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Mercury contamination imposes structural shift on the microbial community of an agricultural soil

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Abstract

Background: The purpose of this study is to use shotgun next-generation sequencing to unravel the microbial community structure of an agricultural soil, decipher the effects of mercury contamination on the structure of the microbial community and the soil physicochemistry and heavy metals content.

Results: The soil physicochemistry after mercury contamination revealed a shift in soil pH from neutral (6.99 ± 0.001) to acidic (5.96 ± 0.25), a decline in moisture content to < 4 %, and a significant decrease in the concentrations of all the macronutrients and the total organic matter. Significant decrease in all the heavy metals detected in the agricultural soil was also observed in mercury inundated SL3 microcosm. Structural analysis of the metagenomes of SL1 (agricultural soil) and SL3 (mercury-contaminated agricultural soil) using Illumina shotgun next-generation sequencing revealed the loss due to mercury contamination of 54.75 % of the microbial community consisting of an archaeal domain, 11 phyla, 12 classes, 24 orders, 36 families, 59 genera, and 86 species. The dominant phylum, class, genus, and species in SL1 metagenome are *Proteobacteria*, *Bacilli*, *Staphylococcus*, and *Sphingobacterium* sp. 21; while in SL3 metagenome, *Proteobacteria*, *Alphaproteobacteria*, *Singulisphaera*, and *Singulisphaera acidiphila* were preponderant. Mercury contamination resulted in a massive upscale in the population of members of the phylum *Planctomycetes* and the genera *Singulisphaera*, *Brevundimonas*, *Sanguibacter*, *Exiguobacterium*, *Desulfobacca*, and *Proteus* in SL3 metagenome while it causes massive decline in the population of genera *Staphylococcus* and *Brachybacterium*.

Conclusions: This study revealed that mercury contamination of the agricultural soil imposed selective pressure on the members of the microbial community, which negatively impact on their population, alter soil physicochemistry, and enriched sizable numbers of members of the community that are well adapted to mercury stress. It also reveals members of microbial community hitherto not reported to be important in mercury detoxification process.

Keywords: Agricultural soil, Soil microcosm, Illumina shotgun sequencing, Mercury contamination

Introduction

The release of heavy metals into the environment through agricultural and industrial operations and the consequences of these pollution on ecosystems and human health are sources of serious concern (Robinson and Tuovinen 1984; Lapanje et al. 2010). In agriculture, mercurial compounds have been used as insecticides, fungicides, herbicides, and bactericides, resulting in severe localized mercury pollution in soils (Bryan and Langston 1992). Other agricultural practices such as application of fertilizers, lime, sludges, and manures are also sources of mercury in soil (Azevedo and Rodriguez 2012). Soil plays an important role in the biogeochemical cycle of mercury acting as both a sink and a source of mercury to biota, atmosphere, and hydrological compartments. However, mercury speciation, accumulation, and transformation and the interaction of the various species with the soil matrix cause changes in solubility, toxicity, and bioavailability of the metal and make it difficult to decipher the effects of mercury contamination

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on the soil microbial community (Nies 1999; Biester et al. 2002; Barkay et al. 2003; Lapanje et al. 2010).

Essential heavy metals such as zinc, copper, iron, and chromium are important to life. They play an integral role in metabolic processes and are essential micronutrients and cofactors of several enzymes, required in redox processes and stabilization of molecules through electrostatic interactions and regulation of osmotic pressure (Bruins et al. 2000; Romaniuk et al. 2018). However, heavy metals with no known biological function such as mercury are very toxic to the cell. In humans, mercury toxicity results in gene expression alteration, kidney damage, tremor, restlessness, anxiety, numbness in hand and feet, total brain damage in early exposure, localized brain damage in late exposure, and death upon exposure to high doses (Weiss et al. 2002; Curtis and Klaassen 2010). In microorganisms, mercury toxicity results in protein denaturation, cell envelope disruption, inhibition of cell division and enzyme activities, destruction of nucleic acids, and transcription inhibition (Khan et al. 2009; Gundacker et al. 2010; Bánfalvi 2011; Wyszkowska et al. 2013; Yuan et al. 2015). In plants, exposure to mercury reduces photosynthesis, transpiration rate, water uptake, and chlorophyll synthesis. It also causes loss of potassium, magnesium and manganese and accumulation of iron (Boening 2000). High affinity of mercury with sulfydryl (SH) groups form the S–Hg–S bridge, which disrupts the stability of the group and affects seed germination and embryo's growth. Furthermore, in plants, mercury is known to affect the antioxidant defense system interfering with the modulation of the non-enzymatic antioxidants glutathione, non-protein thiols, and the enzymatic antioxidants superoxide dismutase, ascorbate peroxidase, and glutathione reductase (Ortega-Villasante et al. 2005; Sparks 2005; Israr et al. 2006).

It is widely believed that mercury contamination in soils impacts negatively on the richness and diversity of the microbial community (Rasmussen and Sorensen 2001; Rasmussen et al. 2008). To validate this claim, we set up an investigation using next-generation shotgun metagenomic approach to decipher the microbial community structure of an agricultural soil (SL1), and to monitor the effects of mercury contamination (250 mg) on the microbial community structure of the agricultural soil (SL3).

Materials and methods

Sampling site description

Soil samples were collected from an agricultural farm at Mandate estate, Ilorin, Nigeria. The coordinates of the sampling site were latitude 8° 28' 24.81″ N and longitude 4° 29' 58.87″ E. Farming history at the sampling site dated back to 20–25 years and crops such as maize, cassava, yam, and vegetables were grown.

Source of heavy metal

Mercury (II) chloride ($HgCl_2$), the source of mercury used in this study was purchased from Sigma Aldrich Corp (St Louis, MO, USA).

Sampling and Microcosm Set up

Soil samples were collected from upper 10–12 cm using a sterile hand trowel after removing the debris from the soil surface. Soil sample was passed through a 2 mm mesh sieve. The sieved soil was thoroughly mixed in a large plastic bag to avoid variability among the results of replicate soil samples and was used without air drying. Sieved soil (1 kg) weighed and placed in an open aluminum pan was designated SL1. The second soil microcosm designated SL3 contain 1 kg of sieved soil amended with 250 mg $HgCl_2$. The two setups (in triplicates) were incubated at room temperature for 4 weeks and flooded weekly with 50 ml distilled water to maintain a moisture content of 25%. Physicochemistry and heavy metal content of the agricultural soil was determined as described previously (Salam et al. 2014).

DNA extraction and Shotgun metagenomics

Total DNA used for metagenomic analysis was extracted directly from the two soil microcosms, SL1 and SL3. Total DNA was extracted from the agricultural soil (SL1) immediately after sampling to determine the microbial community structure of the agricultural soil prior to amendment with mercury. For soil microcosm SL3 containing agricultural soil amended with 250 mg mercury, total DNA was extracted 4 weeks post contamination to determine the effects of the mercury amendment on the microbial community structure. Total DNA was extracted from the sieved soil samples (0.25 g) using ZYMO soil DNA extraction Ki (Model D 6001, Zymo Research, USA) following manufacturer's instructions. Extracted total DNA concentration and quality was ascertained using NanoDrop spectrophotometer and electrophoresed on a 0.9% (*w* / *v*) agarose gel consecutively. Shotgun metagenomics of SL1 and SL3 microcosms were prepared using the Illumina Nextera XT sample processing kit and sequenced on a MiSeq. The protocols for total DNA preparation for Illumina shotgun sequencing were as described previously (Salam 2018; Salam and Ishaq 2019).

Processing of raw reads, quality control, assembly, and taxonomic classification

Processing and quality control of raw reads, assembly, and taxonomic classification were carried out using the analysis tools in EDGE Bioinformatics web server (Li et al. 2017). The pre-processing of the raw Illumina fastq file of the two metagenomes (SL1 and SL3) for quality control check, de novo assembly of the trimmed reads, and assembly validation were carried out using FastQ

Quality Control Software (FaQCs) (Lo and Chain 2014), IDBA-UD (Peng et al. 2012), and Bowtie2 (Langmead and Salzberg 2012), respectively.

Read-based and contig-based classifications in the EDGE Bioinformatics web server were deployed for taxonomic classification of the SL1 and SL3 metagenomes. All the classification tools GOTTCHA (Freitas et al. 2015), Kraken (Wood and Salzberg 2014), MetaPhlAN (Segata et al. 2012), and BWA (Li and Durbin 2009) indicated for read-based classification were deployed (using their default parameters) to decipher the taxonomic affiliation of the metagenomes. Contig-based classification is based on the alignment of the SL1 and SL3 contigs to NCBI's RefSeq database using the BWA-mem aligner. Metagenomic data of SL1 and SL3 have been deposited and made public in EDGE Bioinformatics web server.

Results

Physicochemistry and heavy metals content

The physicochemistry and heavy metal content of the agricultural soil (SL1) and mercury-contaminated agricultural soil (SL3) are shown in Table 1. The pH of the soil, which is very close to neutral (6.99 ± 0.001) in SL1 became slightly acidic in SL3 (5.96 ± 0.25). The moisture content, which is less than 7% (6.83 ± 0.01) in SL1 dropped further to < 4% in SL3 (3.14 ± 0.01). All the other physicochemical parameters also a showed decreasing trend in SL3 (Table 1). In addition, there are significant traces of heavy metals in the soil. While the concentrations of lead (0.05 ± 0.001 mg/kg) and selenium (0.008 ± 0.001 mg/kg) detected in the agricultural soil are significantly low, high concentrations of zinc, iron, copper, and chromium were observed in the agricultural soil. However, the concentrations of the heavy metals substantially decrease in SL3 (Table 1).

Table 1 Dynamics of physicochemical properties and heavy metal content of agricultural soil (SL1) and mercury-inundated agricultural soil (SL3)

	SL1	SL3
Physicochemical parameters		
pH	6.99 ± 0.31	5.96 ± 0.25
Moisture (%)	6.83 ± 0.01	3.14 ± 0.01
Total organic matter (%)	75.62 ± 0.63	43.38 ± 0.58
Total nitrogen (%)	58.49 ± 1.43	17.63 ± 0.80
Phosphorus (mg/kg)	30.49 ± 1.54	9.53 ± 1.35
Potassium (mg/kg)	19.75 ± 0.004	4.03 ± 0.001
Heavy metal content		
Mercury (mg/kg)	ND	52.5 ± 0.003
Lead (mg/kg)	0.05 ± 0.001	ND
Chromium (mg/kg)	5.50 ± 0.003	1.6 ± 0.004
Cadmium (mg/kg)	0.21 ± 0.002	0.035 ± 0.002
Zinc (mg/kg)	16.49 ± 0.003	3.28 ± 0.004
Iron (mg/kg)	13.45 ± 0.003	2.52 ± 0.004
Copper (mg/kg)	13.04 ± 0.004	2.74 ± 0.005
Selenium (mg/kg)	0.008 ± 0.001	ND

General features of the metagenomes

Illumina shotgun next-generation sequencing of the total DNA from the two soil microcosms revealed 46,292 and 27,220 sequence reads for SL1 and SL3, respectively. The SL1 and SL3 metagenomes consisted of 13,787,457 and 7,604,708 bp, mean sequence length of 297.84 ± 27.40 and 279.38 ± 72.10 bp, and mean GC contents of 54.77% ± 7.14 and 52.82% ± 16.09, respectively. After trimming, dereplication, and quality control, sequence reads in SL1 and SL3 reduced to 45,795 (98.93%) and 25,075 (92.12%) with 13,769,735 (99.87%) and 7,529,285 (99.01%) bp, mean sequence lengths of 300.68 ± 1.78 and 300.27 ± 10.25 bp, and mean GC contents of 55.33% ± 4.48 and 57.23% ± 5.34, respectively. Other general features of the metagenomes are indicated in Table 2.

Taxonomic classification of the metagenomes

Inundation of the agricultural soil with mercury significantly alters the structure of the soil microbiome. Taxonomic characterization of the agricultural soil (SL1) revealed 28 phyla with the predominance of the phyla *Proteobacteria* (41.55%), *Firmicutes* (31.46%), *Actinobacteria* (15.00%), *Bacteroidetes* (7.64%), and *Candidatus Saccharibacteria* (1.84%). In mercury-contaminated SL3 microcosm, 17 phyla were recovered with *Proteobacteria* (56.55%), *Planctomycetes* (14.67%), *Actinobacteria* (11.79%), *Bacteroidetes* (11.46%), and *Firmicutes* (5.36%) preponderant. While the population of the phyla *Candidatus Saccharibacteria*, *Acidobacteria*, *Chloroflexi*, and *Firmicutes* decrease by ≥ 90% in SL3 microcosm, there is an exponential increase in the population of the phylum *Planctomycetes* in the mercury-amended soil (Figure 1).

In class delineation, 40 classes were retrieved from SL1 microcosm with the dominance of *Bacilli* (26.55%), *Gammaproteobacteria* (24.12%), *Actinobacteria* (16.23%), *Alphaproteobacteria* (9.72%), and *Betaproteobacteria* (7.80%). In mercury-contaminated SL3 microcosm where 28 classes were recovered, *Alphaproteobacteria* (24.14%), *Gammaproteobacteria* (21.69%), *Planctomycetia* (15.23%), *Actinobacteria* (12.24%), and *Sphingobacteriia* (10.04%) were preponderant (Figure 2). There is a massive decline in the population of members of the class *Bacilli* as it dipped by > 90% in SL3 microcosm, while the population of members of the classes *Gemmatimonadetes* and *Methanomicrobia* (belonging to Archaea domain) completely disappeared. However, there is an exponential and

Table 2 General features of SL1 and SL3 metagenomes

	SL1	SL3
1.Pre-processing		
a. Raw reads		
Reads	46,292	27,220
Total bases (bp)	13,787,457	7,604,708
Mean read length (bp)	297.84 ± 27.40	279.38 ± 72.10
Mean GC content (%)	54.77 ± 7.14	52.82 ± 16.09
b. Quality trimming		
Trimmed reads		
Reads	45,795 (98.93%)	25,075 (92.12%)
Total bases (bp)	13,769,735 (99.87%)	7,529,285 (99.01%)
Mean read length (bp)	300.68 ± 1.78	300.27 ± 10.25
Mean GC content (%)	55.33 ± 4.48	57.23 ± 5.34
Paired reads	45,784 (99.98 %)	24,976 (99.61 %)
Paired total bases	13,766,902 (99.98 %)	7,510,110 (99.75 %)
Unpaired reads	11 (0.02 %)	99 (0.39 %)
Unpaired total bases	2,833 (0.02 %)	19,175 (0.25 %)
2 Assembly and annotation		
a. De novo assembly by idba_ud		
Number of contigs	68	38
N50 (bp)	423	424
Max contig size (bp)	465	458
Min contig size (bp)	326	262
Total assembly size (bp)	27,929	15,691
b. Assembly validation by read mapping		
Number of mapped reads	31,315	15,774
% of Total reads	68.38%	62.91%
Number of unmapped reads	14,480	9,301
% of Total reads	31.62 %	37.09 %
Average fold coverage	276.60 X	275.14 X

significant increase in the population of members of the classes *Planctomycetia* and *Alphaproteobacteria* in the mercury-contaminated SL3 microcosm.

In order classification, 83 orders were recovered in SL1 microcosm. The dominant orders are *Bacillales* (26.37%), *Actinomycetales* (15.02%), *Enterobacteriales* (9.78%), *Micrococcineae* (6.09%), and *Sphingobacteriales* (6.08%). In mercury-contaminated SL3 microcosm where 59 orders were retrieved, *Planctomycetales* (16.17%), *Actinomycetales* (12.44%), *Caulobacterales* (11.45%), *Sphingobacteriales* (10.66%), and *Enterobacteriales* (8.35%), were dominant. In SL3 microcosm, there is a massive decrease by 91% and 98% in the population of members of *Bacillales* and *Xanthomonadales*, respectively, as well as extinction of several other orders. However, massive upscale in sequence reads of *Planctomycetales* and significant increase in the population of members of *Syntrophobacterales* and *Caulobacterales* were observed in SL3 metagenome (Figure 3).

In family delineation, 137 families were recovered from SL1 microcosm. The predominant families are *Staphylococcaceae* (23.11%), *Enterobacteriaceae* (12.54%), *Sphingobacteriaceae* (6.11%), *Bacillaceae* (5.38%), and *Alcaligenaceae* (4.93%). In mercury-contaminated SL3 microcosm where 102 families were retrieved, *Planctomycetaceae* (19.81%), *Caulobacteraceae* (14.03%), *Sphingobacteriaceae* (11.71%), and *Enterobacteriaceae* (10.22%) were dominant. Massive decline in the population of members of *Staphylococcaceae* (99.69%), *Microbacteriaceae* (86.7%), and *Dermabacteraceae* (99.75%) families were observed in SL3. Contrastively, there is a massive upscale in the sequence reads of *Planctomycetaceae* and a significant increase in the population of members of the families *Sanguibacteraceae*, *Caulobacteraceae*, *Phyllobacteriaceae*, *Syntrophaceae*, and few others (Figure 4).

Mercury contamination of the agricultural soil resulted in a significant shift in its microbial community structure. In agricultural soil (SL1) microcosm where 213 genera were recovered, the genera *Staphylococcus* (29.65%), *Sphingobacterium* (4.59%), *Pseudomonas* (4.28%), *Pedobacter* (3.30%), and *Caulobacter* (2.70%) were dominant. However, in mercury-contaminated (SL3) microcosm where 154 genera were retrieved, the genera *Singulisphaera* (16.61%), *Brevundimonas* (10.04%), *Sphingobacterium* (9.48%), *Pedobacter* (7.71%), and *Caulobacter* (7.18%) were preponderant. There is massive reduction in the population of *Staphylococcus* (99.71%) and *Brachybacterium* (99.75%) and several others in SL3. In contrast, mercury contamination massively enriched the population of *Singulisphaera, Proteus, Desulfobacca, Brevundimonas, Sanguibacter, Caulobacter*, and few others in SL3 metagenome (Figure 5).

In species delineation of the metagenomic reads, 242 and 156 species were recovered from SL1 and SL3 metagenomes, respectively. The dominant species in SL1 are *Sphingobacterium* sp. 21 (9.16%), *Pedobacter saltans* (6.57%), *Brevundimonas subvibrioides* (5.04%), and *Brachybacterium faecium* (4.33%). In mercury-amended SL3, the predominant species are *Singulisphaera acidiphila* (22.85%), *Brevundimonas subvibrioides* (13.80%), *Sphingobacterium* sp. 21 (13.04%), and *Pedobacter saltans* (10.34%) (Figure 6).

Contig-based taxonomic classification of the metagenomes (SL1 and SL3) conducted by aligning the SL1 and SL3 contig to NCBI's RefSeq database using the BWA-mem aligner is indicated in Additional file 1: Figs. S1-S6.

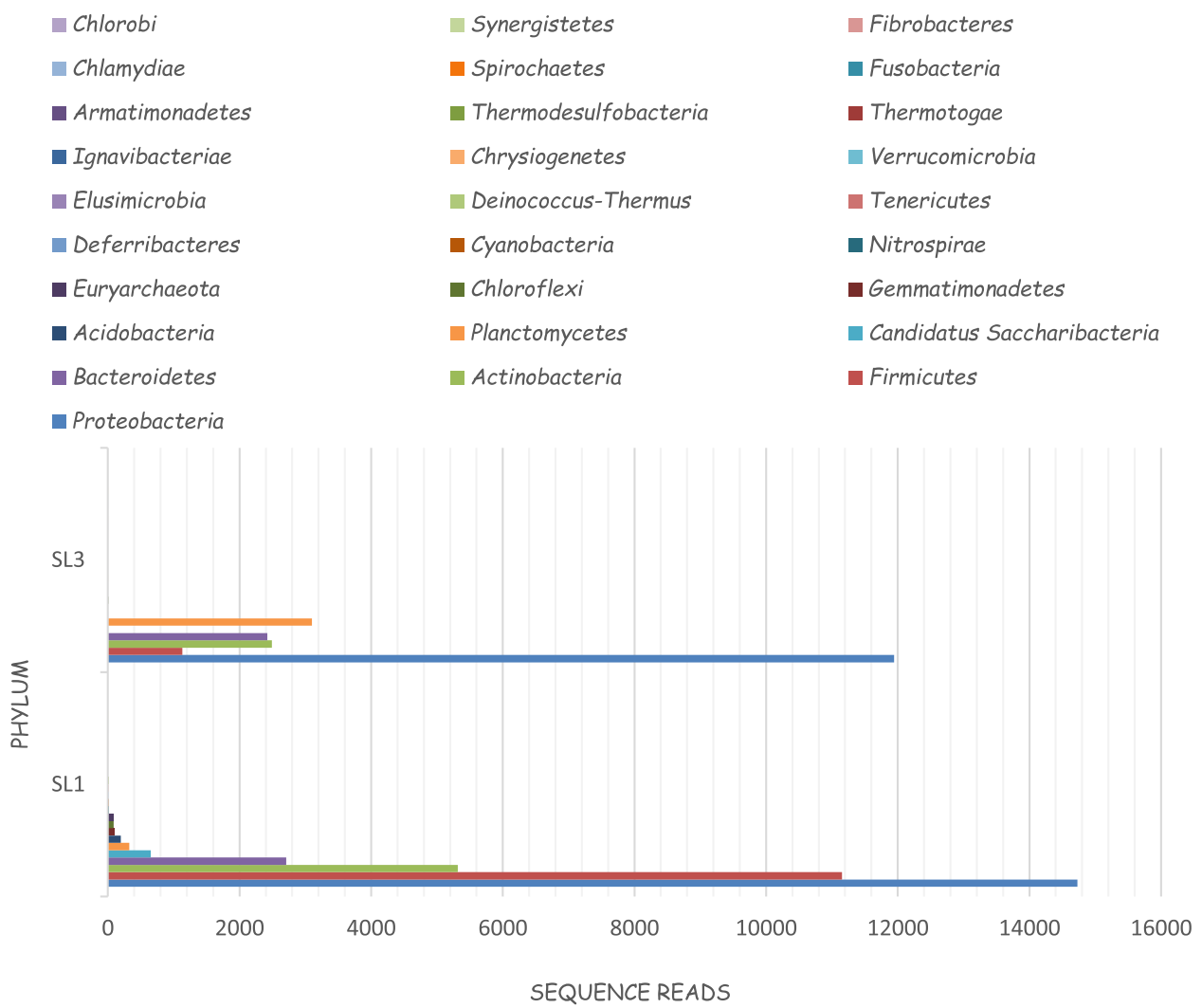

Fig. 1 Phylum classification of SL1 and SL3 metagenomes. Unclassified reads were not used for the analysis. All the phyla detected in SL1 and SL3 metagenomes were used

Discussion

Inundation of agricultural soils with various concentrations of heavy metal ions via waste disposal, bush burning, manure and fertilizer application, as well as pesticide and herbicide application affects soil ecosystem processes, alters soil physicochemistry, reduces microbial richness and diversity, with consequences on biogeochemical cycling and ecosystem balance (Babich and Stozzky 1985; Giller et al. 1998; Lapanje et al. 2010). In this study, all the physicochemical parameters significantly reduce in mercury-amended SL3 microcosm. This may be attributed to mercury contamination. For instance, several authors have averred that organic matter plays a dominant role in the binding of mercury in soils at it often forms stable complexes with organic ligands (Wang et al. 1997; Boszke et al. 2003; Dreher and Follmer 2004) especially where the pH of the soil is < 7 (Gabriel and Williamson 2004). More so, dissolution of metal ions in soil and complexation of the mercury salt with essential soil nutrients such as nitrates and phosphates will lower the pH of the soil and render the nutrients inaccessible to microorganisms (Andrew and Jackson 1996; Salam and Ishaq 2019).

Heavy metal content analysis of the agricultural soil reveals the presence of various heavy metals such as lead, chromium, cadmium, copper, zinc, and selenium though at concentrations not above the permissible threshold for soils (WHO/FAO 2001; UNEP 2013; Toth et al. 2016). This may be attributed to various agricultural practices such as fertilizer application, manure application, bush burning, and pesticide and herbicide application, among others that introduce the heavy metals to the agricultural soil. The concentrations of these metals drastically reduced in SL3 mercury-

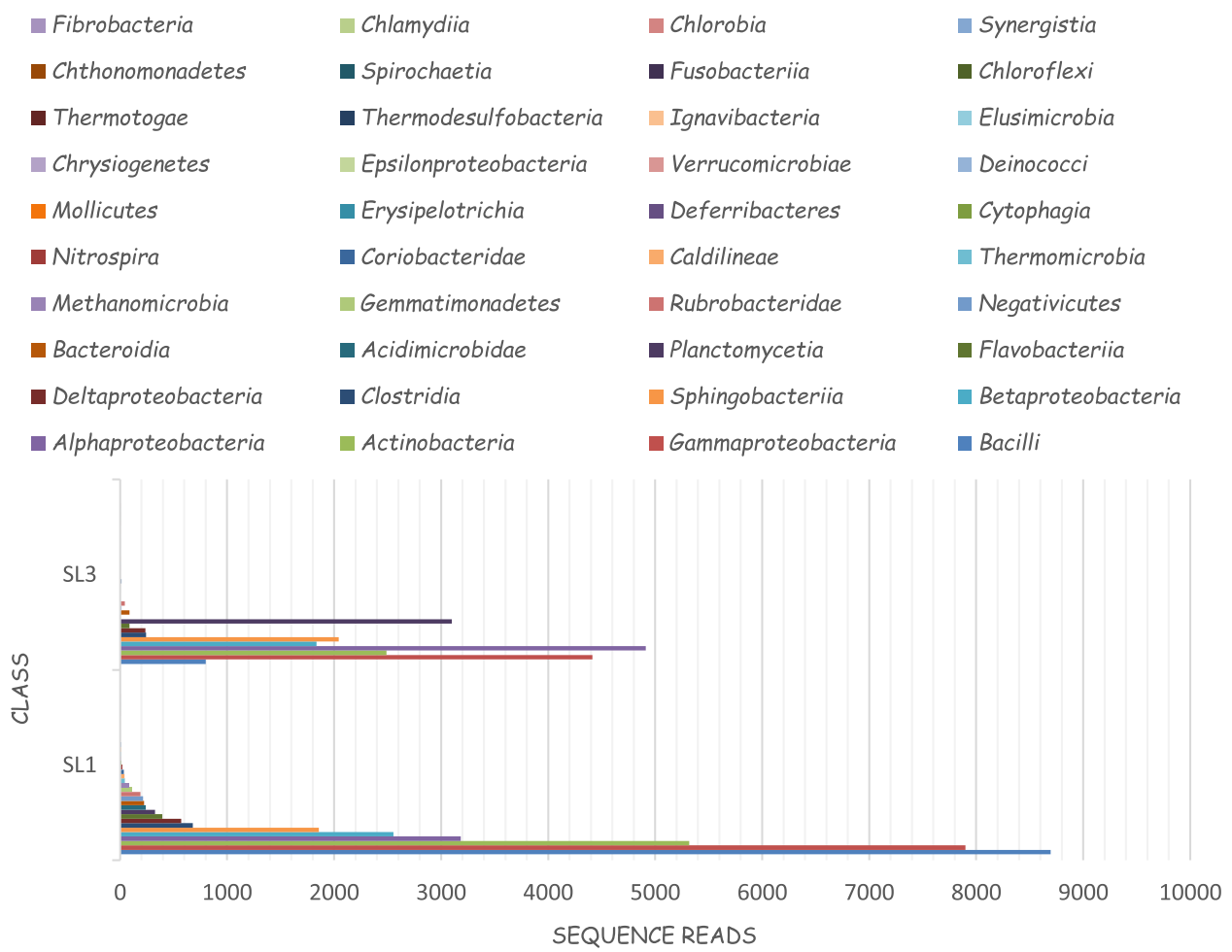

Fig. 2 Class classification of SL1 and SL3 metagenomes. Unclassified reads were not used for the analysis. All the classes detected in SL1 and SL3 metagenomes were used

amended soil. This may be due to heavy metal tolerance/resistance of some members of the microbial community. Heavy metals such as zinc, copper, iron, and chromium are essential micronutrients and cofactors of several enzymes and are important in redox processes, stabilization of molecules through electrostatic interactions, and regulation of osmotic pressure (Bruins et al. 2000). However, non-essential heavy metals with no known biological functions such as lead, cadmium, and mercury are very toxic with deleterious effects on microorganisms such as protein denaturation, cell envelope disruption, inhibition of cell division and enzyme activities, destruction of nucleic acids, and transcription inhibition (Khan et al. 2009; Gundacker et al. 2010; Banfalvi 2011; Wyszkowska et al. 2013; Yuan et al. 2015). The ability of the microbial community in SL3 to maintain intracellular homeostasis of the essential heavy metals and normalize resistance against toxic heavy metals is predicated on diverse resistance mechanisms such as chromosomal/plasmid mediated efflux systems that pump the toxic metal ions from microbial cells, enzymatic biotransformation of metals to less toxic species, and incorporation of heavy metals into complexes by metal-binding proteins, which makes them less toxic to the cell (Nies and Silver 1995; Silver and le Phung 2005; Dziewit and Drewniak 2016).

Mercury contamination of SL1 agricultural soil resulted in the loss of 54.75% of the microbial community consisting of an archaeal domain, 11 phyla, 12 classes, 24 orders, 36 families, 59 genera, and 86 species. Previous reports have indicated that exposure of microbial communities to mercury contamination results in an initial decline in microbial numbers followed by enrichment and rapid growth of mercury-resistant subpopulations with relatively low genetic diversity of mercury-adapted communities as consequence (Rasmussen and Sorensen 2001; Rasmussen et al. 2008). Mercury compounds (Hg^{2+}), on gaining access to the cell, form covalent bond with cysteine residues of proteins and deplete cellular antioxidants (Valko et al. 2006). This results in oxidative stress to microbial cells

Fig. 3 Order classification of SL1 and SL3 metagenomes. Unclassified reads were not used for the analysis. All the orders detected in SL1 and SL3 metagenomes were used

due to imbalance between pro- and anti-oxidant homeostasis (Fashola et al. 2016). This perhaps explains the massive reduction in the population of dominant genera in agricultural soil SL1 such as *Staphylococcus*, *Geobacillus*, *Streptococcus*, *Brachybacterium*, *Flavobacterium*, and many others in mercury-contaminated SL3.

The predominance of the phyla *Proteobacteria* and *Firmicutes* in the agricultural soil is expected as these phyla are replete with members that are well adapted to agricultural soils (Cheema et al. 2015; Trivedi et al. 2016; Salam et al. 2017; Yin et al. 2017). This is due to their huge physiological, morphological, and metabolic diversity; ability to survive in oligotrophic environments; ability to utilize diverse low molecular weight and high molecular weight recalcitrant carbon compounds prevalent in soils; and ability to survive adverse environmental conditions and resist desiccation caused by sharp variation in soil surface temperature (Fierer et al. 2007; Spain et al. 2009; Eilers et al. 2010; Goldfarb et al. 2011; Aislabie and Deslippe 2013; Montecchia et al. 2015). While the phylum *Proteobacteria* loses 19% of its members due to mercury contamination in SL3, it still constitutes the highest population (57%) in SL3 metagenome. Interestingly, 90% of the members of the phylum *Firmicutes* in SL3 were lost, while surprisingly, those of the phylum *Planctomycetes* were massively enriched in SL3 metagenome. The massive increase

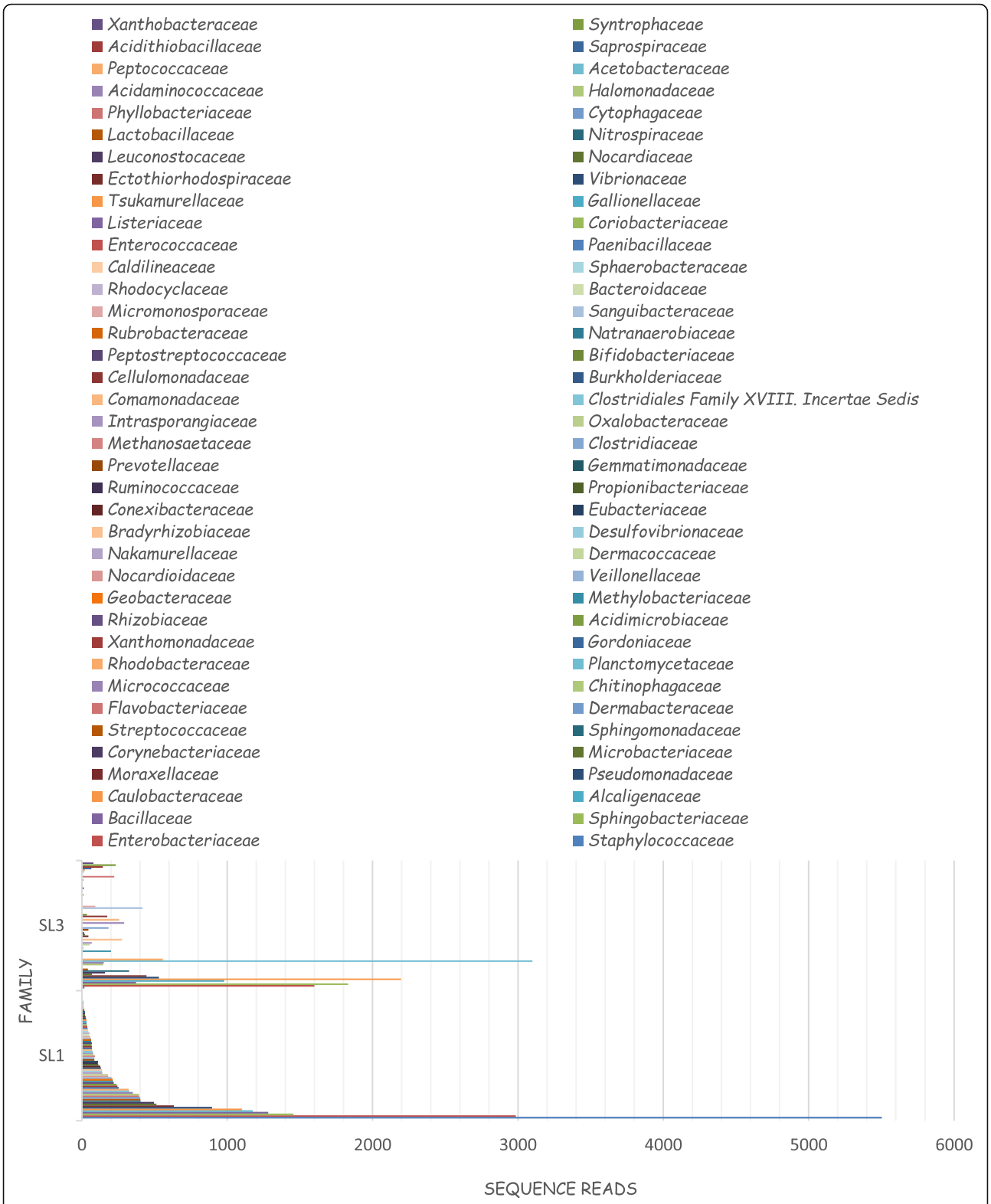

Fig. 4 Family classification of SL1 and SL3 metagenomes. Unclassified reads were not used. Only families with ≥ 10 sequence reads were used for the analysis

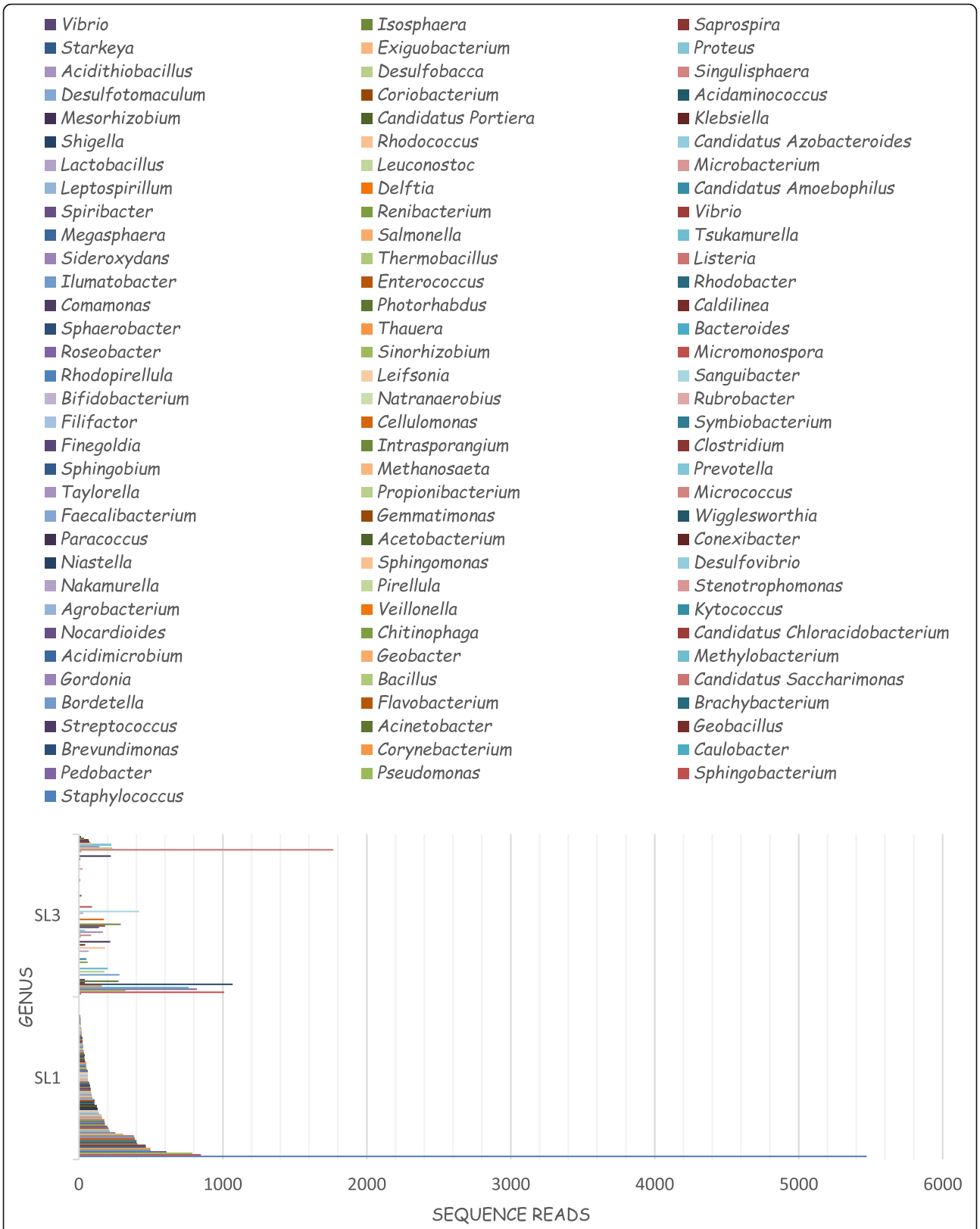

Fig. 5 Genus classification of SL1 and SL3 metagenomes. Unclassified reads were not used. Only genera with ≥ 10 sequence reads were used for the analysis

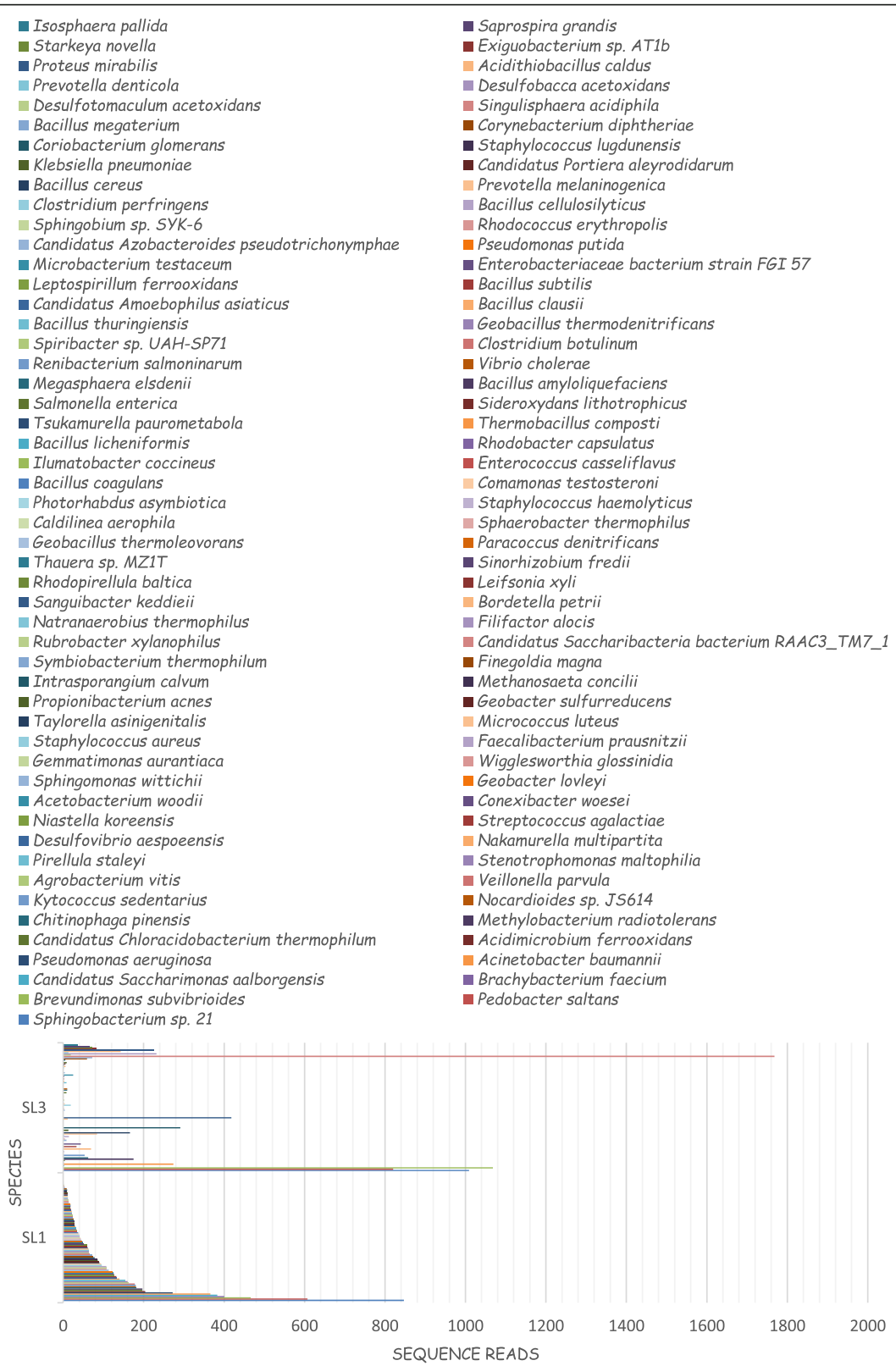

Fig. 6 Species classification of SL1 and SL3 metagenomes. Unclassified reads were not used. Only species with ≥ 10 sequence reads were used for the analysis

in the population of *Planctomycetes* may be attributed to the acidic pH observed in SL3 due to mercury contamination, heavy metal encrustation on the surface of the stalks of some members of *Planctomycetes*, and the detection of heavy metal resistance genes (Schmidt et al. 1981; Schmidt et al. 1982; Kulichevskaya et al. 2008; Guo et al. 2012).

Though the massive reduction in the concentration of the added inorganic mercury may be connected to the sorption of Hg^{2+} to iron oxides and organic matter in the soil (Selin 2009), the conversion of Hg^{2+} to elemental mercury (Hg^0) via the activities of members of the microbial community possessing the mercury resistant *mer* gene may have also played a prominent role in the reduction (Gabriel and Williamson 2004). This is because in Hg-resistant microorganisms, the Hg^0 evaporates from the microbial cells (Barkay et al. 2003) and the mercury concentration of the soil containing Hg-resistant microorganisms becomes progressively reduced (Wagner-Dobler et al. 2000).

The predominance of the genus *Singulisphaera* belonging to the phylum *Planctomycetes* in SL3 metagenome is interesting. Aside from the fact that the pH of SL3 microcosm (5.96 ± 0.25) falls within the range of optimum pH (5.1–6.2) of the species *S. acidiphila* (Kulichevskaya et al. 2008), which perhaps contribute to the dominance of the genus and species in SL3 metagenome, the detection of heavy metal-translocating P-type ATPase in the draft genome of the species *S. acidiphila* (Guo et al. 2012) may have played a prominent role in the predominance of these species in the mercury-amended SL3 metagenome.

The genera, *Brevundimonas* and *Sphingobacterium* are the second and third dominant genera in SL3 metagenome, respectively. The preponderant of these genera in mercury-contaminated environments has been reported by various workers. The genus *Brevundimonas*, which is significantly enriched in SL3 metagenome has been recovered by several workers from mercury-contaminated environments. Irawati et al. (2012) isolated two highly Hg-resistant (MIC 575 ppm) species, *Brevundimonas* sp. HgP1 and *Brevundimonas* sp. HgP2 from a gold mine in Pongkor village, Indonesia. Similarly, Chasanah et al. (2018) reported the isolation of *Brevundimonas vesicularis* among the mercury-resistant bacterial strains recovered from small-scale gold mine tailings in West Lombok, Indonesia. In addition, using a 10-phylum-specific MerA primer set, Møller et al. (2014) detected the *MerA* gene from *Sphingobacterium spiritivorum* isolated from freshwater samples.

Other species with significant presence in SL3 metagenome such as *Pedobacter saltans*, *Sanguibacter keddieii*, *Proteus mirabilis*, *Exiguobacterium* sp. AT1b, *Bacillus megaterium*, *Desulfobacca acetoxidans*, and *Acidithiobacillus caldus* strain SM-1 have also been recovered from mercury-contaminated matrices, thus, indicating that their presence is not fortuitous. For instance, resistance of *Proteus mirabilis* isolated from gold-processing mercury-contaminated sites to 40 mg/l phenyl mercury (Fatimawali et al. 2019) and possession of glutathione S-transferase involved in mercury and other heavy metal resistances by *Proteus mirabilis* (Zhang et al. 2013) have been reported. Also, Huang et al. (1999 a, b) isolated *Bacillus megaterium* MB1 from the Minamata bay sediment in Japan that harbors a transposon TnMER11 on its chromosome encoding *merR, merT, merP, merA*, and *merB* genes for metal-specific activator-repressor, transport, extracellular metal ion binding, mercuric reductase, and organomercurial lyase, respectively. In addition, while Karami et al. (2011) reported the isolation of mercury-resistant *Exiguobacterium* sp. AT1b, which tolerated 50–75 ppm mercury (supplied as mercury chloride) from coastal waters in the Persian Gulf, Castro-Severyn et al. (2017) isolated *Exiguobacterium* sp. SH31 from an altiplanic shallow athalassohaline lake in Chile encoding a wide repertoire of proteins required for cadmium, copper, mercury, tellurium, chromium, and arsenic resistance. Furthermore, Acuna et al. (2013) conducted a genomic analysis of *Acidithiobacillus caldus* strain SM-1, which revealed a truncated version of the mercuric reductase encoding operon merTPAB, which confers the strain with increased mercury detoxification capacity.

Conclusions

In summary, this study has revealed that mercury contamination of the agricultural soil impacted negatively on the richness and diversity of the microbial community structure, significantly altered the soil physicochemistry, and massively enriched members of the phylum *Planctomycetes* and others hitherto not reported to be involved in mercury detoxification.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42269-019-0208-5.

Additional file 1: Figs. S1-S6. Contig-based taxonomic classification of the metagenomes (SL1 and SL3).

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Authors' contributions

LBS designed, the work, analyzed the data, and wrote the manuscript. HS, ZU, and FB performed the experiments. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interest.

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