned into 1,271 genera of 50 bacterial phyla. The α-diversities of the soil microbiomes, *i.e.*, Shannon indexes, were evaluated using those data (Figure 2a; other indexes, *i.e.*, Ace, Chao1 and Simpson indexes, in Table S1).

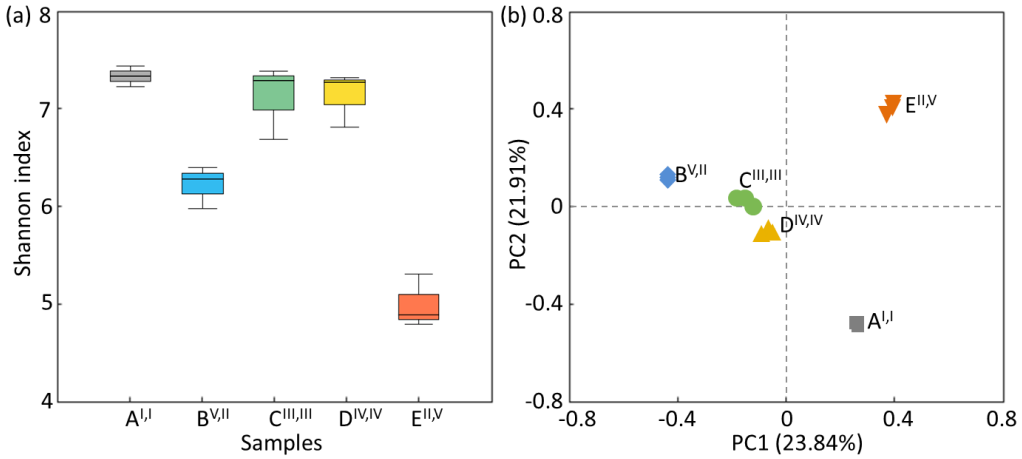

Figure 2. The α-(a) and β-(b) diversities of soil microbiomes. Symbols of the sampling sites are explained in Figure 1. Roman numbers refer to ∑HMs (the first superscript) and ∑PAHs (the last superscript) in the soil samples increased successively from 'I' (low) to 'V' (high).

The Shannon indexes of samples A, C and D (7.10~7.33) were not significantly different from each other ($p > 0.05$), but they were significantly higher than those of samples B (6.31±0.15) and E (5.01±0.20) ($p < 0.05$), indicating that the richness of soil microbiomes in samples B and E was apparently lower than that in samples A, C and D. Interestingly, samples B and E had the highest ∑HMs and ∑PAHs, respectively, among all samples. This indicated that soil microbial diversity was decreased by high ∑HMs in sample B (16,780.08±1,278.91 mg/kg) or high ∑PAHs in sample E (12,558.06±611.19 mg/kg). In addition, the Shannon index of sample B was significantly higher than that of sample E ($p < 0.05$). This could indicate that PAHs represent a bigger threat to soil microbiomes than HMs. The β-diversity of the soil

microbiomes was evaluated by means of PCoA (Figure 2b). Samples were distant from each other, indicating that the community structures of the soil microbiomes were affected by the concentration of HMs or PAHs.

3.3. Features of soil microbial community at phylum and genus levels

The microbial communities were then analyzed at phylum and genus levels (Figure 3). There were twelve bacterial phyla with relative abundance more than 1% in the soil samples (Figure 3a). The top ten phyla found in the soil samples were *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, *Bacteroidota*, *Chloroflexi*, *Planctomycetota*, *Myxococcota*, *Gemmatimonadota*, *Firmicutes* and *Cyanobacteria*. The *Actinobacteriota* (21.49%) and *Firmicutes* (4.31%) were relatively abundant in sample A but less in the other samples. *Acidobacteriota* (24.55%) and *Myxococcota* (6.90%) showed the highest relative abundances in sample B with the highest ∑HMs. The relative abundance of *Proteobacteria* increased significantly from 28.24% to 78.69% along with the increasing ∑PAHs from sample A (4.4±0.84 mg/kg) to sample E (12,558.06±611.19 mg/kg). These results were similar to those of previous studies reporting that *Acidobacteriota* and *Myxococcota* are more frequently detected in HMs-heavily contaminated environment (Dell'Anno *et al.* 2021) and that *Proteobacteria* were more abundant when ∑PAHs increased (Liu *et al.* 2019).

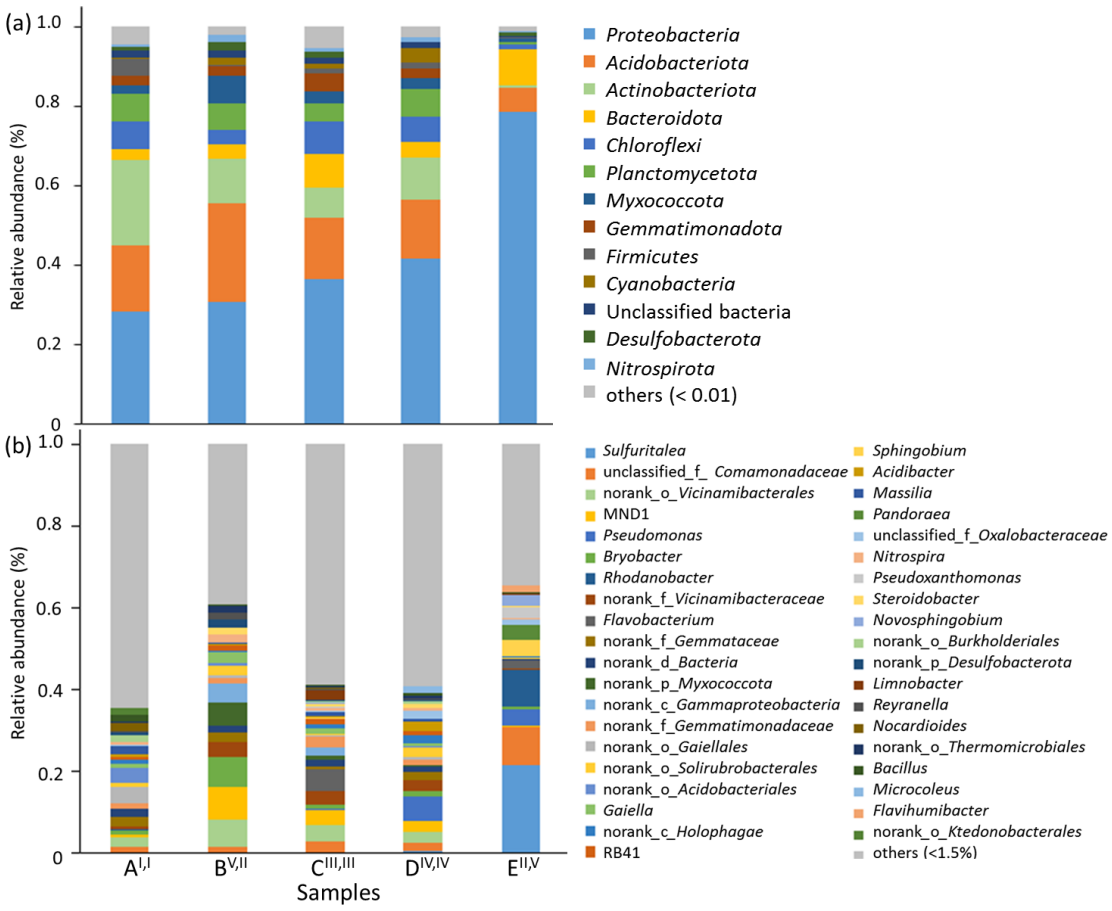

Figure 3. Bacterial phyla (a) and genera (b) in the soil samples. Unclassified genera in different domains (d), phyla (p), classes (c), orders (o) and families (f) are given. Symbols of the sampling sites are explained in Figure 1. Roman numbers refer to ∑HMs (the first superscript) and ∑PAHs (the last superscript) in the soil samples increased successively from 'I' (low) to 'V' (high).

Thirty-nine genera had relative abundances above 1.5%, including twenty-two classified genera (56.41%) and seventeen unclassified genera (43.59%) (Figure 3b). Such a high proportion of unclassified genera showed that many bacteria still need to be characterized in the co-contaminated sites. The genera in sample A were quite diverse, and each of them was present with a similar relative abundance. In sample B, MND1 (7.95%), *Bryobacter* (7.54%) and an unclassified genus of *Vicinamibacteriales* order (6.66%) showed high relative abundances. *Flavobacterium*

genus showed the highest abundance in sample C (5.39%) and *Pseudomonas* genus (6.01%) was well represented in sample D. *Sulfuritalea* genus was detected in samples D and E but barely in samples A-C. The relative abundance of *Sulfuritalea* genus in sample E was high, *i.e.*, 21.34%. Besides, *Rhodanobacter* (9.06%), an unclassified genus of the *Comamonadaceae* family (9.35%), *Sphingobium* (4.03%), *Pandoraea* (3.70%) and *Pseudoxanthomonas* (2.92%) showed higher relative abundances in sample E.

LEfSe (linear discriminant analysis effect size) analysis with LDA (linear discriminant analysis) score >4.0 was useful to identify the main genera which were differently represented in the soil microbiomes of the various places of sampling (Figure 4). There were eight and six typical genera with LDA score >4.0 in samples B and D, respectively, but only three in samples A, C and D. Unclassified genera of *Gaiellales* (3.93%) and *Acidobacteriales* (3.85%) orders and *Massilia* (2.18%) mainly existed in sample A and all of them showed relative abundances <1% in the other samples. The three genera might be sensitive to HMs and PAHs. Genera such as MND1, *Bryobacter* and an unclassified genus of *Vicinamibacteriales* order showed the highest relative abundances in sample B (6~8%). Genera including *Sulfuritalea*, *Rhodanobacter* and an unclassified genus of *Comamonadaceae* family were the highest in sample E (9% to 22%), whereas the three genera showed relative abundances ranging from 0.20% to 4.15% in samples A-D and decreased along with the increase of ∑PAHs. The results suggested that bacteria tolerant to high ∑HMs

existed widely in the soil but failed to colonize soils with high concentration of PAHs. Differently, bacteria tolerant to high ∑PAHs dominated in PAHs-polluted soils but were hardly found in HMs-polluted soils. Besides, genera such as *Flavobacterium* in sample C (5.39%) and *Pseudomonas* in sample D (6.01%) may perform better than other genera in the soil polluted by HMs and PAHs at moderate concentration.

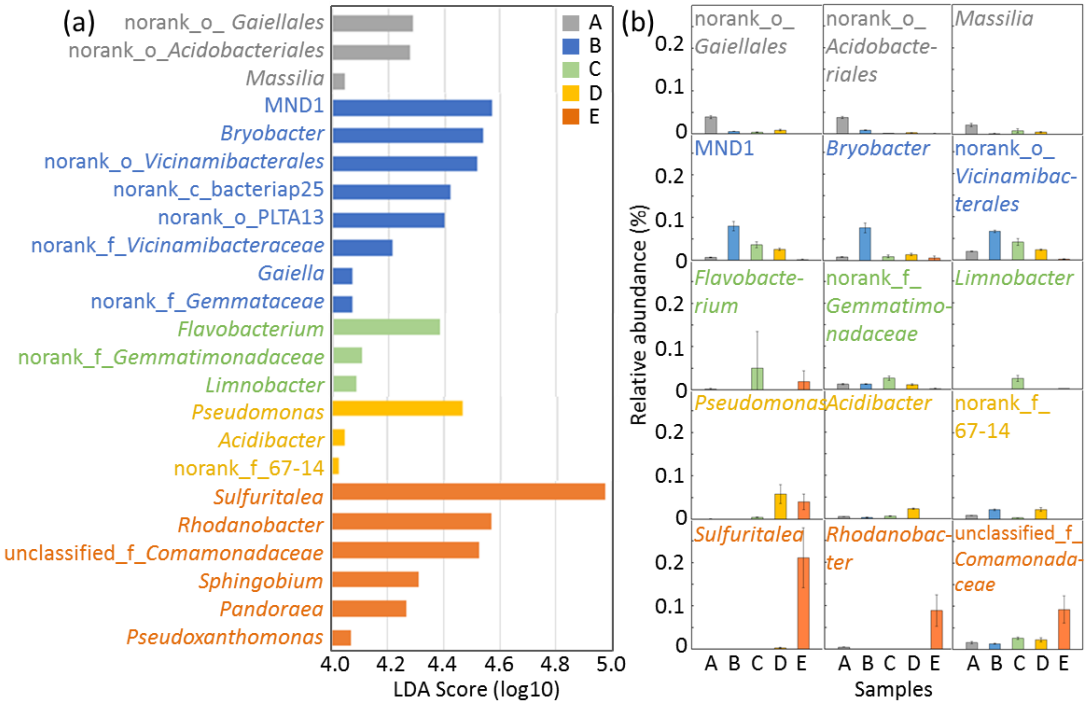

Figure 4. LEfSe results of bacterial genera (a) and relative abundances of typical genera in samples (b). LEfSe was performed with LDA score > 4.0 and $p < 0.05$. Symbols of the sampling sites are explained in Figure 1.

3.4. HMs and PAHs impact differently the soil microbiome composition

The impact of HMs and PAHs on the soil microbiome composition was evaluated with CCA (canonical correlation analysis) (Figure 5a and Table S2). Results showed that microbial community structures of these soil samples were mainly influenced by eight congeners of PAHs (*i.e.*, ACY, FLO, BgP, PYR, DhA, BbF, FLA

and IcP) and four HMs (*i.e.*, Sr, Mn, As and Ni). Sample A with low concentration of HMs and PAHs was far from the others in Figure 5a. The microbiomes of samples B, C and D were mainly influenced by HMs, and the microbiome of sample E was highly impacted by PAHs. The result of CCA coincided with the concentration of HMs and PAHs in the soil samples. Sample B and E were highly contaminated by either HMs (16,780.08±1,278.91 mg/kg) or PAHs (12,558.06±611.19 mg/kg). The microbial community structures of them were therefore determined by HMs and PAHs, respectively. For samples C and D polluted by HMs (2,124.21 to 3,118.97 mg/kg) and PAHs (208.00 to 698.74 mg/kg) at moderate concentration, the microbial community structures were mainly shaped by HMs.

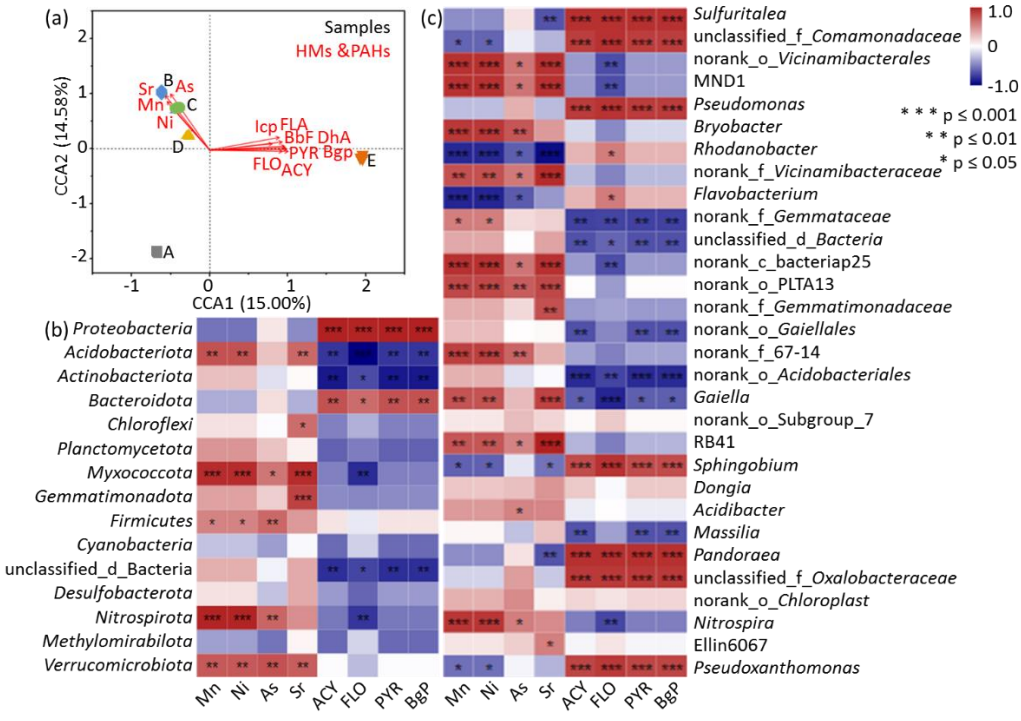

Figure 5. Canonical correlation analysis (CCA) showed reverse influences of HMs and PAHs on the microbial community structure (a) and correlation between the main kinds of contaminants and

bacterial phyla (b) and genera (c). Symbols of the sampling sites and the abbreviations of PAHs (*e.g.*, ACY, FLO and PYR) are explained in Figure 1.

The influences of four HMs (*i.e.*, Mn, Ni, As and Sr) and PAHs (*i.e.*, ACY, FLO, PYR and BgP) on bacterial phyla (Figure 5b) and genera (Figure 5c) were analyzed further. Interestingly, bacteria in soil polluted with high concentration of HMs or PAHs almost separated in two groups. At phylum level, the abundance of *Acidobacteriota*, *Myxococcota* and *Nitrospirota* were positively correlated to the concentration of HMs but negatively to that of PAHs, while the abundance of *Proteobacteria* and *Bacteroidota* were positively related to the concentration of PAHs and indistinctively related with that of HMs. At genus level, the abundance of MND1, *Bryobacter*, *Gaiella*, RB41, *Acidibacter* and *Nitrospira* were positively related to the concentration of HMs and partially negatively to that of PAHs, whereas the abundance of *Sulfuritalea*, *Pseudomonas*, *Rhodanobacter*, *Flavobacterium*, *Sphingobium*, *Pandoraea* and *Pseudoxanthomonas* were positively related to the concentration of PAHs and partially negatively to that of HMs. The correlation analysis results shown in Figure 5 was in accordance with LEfSe results shown in Figure 4. To our knowledge, this is the first study reporting that soil microbiomes in highly HMs- or PAHs- contaminated soils change in opposite directions, and that bacteria tolerant to high concentration of HMs or PAHs were completely different. The different abilities of bacteria to cope with HMs and PAHs may be related to their properties of cell membranes and enzymes. For example, many stains of

Cyanobacteria may produce negatively charged extracellular polymeric substances, which are considered as the first protective barrier against metal stress (Cassier-Chauvat and Chauvat 2014). In contrast, *Sphingobium* spp. were usually characterized by high cell surface hydrophobicity due to their rich polysaccharidic content and hence tend to adsorb PAHs (Garcia *et al.* 2018).

In previous studies, genera such as *Bryobacter*, *Gaiella* and *Nitrospira* were reported to resist to HMs (Hao *et al.* 2018, Kulicheyskaya *et al.* 2014, Li *et al.* 2021a) and *Pseudomonas*, *Sphingobium* and *Pseudoxanthomonas* were largely reported to be able to degrade PAHs (Liang *et al.* 2021, Madueno *et al.* 2018b, Shinde *et al.* 2020). Among these bacteria, many strains of *Pseudomonas* and *Sphingobium* were reported to degrade PAHs and resist to HMs (Afegbua and Batty 2019, Madueno *et al.* 2018a). Few strains of *Sulfuritalea* and *Rhodanobacter* were reported to be able to degrade PAHs (Li *et al.* 2017, Sperfeld *et al.* 2019). Knowledge of genera such as *Bryobacter*, *Flavobacterium* and *Pseudoxanthomonas* is still limited to the bacterial community level, and scarce strains were obtained and studied at laboratory level (Li *et al.* 2021a, Miao *et al.* 2020) (Wang *et al.* 2020). There are still many HMs-tolerant and PAHs-degrading strains to be studied. This study represents a contribution to the application of PAHs-degrading strains for the remediation of sites polluted with HMs and PAHs. For example, *Sphingobium* may be used to remediate soils severely polluted with PAHs (10^3~10^4 mg/kg) but only slightly with HMs, while *Pseudomonas*

could be applied to soil contaminated by both HMs and PAHs at moderate concentration (10^2 ~10^3 mg/kg).

4. Conclusions

Fifteen HMs and fifteen PAHs were analyzed in contaminated soil samples from an e-waste dismantling plant and a coking plant. Results showed that the microbiomes of these samples were mainly influenced by four HMs (*i.e.*, Sr, Mn, As and Ni) and eight PAHs (*i.e.*, ACY, FLO, BgP, PYR, DhA, BbF, FLA and IcP). The abundance of bacterial taxa such as MND1, *Bryobacter* and *Galiella* were positively related to the concentration of HMs, whereas the abundance of genera such as *Sulfuritalea*, *Pseudomonas* and *Sphingobium* were positively related to the concentration of PAHs. These results demonstrated that the soil microbiomes of the contaminated sites were driven in different way by HMs and PAHs.

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Author Contributions

Methodology, data analysis and original draft preparation, Z. N. Yang and Z. S. Liu; measurements of PAHs and HMs, K. H. Wang and Z. L. Liang; sample preparation, DNA extraction and bacterial analysis, R. Abdugheni, Y. Huang, R. H. Wang, H. L. Ma, X. K. Wang, M. L. Yang and B. G. Zhang; review and editing, C. Y. Jiang, D. F. Li, P.F.-X. Corvini and S. J. Liu; funding acquisition, S. J. Liu. All authors have read and agreed to the published version of the manuscript.

Abbreviations

E-waste, electronic waste; HMs, heavy metals; PAHs, polycyclic aromatic hydrocarbons; NAP, naphthalene; ACY, acenaphthylene; FLO, fluorene; PHE, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; BaA, benzo[A]anthracene; CHR, chrysene; BbF, benzo[b]fluoranthene; BkF, benzo[k]fluoranthene; BaP, benzo[a]pyrene; DhA, dibenzo[a,h]anthracene; BgP, benzo[ghi]perylene; IcP, indeno[1,2,3-cd]pyrene; 16S rRNA, 16S ribosomal RNA; ASV, amplicon sequence variants; PcoA, principal co-ordinates analysis; LEfSe, linear discriminant analysis (LDA) coupled with effect size measurements; CCA, canonical correlation analysis.

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Figure 1

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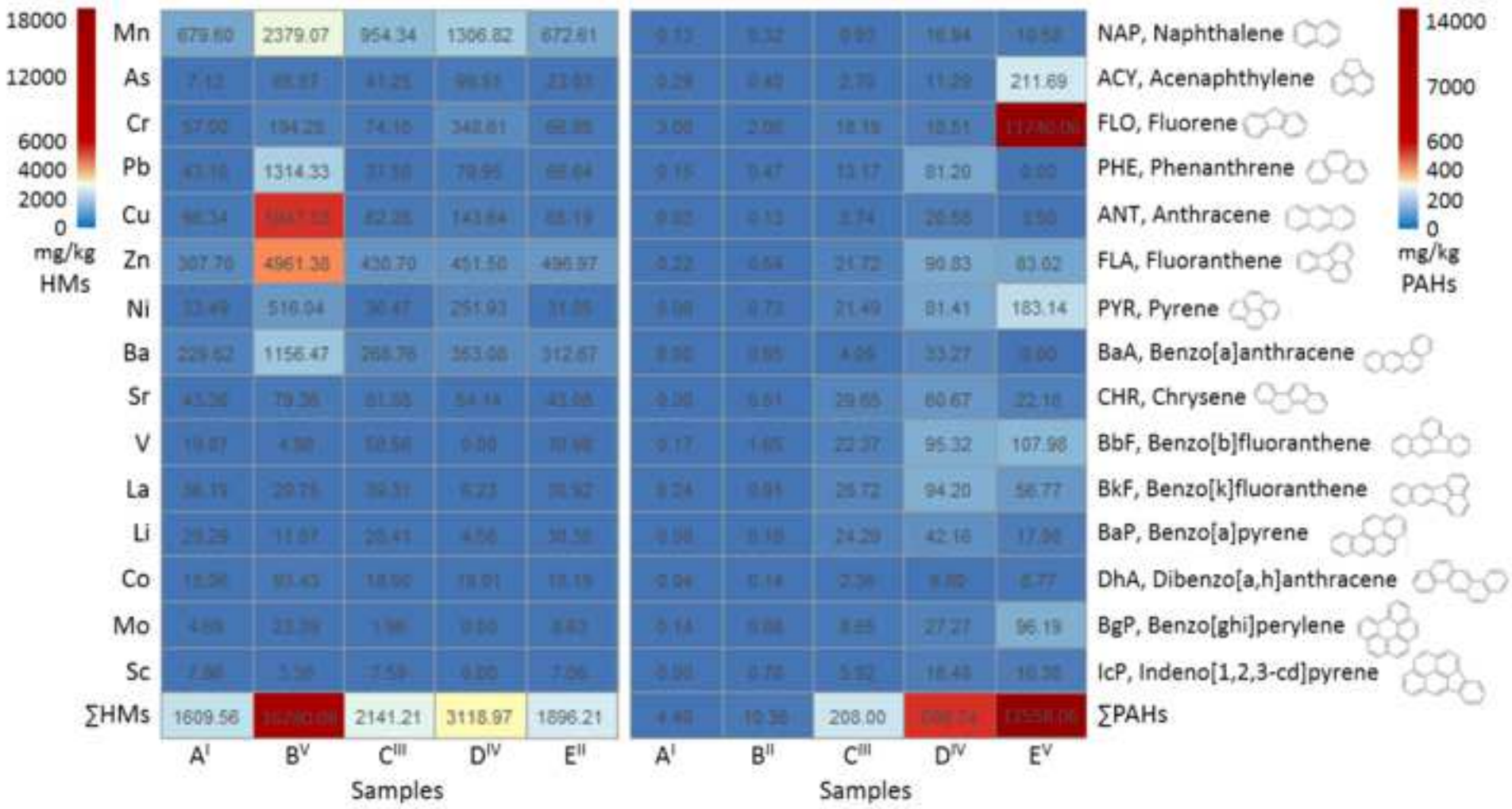

Figure 2a

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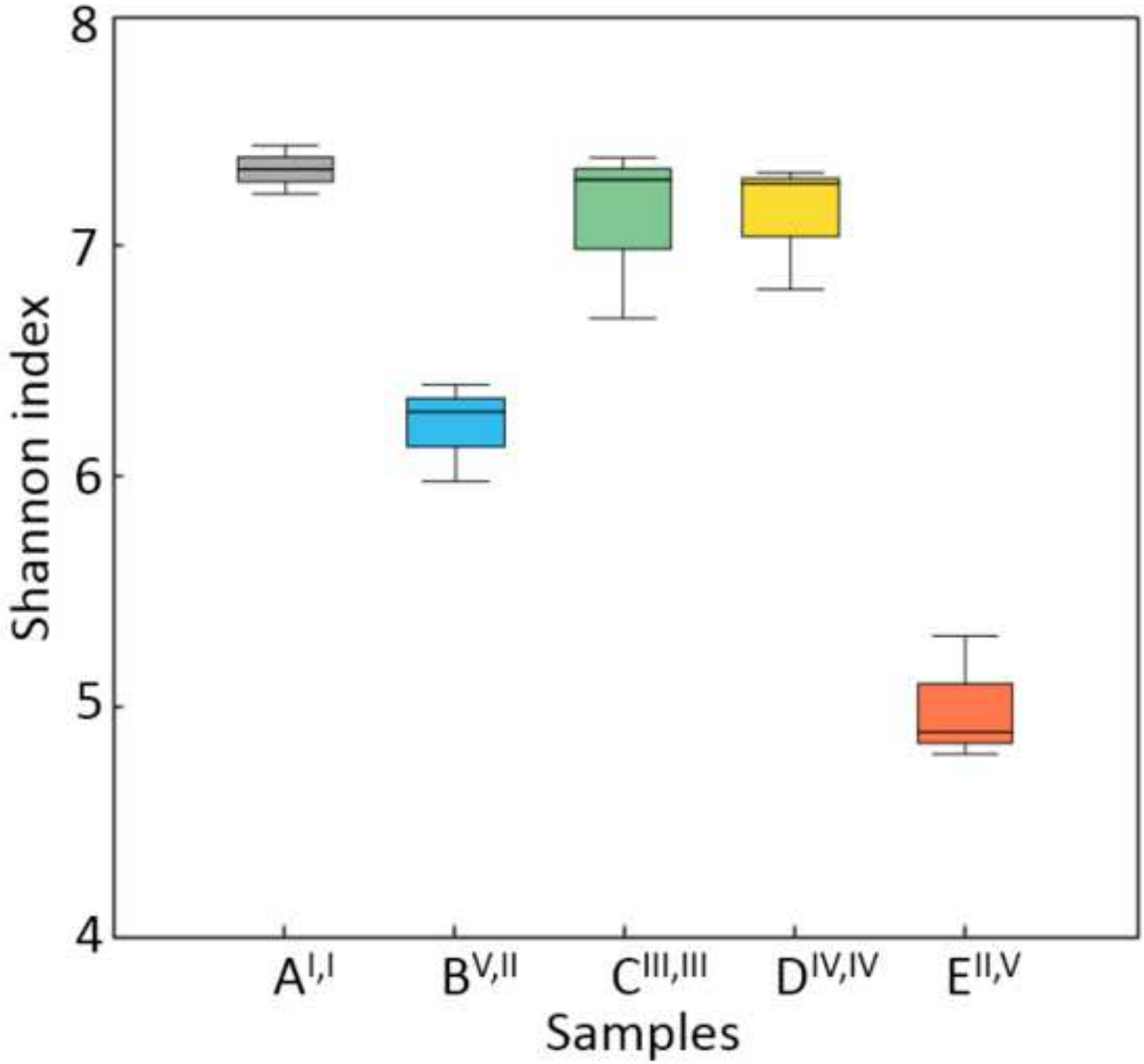

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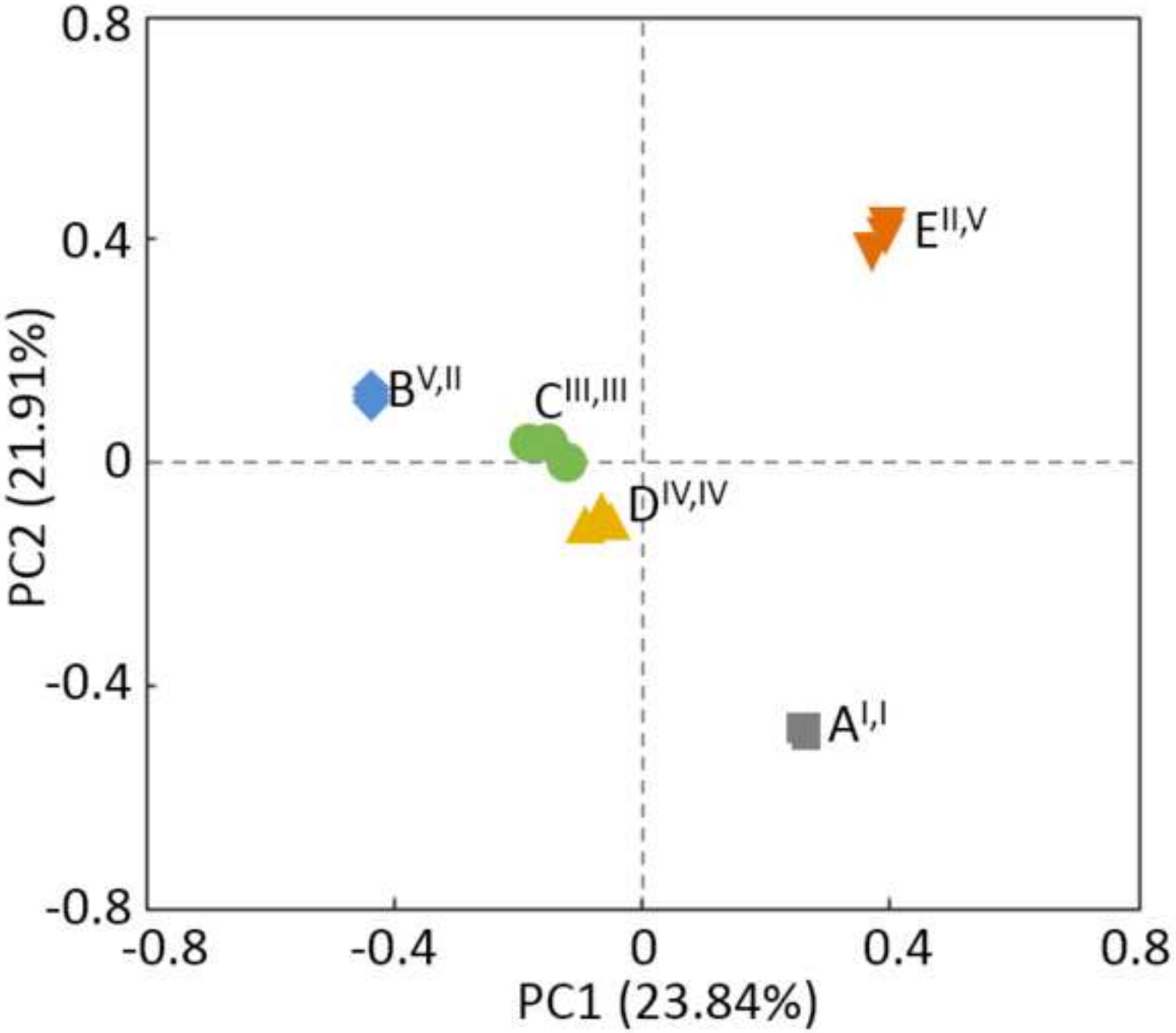

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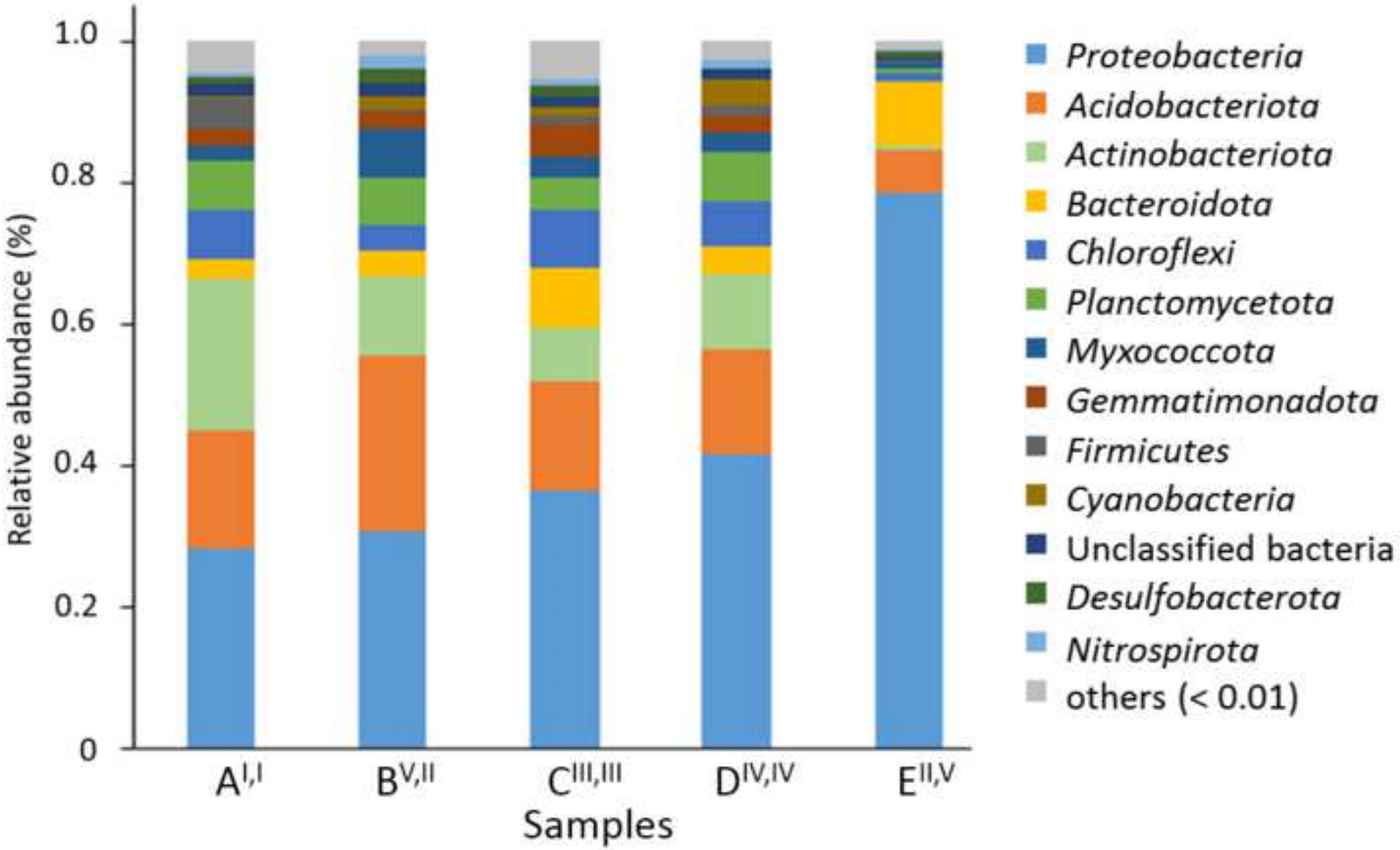

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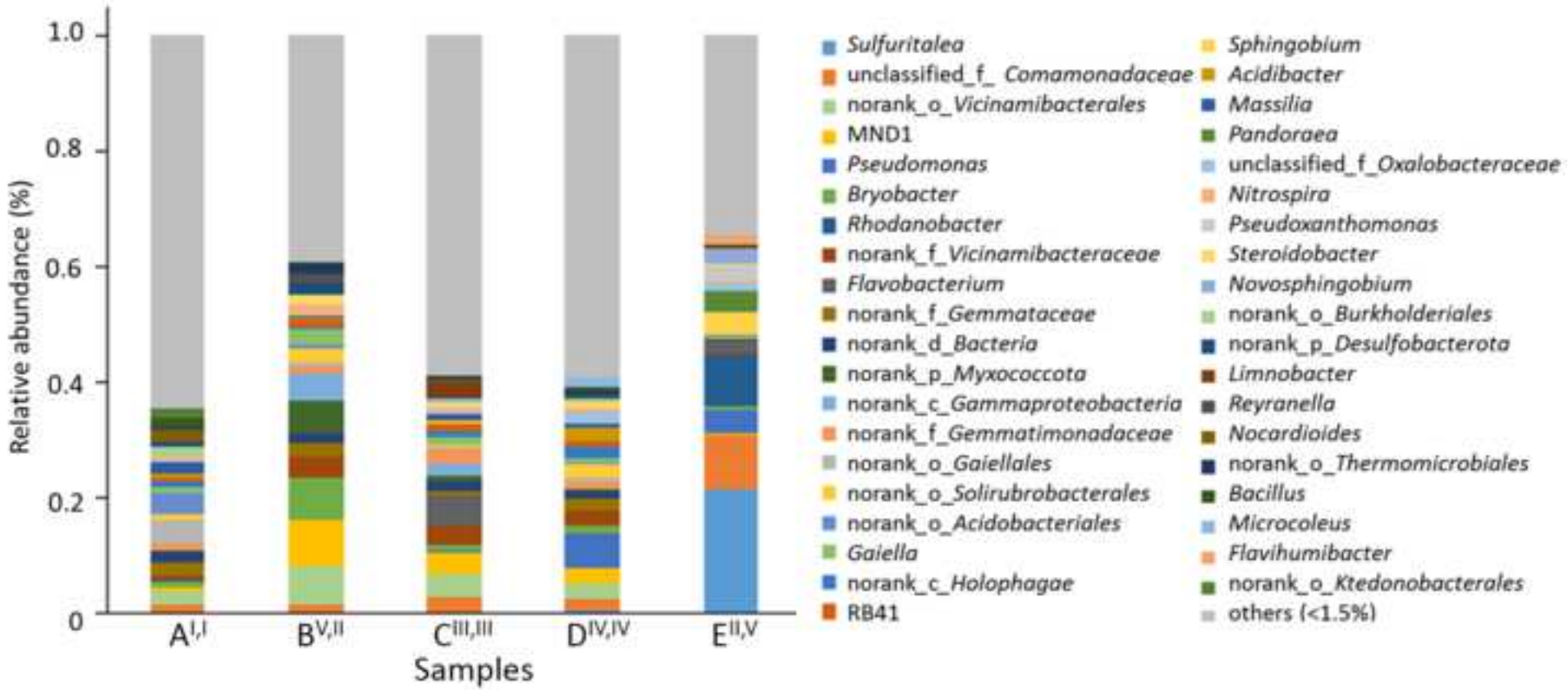

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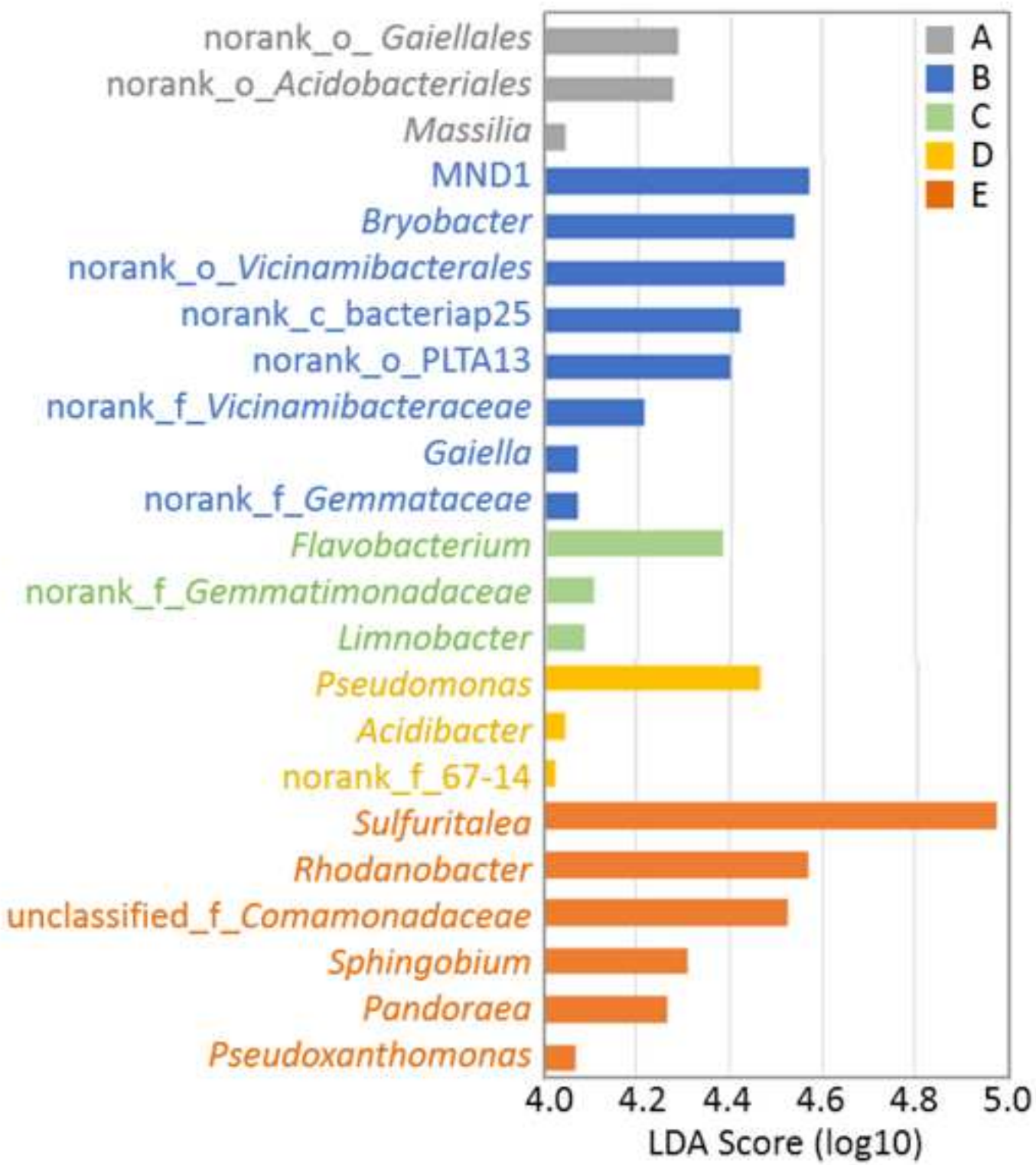

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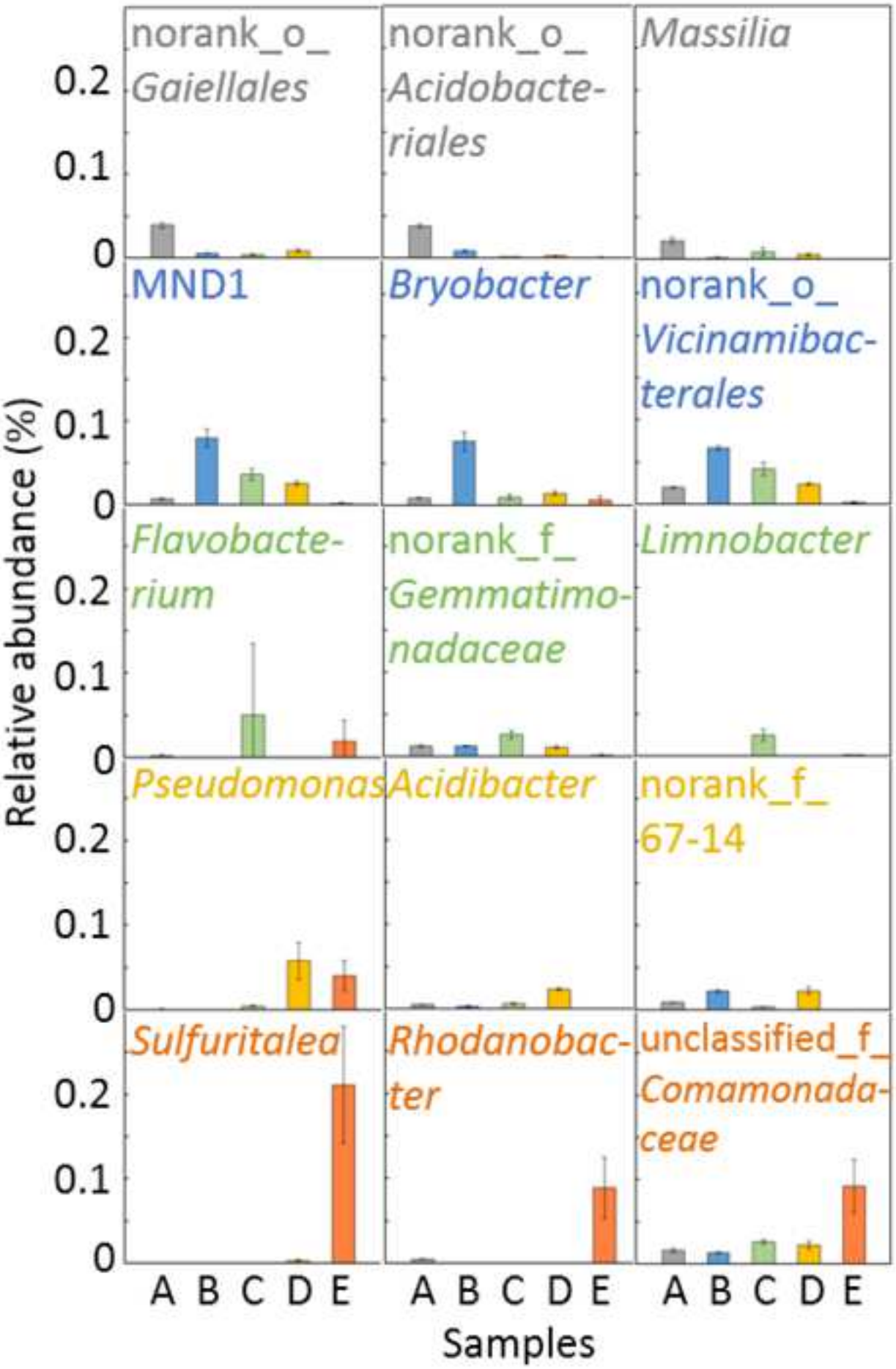

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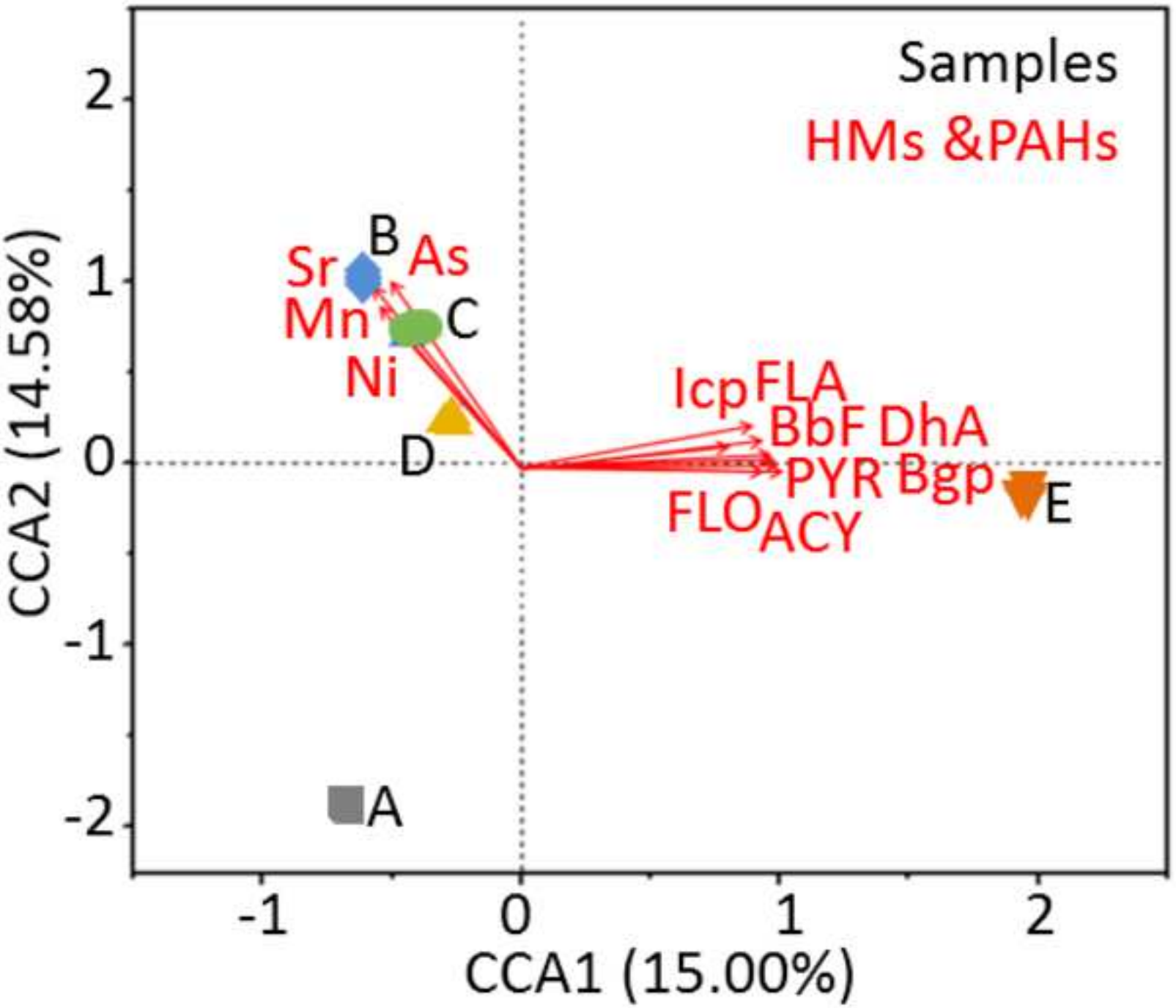

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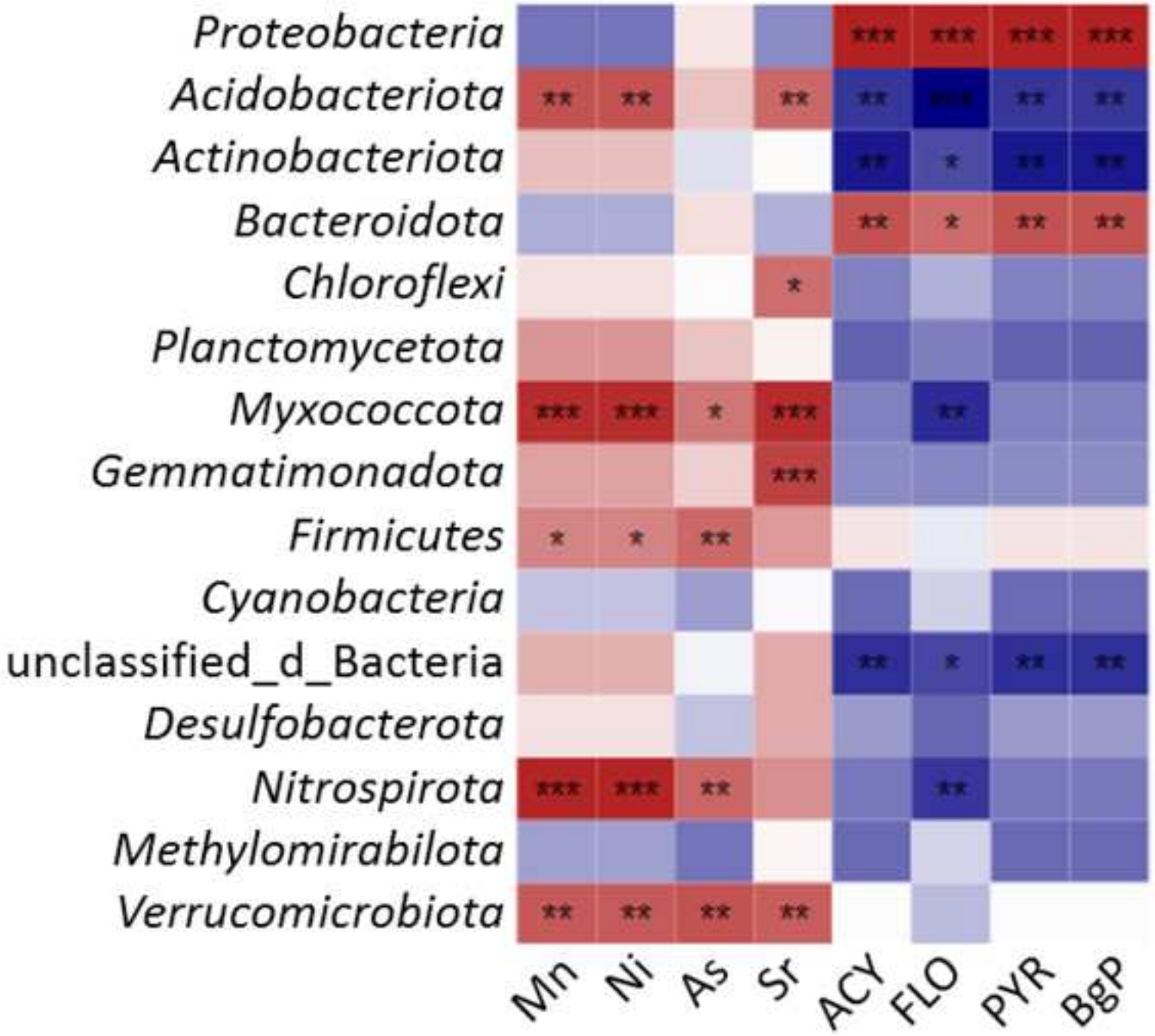

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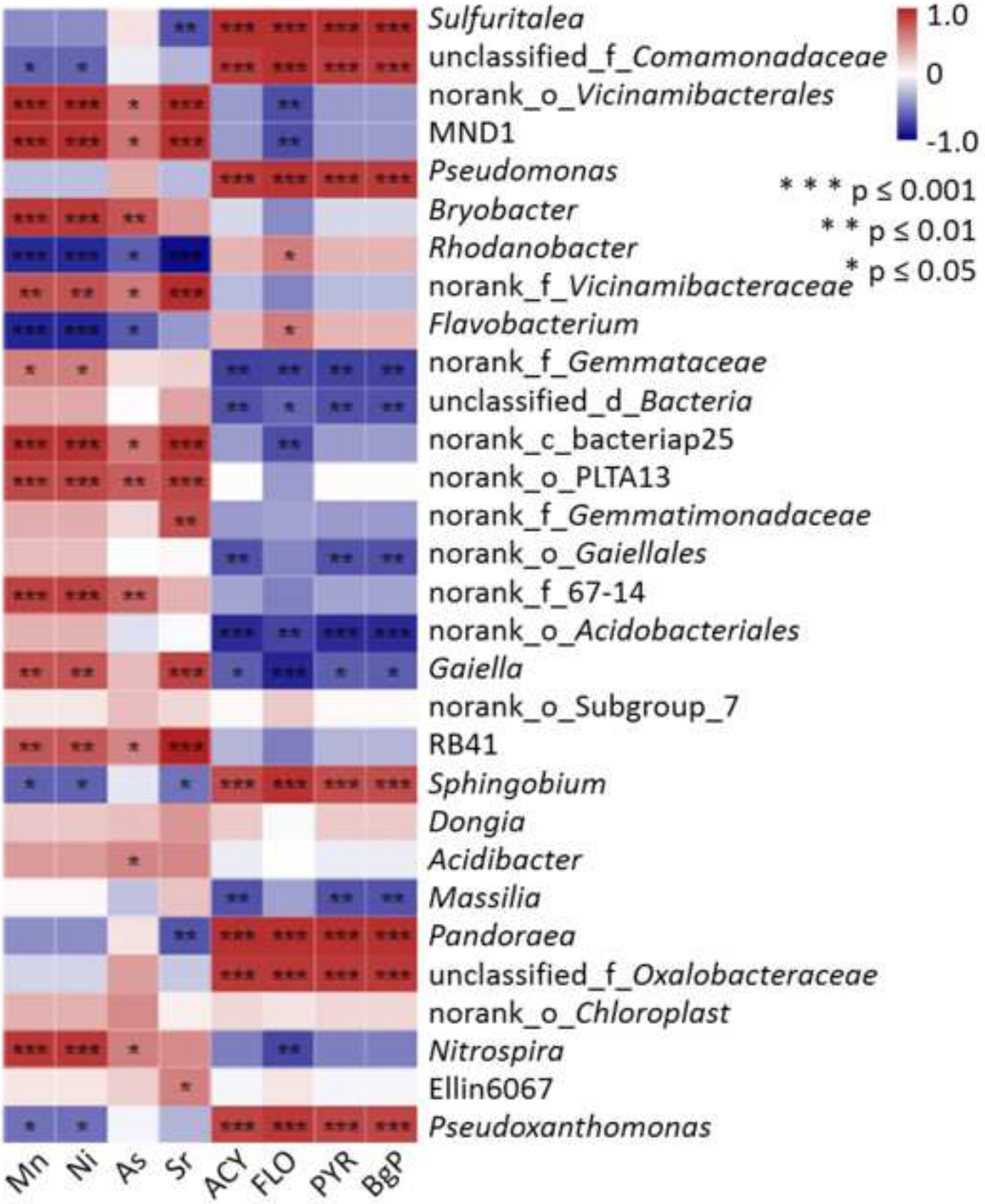

Highlights

1. Mixed contamination (HMs+PAHs) was detected in soil from the e-waste dismantling plant and the coking plant.

2. The e-waste dismantling plant site was featured by heavy contamination of HMs but minor PAHs.

3. The coking plant was featured by severe contamination of PAHs and moderate HMs.

4. The abundance of bacterial genera such as *Sulfuritalea*, *Pseudomonas* and *Sphingobium* increased with severe pollution of PAHs.

5. The abundance of bacterial genera such as *Bryobacter*, *Galiella* and *Nitrospira* increased in soil contaminated with HMs.

Graphical Abstract (for review)

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