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The metaphenome of a calaminiferous soil

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ABSTRACT

The aim of the present research was to determine the metaphenome (the expressed microbial functions) of a calaminiferous soil mostly contaminated with Zn and Pb. For that, three 'omics approaches were combined using soil samples: shotgun metagenomics (MG), metatranscriptomics (MT) and shotgun metaproteomics (MP). Results show that several indexes of bacterial diversity were significantly reduced. The soil was dominated by Acidobacteria, Chloroflexi and Gemmatimonadetes. Although many of the species were not identified, the mean percentages of reads for Candidatus *Solibacter*, *Bryobacter, Rhodomicrobium* and *Niastella* were significantly more elevated in the contaminated soil when compared to a control soil. For functionality, the identified proteins were classified in 28 functional SEED categories. The MG approach detected many genes that may be used to degrade various types of organic materials, among which chitin, N-acetylglucosamine, cellulose and glycogen. This suggests that the important carbon cycle functions of the heterotrophic communities are maintained in the soil. For metal resistance, the most abundant proteins were P-type ATPases efflux systems.

1. Introduction

Calaminiferous soils, or calaminarian grasslands, are open plant formations (the substrate is frequently visible) which colonize soils rich in Cd and Zn, sometimes with other metals such as Pb (Baker et al., 2010). They take their name from the town of La Calamine, in the east of Belgium, where various ores rich in Zn and Pb were extracted. Calamine is also a historic name for several ores of zinc such as smithsonite ($ZnCO_3$) and hemimorphite ($Zn_4Si_2O_7(OH)_2{\cdot}H_2O$). Calaminarian grasslands are naturally present all over the world, for example on rocky outcrops containing high proportions of zinciferous ores. Today, natural calaminarian grasslands have practically disappeared as a result of the exploitation of the metal deposits. However, they are still found on waste dumps, along the edge of some rivers, and on soils poisoned by the fallout of fumes from zinc factories and aerosols from waste dumps such as in Auby in northern France (Lemoine, 2012) and also in Poland (Gołębiewski et al., 2014) and Spain (Epelde et al., 2010). Metals have a significant influence on these soils and many organisms resistant to high concentrations of metals are found (Baker et al., 2010). Surprisingly, very few studies on the microbiology of such soils have been published (Epelde et al., 2010; Gołębiewski et al., 2014; Beattie et al., 2018; Olenska et al., 2020), and only two of them have used 16S rRNA gene analysis to assess bacterial diversity (Gołębiewski et al., 2014; Beattie et al., 2018). It was found that the core set of OTUs comprised members of such taxa as *Sphingomonas*, Candidatus *Solibacter*, or *Flexibacter* (Gołębiewski et al., 2014). Seven phyla were found to significantly correlate with at least one metal including Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Proteobacteria, and Verrucomicrobia. Within these phyla, multiple genera were significantly and positively correlated with metals including *Streptomyces, Flavisolibacter, Sphingomonas, Geobacter, Gemmata* and *Planctomyces* (Beattie et al., 2018). However, none of these studies used proteomics, shotgun metagenomics or transcriptomics to focus on active soil microorganisms and discover their metal-resistance mechanisms. Therefore, the functioning of microbial communities in calaminiferous soils is still largely unknown.

The aim of the present research was to determine the metaphenome of a calaminiferous soil located in North of France (Auby, Péru Parc). The metaphenome may be defined by the expressed functions encoded in a metagenome found in a particular environment (Jansson and Hofmockel, 2018). The metaphenome thus encompasses the entire 'omics' field, including the metagenome, the metatranscriptome (expressed genes), the metaproteome (proteins resulting from translation) but also the metabolome. In the present research we will mainly focus on taxonomy (who's there? who is active?), functionality, and the capacity of metal resistance. For that three 'omics approaches will be combined: metagenomics (MG), metatranscriptomics (MT) and metaproteomics (MP). A targeted MT approach, based on the 16S rRNA gene, was first

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used to compare the bacterial communities in the calaminiferous soil and a control soil, to identify the active bacterial clades that were enriched in Auby. We then focused on the metal-contaminated soil using a shotgun MP approach to confirm the active soil microbes found by MT. The main functions in the soil were also provided by a shotgun MG approach. The combination of these 'omics approaches gave valuable insights on both taxonomy and functionality of the most abundant and active microorganisms in the calaminiferous soil.

2. Materials and methods

2.1. Collection of soil samples

Samples of surface soil (0–5 cm) were collected in the Péru parc (Auby, France, 50°24'26"N, 03°04'59"E; 50.407222 N, 3.083056 E), a calaminiferous soil in the vicinity of an active Zn factory. Samples of soil were also obtained in a control soil (garden soil of the Mons University, 50°27'46"N, 03°57'16"E, located 82 km at the east of Auby). The Auby soil featured metallophytes (*Arabidopsis thaliana* and *Viola calaminaria*), and these plants were absent from the control soil.

For MT, samples were collected in both sites on the 23d September 2021 and placed in sterile 50 mL Falcon tubes in triplicate (n = 3). For MP and MG only the calaminiferous soil was investigated. For MG the soil was collected on October 25th, 2018, and samples from several points separated by about 2 m from each other were combined and homogenized. A total of 165 g of mixed soil was obtained, and 5 g of the soil mixture was then used for DNA extraction. For MP, 4 soil samples of 5 g (separated by about 2 m from each other) were obtained on 27th August 2019.

2.2. Total metals and soil pH

For total metals, soil samples were obtained in October 2022 (n = 8 for Auby, n = 4 for controls). The samples were first dried for 48 h at 60 °C. Aliquots of ca. 2 g of soil were then mineralized by a 1 h treatment with hot (80–100 °C) HNO_3^- (20 mL, 65%), H_2O_2 (10 mL) and HCl (30 mL, 37%). The solution was then filtered by gravity (Schleicher & Schüll filters, n°589/2, porosity 4–12 μm) and the glassware was rinsed with MilliQ water, up to a final volume of 20 mL. Metals were then quantified by the ICP-AES approach (Iris Intrepid II XSP) using a calibration curve and certified reference materials (ICP multi-element standard solution IV, Cetripur, Merck). Soil pH was determined with fresh soil samples (250 mg) placed in 10 mL of a solution of 1 M KCl and using a laboratory pH-meter (Mettler-Toledo FiveEasy).

2.3. Metatranscriptomics (MT)

Nucleic acids extraction. Six soil subsamples of 500 mg (3 tubes for the Auby metal-contaminated soil, 3 tubes for the control soil) were placed in 2 mL tubes with glass beads (Lysing Matrix E, MP-Biomed). A volume of 500 μL of CTAB buffer was then added to the six tubes. This buffer contained (for 200 mL) 4.09 g of NaCl and 10 g of hexadecyltrimethylammonium bromide dissolved in a final volume of 100 mL of DEPC-treated water; then a volume of 100 mL of a potassium phosphate buffer (240 mM, pH 8.0) was added. Phenol-chloroform-isoamyl alcohol (25:24:1; pH 8.0) was added to the six tubes (500 μL). The tubes were then placed in a bead-beater (Precellys 24, Bertin technologies) and agitated at 6500 rpm for 45 s at room temperature. Tubes were then briefly placed on ice and centrifuged at 16000*g* for 5 min at 4 °C. The top aqueous phase was recovered (450 μL) in an Eppendorf Protein LoBind tube (certified RNase and DNase free) and combined with the same volume of chloroform-isoamyl alcohol (24:1). Tubes were vortexed a few seconds and centrifuged as before. The top aqueous phase was then recovered (400 μL) and combined with two volumes (800 μL) of a PEG-NaCl buffer. Buffer PEG-NaCl contained 30% (w/v) of polyethylene glycol 6000 in 1.6 M NaCl. Tubes were then manually agitated by inversions and left for 2 h at room temperature to precipitate nucleic acids. Tubes were then centrifuged (20 min at 16000*g* at 4 °C), the supernatant was eliminated, and the pellet formed was then washed with 1 mL of 70% ethanol in DEPC-treated water (tubes were inverted several times). Tubes were then centrifuged for 10 min (16000*g*, 4 °C) and the 70% ethanol was slowly eliminated with a pipet to leave the pellet undisturbed. The pellet was then dried 5–10 min at ambient temperature (tube caps opened). The dried pellet was resuspended in 40 μL of THE RNA storing solution (ThermoFischer) and the nucleic acids were stored at −80 °C.

RNA purification and reverse transcription. The DNA in the six tubes was eliminated using the RQ1 RNase-free DNase kit (Promega). For that, 38 μL of nucleic acids were combined with 7.6 μL of reaction buffer and 30.4 μL of DNase. The tubes were incubated 30 min at 37 °C, then 7.6 μL of stop solution was added and then tubes were incubated 10 min at 65 °C. The RNA was then purified using the MEGAclear Transcription clean-up kit (ambion, Life technologies) and then eluted using the elution solution provided by the kit (2 tubes of 50 μL). The RNA was quantified using the Qubit 3.0 method, and the tubes with the maximal quantity of RNA were selected. The first strand of the reverse transcription process was then generated using the SuperScript IV first-strand cDNA synthesis kit (ThermoFischer) with random hexamers from Sigma (pd(N)6, potassium salt, ref 11034731001). For that 22 μL of RNA was combined with 2 μL of hexamers (final concentration of 178 μM) and 2 μL of dNTP. The mixture was incubated 5 min at 65 °C then 8 μL of SSIV buffer (5x), 2 μL of 100 mM DDT, 2 μL of an RNase inhibitor and 2 μL of SuperScript IV reverse transcriptase (200 U/μL) were added. The tubes were incubated 10 min at 23 °C, 2 h at 52 °C and 10 min at 80 °C. The second strand cDNA synthesis kit (Invitrogen) was then used following the protocol given by the manufacturer, except that the tubes were incubated 2 h at 16 °C. The DNA produced was purified using the GeneJET PCR purification kit (ThermoScientific) and the DNA was eluted in 50 μL.

PCR amplification of the 16S rRNA gene and sequencing. DNA from the previous reverse transcription step was used with primers 515 F-Y (5′-GTG YCA GCM GCC GCG GTA A-3′) and 926 R (5′-CCG YCA ATT YMT TTR AGT TT-3′) (Parada et al., 2016) to amplify a 411 bp fragment of the 16S rRNA gene by PCR (V4 and V5 region of the 16S rRNA). The Red'y StarMix PCR kit from Eurogentec was used with a final concentration of primers of 0.1 μM. The following cycle was used: 3 min at 95 °C, then 25 cycles with 45" at 95 °C, 45" at 50 °C and 90" at 68 °C. The final extension step was 5 min at 68 °C. PCR amplicons were visualized by electrophoresis on agarose 1.5% with Gel Red staining. Several PCR amplifications were performed in parallel to produce enough DNA for sequencing. PCR amplicons were finally combined and purified using the GeneJET PCR purification kit. The quantity of DNA obtained was comprised between 150 and 550 ng. The DNA was then sequenced by the Illumina method on a NovaSeq platform using a 250 bp paired-end method (sequencing performed by Novogene, PE250, Q30 $\geq$ 75%). For that the DNA was randomly sheared into short fragments. Then DNA fragments were narrowly size selected with sample purification beads. The obtained fragments were end-repaired, A-tailed and further ligated with Illumina adapters. After these treatments, the fragments were filtered with beads again. The library was checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced according to the effective library concentration and data amount required (1 G raw data per sample). The number of raw reads obtained varied between 4 and 5.10^9 for each of the 6 samples with Q20 > 95% and Q30 > 90% (Base count of Phred value > 20 or 30) / (Total base count).

Bioinformatic treatments. The Cutadapt software (https://github.com/marcelm/ cutadapt) was first used in paired-end mode to remove PCR primers from the raw reads and sequences without primers were eliminated. The DADA2 pipeline (Callahan et al., 2016) was then used within the R environment for all the bioinformatic treatments (package version 1.23.0). Sequences of low quality or featuring unidentified

nucleotides (N) were eliminated using the filterAndTrim command (forward reads were trimmed at 240 bases, and reverse reads at 225 bases). Reads were dereplicated (derepFastq command) and error rates were calculated (learnErrors command). The dada command was then used with OMEGA_A = 1e–30 and contigs were generated using the mergePairs command. Only unique contigs were kept for the analysis. At this point, chimeric sequences were removed (removeBimeraDenovo command) and contigs were aligned to the SILVA database (version silva_nr_v132). Then, sequences homolog to chloroplasts and mitochondria were removed, as well as sequences featuring more than 8 identical nucleotides (homopolymers). Finally, a last check with UCHIME2 was performed to remove remaining chimeric sequences (Edgar et al., 2011; Edgar, 2016). Tables with the percentage of reads at the phylum, class, order, family, and genus levels were then generated. Non-parametric statistics were then used to analyze these tables (Mann Whitney U test). For alpha diversity, the Shannon (H), Chao1 and Simpson indexes (D) were calculated using the list of bacterial genera in Excel as described in Kim et al. (2017). Sequences were then deposited at the NCBI (Auby: BioProject PRJNA929332; UMons: BioProject PRJNA929480).

2.4. Shotgun metagenomics (MG)

The purpose of the shotgun MG approach was to assess soil functionality but also to construct a gene database to be used for metaproteomics (MP). For DNA extraction, the 5 g of mixed soil obtained above was first washed successively with three sterile buffers differing in EDTA concentration in order to remove as much metals and sequencing inhibitors as possible (Fortin et al., 2004). Total DNA was then extracted using the DNeasy PowerSoil Kit (QIAGEN) according to the manufacturer's instructions. For that 6 × 250 mg of soil was used (the maximum quantity of soil in one tube). The DNA quantity and quality were then determined by the PicoGreen approach and the quality was also verified by 1% agarose gel electrophoresis.

Sequencing was then performed using an Illumina PE150, NovaSeq platform (paired ends, 2 ×150 bp). The obtained raw FastQ files were uploaded to the Metagenomics Rapid Annotation using Subsystem Technology (MG-RAST, v4.0.3) server for taxonomic and functional analysis (Meyer et al., 2008). The default settings of the MG-RAST automated processing pipeline were used. Briefly, the low-quality reads were trimmed by the Skewer software, the clean reads were then aligned by Bowtie 2, coding regions were predicted by FragGeneScan, and protein clusters at 90% identity level were built using CD-HIT. For taxonomic assignments, sequences were searched against the RefSeq database. For protein identification and annotation, the putative sequences were subjected to BLAST against the M5NR (non-redundant protein database) in the MG-RAST server to annotate their function. The M5NR is an integrated database containing the NCBI GenBank, Clusters of Orthologous Groups (COGs), Kyoto Encyclopedia of Genes and Genomes (KEGG) and SEED in a single searchable database (Meyer et al., 2008). The Cut-off parameters were as default in MG-RAST for taxonomic and functional analysis, included an E-value < 1.00e-05, identity of 60%, and a minimum alignment length of 15.

An MG-RAST independent analysis was also performed for metal-resistance genes. For that, the illumina paired-end reads were first assembled with PEAR v0.9.11 (Zhang et al., 2014). A total of 41,687,329 assembled reads were thus obtained. These reads were then compared to the BacMet database (version 2.0), a manually curated database of 753 metal and biocide-resistance genes, with experimentally confirmed resistance function (Pal et al., 2014). To perform this comparison DIAMOND v2.1.8.162 was used with the blastx option (Buchfink et al., 2021). The scoring parameters were Matrix=BLOSUM62, Lambda= 0.267, K= 0.041, Penalties= 11/1. The tsv-file produced was then searched for matches (identities >80% and an Evalue <1.00e-05) using tsv-filter (https://opensource.ebay.com/tsv-utils/).

The raw sequences data (FASTQ format) acquired through the shotgun sequencing were deposited at NCBI SRA databases under BioProject accession number PRJNA782887.

2.5. Shotgun metaproteomics (MP)

Protein extraction of the 4 unmixed soil samples (n = 4) was performed as previously described (Gillan et al., 2015; Bertin et al., 2011). A Nycodenz gradient was first used to recover microbial cells from the soil. Five grams of fresh soil was first mixed with 15 mL of recovery solution (0.15 g/L $Na_2SO_4$0.10 H_2O; 0.45 g/L $(NH_4)_2SO_4$, 0.05 g/L KCl; 0.5 g/L $MgSO_4$0.7 H_2O; 0.05 g/L KH_2PO_4; 0.014 g/L $Ca(NO_3)_2$0.4 H_2O) and agitated for 60 h at 4 °C in order to detach microbial cells from the soil particles. The solution was vortexed, sonicated and centrifuged at low speed (300 g) and 8 mL of supernatant was collected and then replaced by 8 mL of fresh recovery solution, every 12 h. The individual supernatants were finally combined. The combined supernatants were then mixed with a Nycodenz solution with a ratio of 1:2.33 (v/v), and then centrifuged 30 min at 9000*g*. The interfaces containing the bacterial cells were collected gently, diluted with two volumes of recovery solution, then combined. Finally, the solution was centrifuged at 16,000 g for 30 min and the bacterial pellets were used for protein extraction. The bacterial pellet was resuspended in 40 μL of Laemmli buffer 1% and sonicated 30 s, then heated 3 min at 95 °C, centrifuged at 14,000 g for 5 min. The proteins in supernatant were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 4–20% Mini-PROTEAN TGX Precast Gel (Bio-Rad Laboratories). The gel was stained with a solution containing 0.25% Coomassie Brilliant Blue R-250, 10% acetic acid, and 50% methanol. Each gel lane (four lanes) was cut into 25 pieces of 1 mm. Subsequently, the gel bands were washed, and the proteins were reduced by dithioerythritol (DTE), alkylated by iodoacetamide and finally precipitated using acetonitrile (ACN). The obtained protein pellets were then solubilized using 25 mM ammonium bicarbonate (NH_4HCO_3) containing trypsin (final concentration 10 μg mL^{-1}) and incubated overnight at 37 °C. Finally, 0.1% formic acid (v/v, final concentration) was added to the digestion solution and stored them at −20 °C for LC-MS/MS analysis.

Protein identifications were performed following a label-free strategy on an UHPLC-HRMS platform (EkspertTM nanoLC 425- AB SCIEX TripleTOFTM 5600) (Géron et al., 2019). Peptides were separated on a 75 μm x 25 cm C18 column using a linear acetonitrile (ACN) gradient [3–35% (v/v), in 30 min] in water containing 0.1% (v/v) formic acid at a flow rate of 300 nL min^{-1}. Data were acquired in a data-dependent acquisition mode (DDA). The acquired MS/MS data was searched against three home-made protein databases using ProteinPilot software (v5.0.1, AB SCIEX) with Paragon algorithm. The three home-made databases include a metagenome database (DB1) which was constructed using the predicted coding regions obtained by MG analysis (unassembled reads), a metal resistance protein database (DB2) constructed by collecting all the proteins associated with metal resistance from UniProt, and a combined database (DB3) using DB1 and DB2. The searching parameters were as follows. Sample Type: Identification; Cysteine Alkylation: Iodoacetamide; Digestion: Trypsin; Search Effort: Thorough ID; ID Focus: Biological modifications and Amino acid substitutions; Detected Protein Threshold [Unused ProScore (Conf)]> : 0.05(10%). The FDR threshold of 1% was used for each protein searches. Peptides and proteins lists were then exported from ProteinPilot to the ProteinPilot descriptive statistics template (PDST, AB SCIEX) for data management and additional analysis. The protein annotation was performed using metaProteomics in environmental sciences (mPies) software (Werner et al., 2019). For taxonomic and functional annotation, mPies used Diamond to align the non-redundant NCBI database and the UniProt database (Swiss-Prot), respectively, and retrieved up to 20 best hits based on alignment score (>80). For taxonomic annotation, mPies uses returned the LCA among the best hits via MEGAN (bit score >80). For functional annotation, mPies returned the most frequent protein name, with a consensus tolerance threshold > 80% of similarity among

the 20 best blast hits. Proteins annotated with a score below this threshold were manually validated (Géron et al., 2019).

3. Results

3.1. Metals and soil pH

Total metals in the two types of soils are shown in Table 1. It may be seen that the calaminiferous soil (Auby) is much more metal-contaminated that the control soil (Cd, 395 times; Pb, 15 times; Zn, 83 times). Cu levels were below the detection limit in the control soil. The pH of the Auby soil was significantly lower than the pH of the control soil (Mann-Whitney, $\alpha = 0.05\%$).

3.2. Number of sequences obtained

The number of raw sequences obtained in the targeted MT approach (based on the 16S rRNA PCR amplification after the extraction of the total RNA) varied between 1.7 and 2.6 $\times$ 10^6 sequences. After cleaning by the DADA2 pipeline (dereplication, merging, removal of chimera, eukaryotes and homopolymers), and the removal of chimeric sequences by UCHIME2, the number of unique 16S rRNA partial sequences obtained was comprised between 173 and 220 sequences (Table S1).

For MG (only in Auby), a total of 38,159,653 raw sequences were obtained, with an average read length of 252 bp and a GC percentage of 61% (Table S2). Following quality trimming, a total of 33,738,709 (88.41% of the total) sequences remained, including 46,014 sequences (0.14%) of ribosomal RNA genes, 17,082,540 sequences (50.63%) of predicted proteins with known functions, and 16,610,155 sequences (49.23%) of predicted proteins with unknown function.

For MP (only in Auby), four independent samples were collected from the soil (n = 4) and three home-made protein databases (DB1, DB2, DB3) were used for protein identification. The mean number of identified proteins were 1785, 48 and 1790, based on DB1, DB2 and DB3, respectively (Table S2).

3.3. Bacterial diversity

The diversity of reads obtained by MT was assessed using the Shannon, Chao1 and Simpson indexes (Table 2). These indexes were significantly more elevated in the control soil (U1–U3) when compared to the Auby metal-contaminated soil (Mann-Whitney, $p < 0.05$).

3.4. Taxonomy at the phylum level

MG analysis indicated that Bacteria were dominant in Auby (98% of the MG reads) with only 1.1% of the reads that were affiliated to Eukaryota and 0.9% to the Archaea. At the phylum level, MT analysis indicated that both soils were dominated by Proteobacteria, Actinobacteria and Acidobacteria (Fig. 1, Table S3). The mean percentages of reads affiliated to Proteobacteria were significantly less abundant (1.5x) in the Auby soil when compared to the control soil ($p < 0.05$). On the contrary, for Acidobacteria, Chloroflexi and Gemmatimonadetes, the mean percentages of reads were significantly more elevated in the Auby soil when compared to the control soil (Table S3). Acidobacteria levels were increased 2.1 times, Chloroflexi levels 3.3 times, and Gemmatimonadetes 3.6 times.

MP confirmed the dominance of Proteobacteria and Acidobacteria in Auby (Fig. 1). However, variability was larger with that approach. For instance, Bacteroidetes represented 15—32% of the reads in two samples, and only 1–3% in the two other samples. The situation was similar for Actinobacteria (15% in one sample, 3–4% in the three other samples) or Gemmatimonadetes (11–17% in two samples, 0.5–1% in the two other samples). Verrucomicrobia were almost not represented with MP (0.1–0.9% of the reads) but well represented with MT (2.9–5.4%) and MG (2.4%).

Table 1
Total metals (mean ± SD, in μg g^{-1} of dry weight) and soil pH in the calaminiferous soil (Auby, n = 8) and the control soil (Mons, n = 4). n.d., not detected.

	Auby	Mons
Cd	79 ± 97	0.2 ± 2E–05
Cu	5 ± 8	n.d.
Pb	1638 ± 986	107 ± 28
Zn	12,125 ± 9431	146 ± 28
pH	5.8 ± 0.3	6.4 ± 0.2

Table 2
Values of the diversity indexes (Shannon, Chao1 and Simpson) obtained after metatranscriptomics. A1–A3, Auby metal-contaminated soil samples; N, number of reads analyzed; N(id), number of reads identified at the genus level; S, number of unique genera; U1–U3, control samples.

	N	N (id)	S	Shannon (H)	Chao1	Simpson (D)
U1	211	90	67	15.5	151	0.030
U2	173	65	48	6.2	83.2	0.029
U3	202	97	63	5.6	158	0.020
A1	220	93	42	– 10.5	53	0.014
A2	202	82	43	– 4.7	57	0.018
A3	127	55	31	– 19.7	37.5	0.013

3.5. Taxonomy at the class level

Analysis of the MT sequences at the class level (Fig. 2, Table S4) show that the mean percentages of reads affiliated to Deltaproteobacteria was significantly less abundant in the Auby soil when compared to the control soil (1.5x; $p < 0.05$). In addition, several classes present in the controls were not detected in Auby, such as Bacilli, Nitrososphaeria and Entotheonellia. On the contrary, for three classes (Acidobacteriia, Gemmatimonadetes and Chloroflexia), the mean percentages of reads were significantly more elevated in the Auby soil when compared to the control soil (Table S4). The most important increase was observed for Acidobacteriia (7.1x).

At the class level, some results obtained by MT were confirmed by MP. For instance, both methods identify Planctomycetacia as one of the dominant bacterial class in Auby. However, large differences were observed for the other groups. For instance, Gamma- and Deltaproteobacteria classes were largely underrepresented by MP (<3.7% and <0.6% respectively). And some classes were even not found by MP (Bacteroidia, Subgroup_6, Verrucomicrobiae, Acidimicrobiia, Nitrososphaeria, Phycisphaerae, Entotheonellia, BD7–11, Blastocatellia_Subgroup_4, and NC10).

3.6. Taxonomy at the order, family and genus levels

Analysis of the MT sequences at the order level (Table S5) showed that the mean percentages of reads affiliated to Myxococcales, Rhizobiales, Chthoniobacterales, Micrococcales, Cytophagales, Pirellulales, and Rhodobacterales were significantly less abundant in the Auby soil when compared to the control soil ($p < 0.05$). On the contrary, for the orders Chitinophagales, Solibacterales and Gemmatimonadales, the mean percentages of reads were significantly more elevated in the Auby soil when compared to the control soil. The most important increases were observed for the orders Solibacterales (6.4x) and Gemmatimonadales (4.5x).

At the family level (Table S6), the mean percentages of MT reads affiliated to Pirellulaceae, Rhodobacteraceae, Xiphinematobacteraceae, Beijerinckiaceae and Rhizobiaceae were significantly less abundant in the Auby soil when compared to the control soil ($p < 0.05$). On the contrary, for the orders Chitinophagaceae, Solibacteraceae_Subgroup_3 and Gemmatimonadaceae the mean percentages of reads were significantly more elevated in the Auby soil when compared to the control soil.

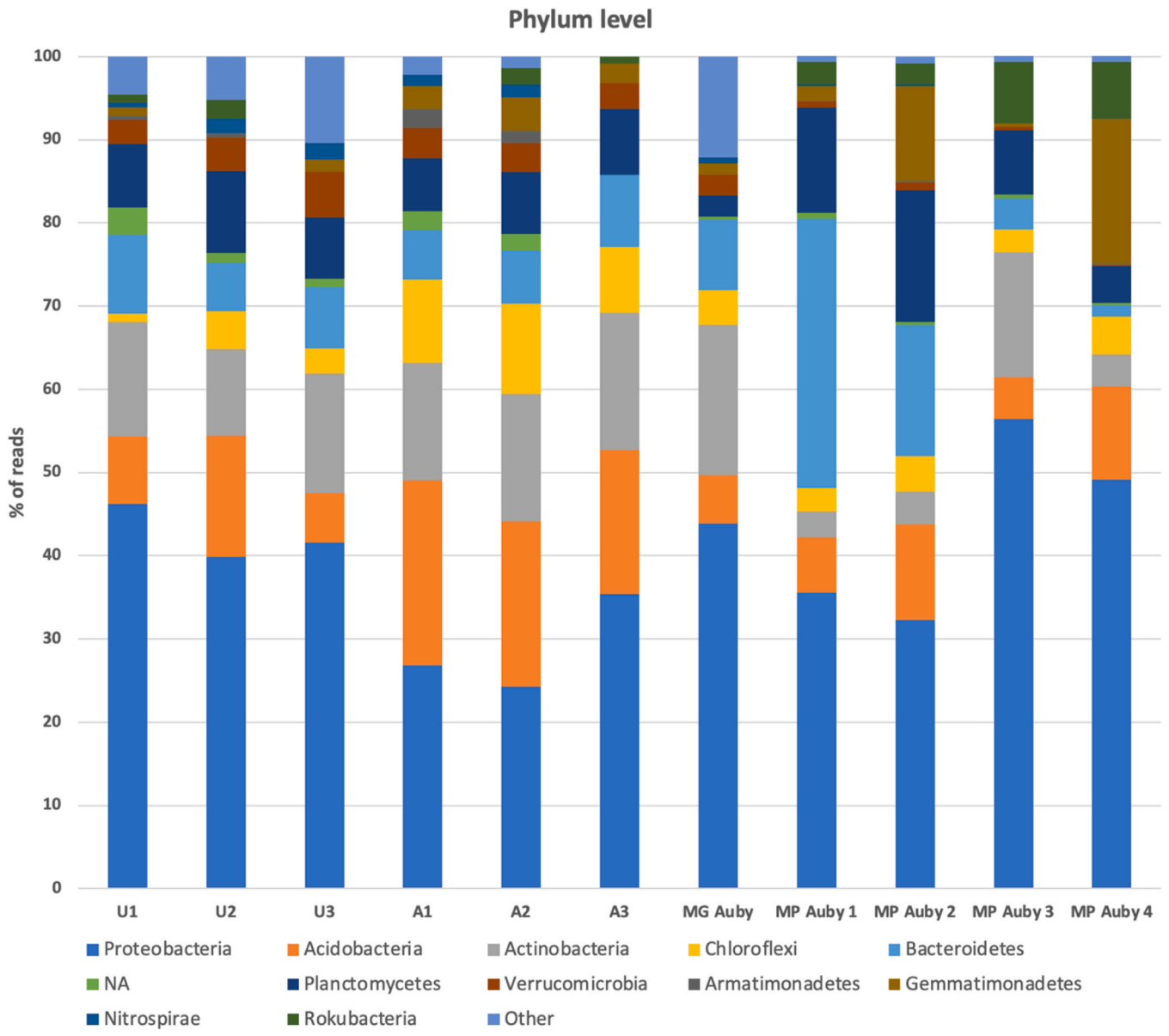

Fig. 1. Results of metatranscriptomics (controls, U1–U3; Auby soil, A1–3), metagenomics (MG Auby) and metaproteomics (MP Auby 1–4) at the phylum level.

The most important increase was observed for the family Solibacteraceae_(Subgroup_3) (6.4x).

Finally, 55–97 bacterial genera could be identified by MT analysis in both sites (N(id) in Table 2). However, genera with an abundance > 1% were less numerous (5–10 in the controls, 9–13 in the Auby soil) (Table S7). The mean percentages of reads affiliated to Candidatus_*Xiphinematobacter*, *Microvirga, Iamia, Skermanella* and OM27_clade were significantly less abundant in the Auby soil when compared to the control soil ($p < 0.05$). On the contrary, for the genus Candidatus_*Solibacter*, *Bryobacter, Aridibacter, Rhodomicrobium* and *Niastella* the mean percentages of reads were significantly more elevated in the Auby soil when compared to the control soil. The most important increases in the Auby soil were observed for the genera *Rhodomicrobium* (9.8x), Candidatus_*Solibacter* (7.7x) and *Niastella* (8x).

The findings of the MT approach at various taxonomic levels are summarized in Table 3. This table shows that reads belonging to three bacterial phyla were significantly enriched in the metal-contaminated Auby soil (Acidobacteria, Chloroflexi and Gemmatimonadetes) and that several of their subgroups (at the class, order, family or genus levels) were also significantly enriched in comparison to the control soil.

Of the bacterial genera identified by MT, only 8 were retrieved by MP. Their relative abundances are given in Fig. 3. MP analysis suggested that the genus *Bradyrhizobium* (Alphaproteobacteria) is an important member of the community in Auby (2.3–4.4% of the proteins), however it only represented 0–1.4% of the MT reads. On the contrary, MT analysis suggested that the genera Candidatus_*Solibacter*, *Rhodomicrobium* and *Niastella* are important members of the community in Auby (1.6–3.6%, 1.5–1.8%, 1.0–1.6% of the MT reads, respectively), however they only represented 0.0–0.5% of the identified proteins in Auby.

3.7. Functional profile

For the MG and MP approaches (only in the Auby soil), a shotgun strategy was used and it was therefore possible to analyze the functional profile of the community. For shotgun MG, 13.7 10^6 of protein features were identified by MG-RAST and could be classified in the 28 functional SEED categories at level-1 (Fig. S1). The carbohydrates SEED category (13.2%, with 1,240,941 reads) was the most represented and the photosynthesis category (0.1%) was the least frequent.

For MP, only 1790 proteins were identified and therefore some SEED

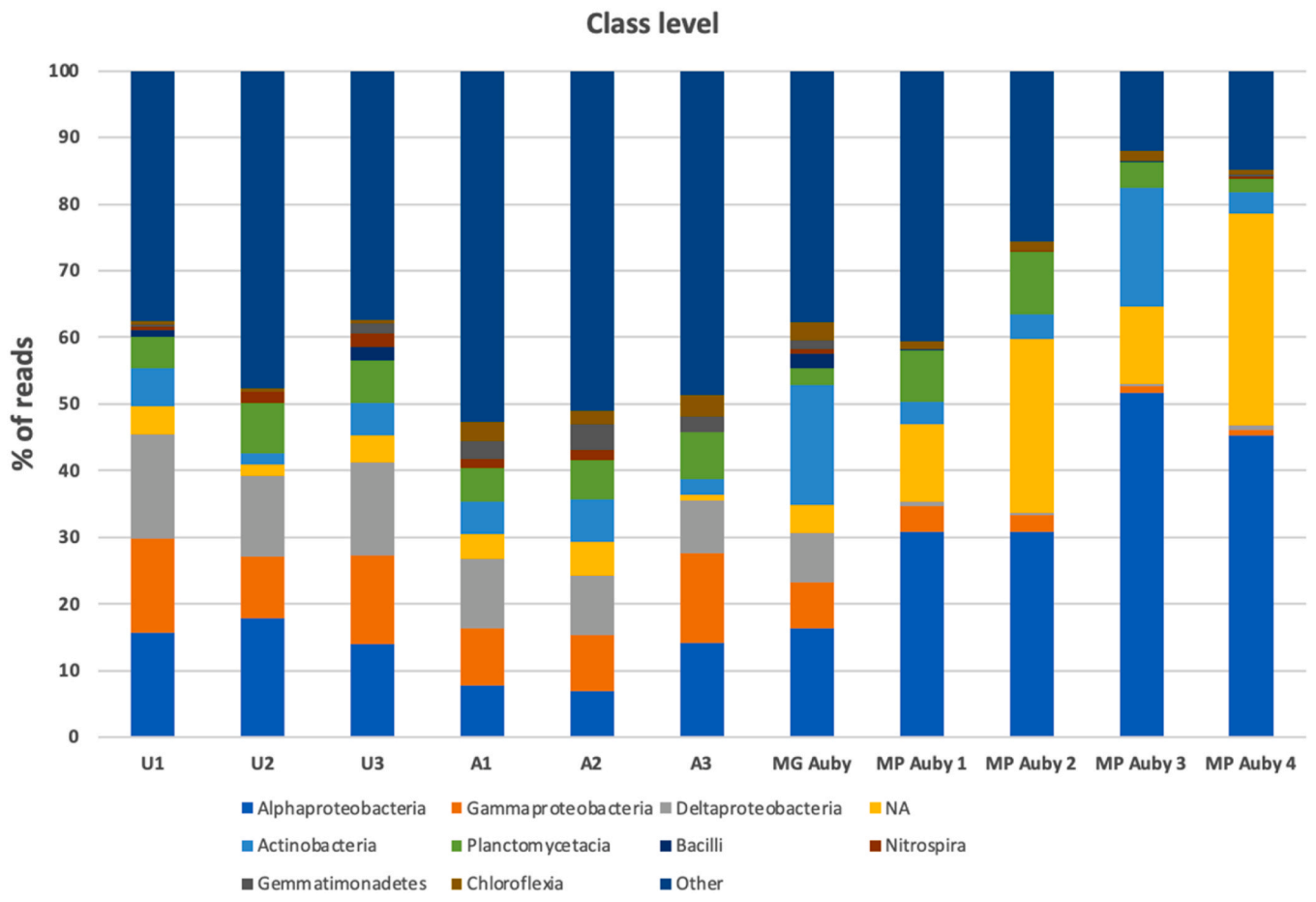

Fig. 2. Results of metatranscriptomics (controls, U1–U3; Auby soil, A1–3), metagenomics (MG Auby) and metaproteomics (MP Auby 1–4) at the class level.

Table 3
Summary of the MT approach. Taxa in bold were significantly enriched in the metal-contaminated Auby soil.

Phylum	Class	Order	Family	Genus
Acidobacteria	**Acidobacteriia**	**Solibacterales**	**Solibacteraceae_(Subgroup_3)**	**Candidatus_Solibacter**
				Bryobacter
	Blastocatellia_(Subgroup_4)	Blastocatellales	Blastocatellaceae	***Aridibacter***
Chloroflexi	**Chloroflexia**			
Gemmatimonadetes	**Gemmatimonadetes**	**Gemmatimonadales**	**Gemmatimonadaceae**	
Bacteroidetes	Bacteroidia	**Chitinophagales**	**Chitinophagaceae**	***Niastella***
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	***Rhodomicrobium***

categories were not represented. Only 10 proteins had a relative abundance above 1% (Fig. 4). The dominant protein identified was a 60 kDa chaperonin protein (7.6–7.8%). We then find an ABC transporter (ca. 2.6%), the elongation factor Tu (1.9%), the beta subunit of the ATP synthase and an outer membrane protein of the SusC/RagA family. The complete list of proteins in each replicate sample is given in Supplementary File SF1 (using DB3).

3.7.1. *Metal resistance*

For the MG-RAST analysis, the "Resistance to antibiotics and toxic compounds" SEED category featured most of the MG reads involved in metal resistance (327,010 reads). In that group we find (Table S8): Cobalt-zinc-cadmium resistance (101,302 reads), Copper homeostasis (35,859 reads), Zinc resistance (14,807 reads), Arsenic resistance (8126 reads), and Resistance to chromium compounds (3711 reads).

According to MG-RAST, the most abundant genes in the Cobalt-zinc-cadmium_resistance category (101,302 reads) were tripartite RND efflux transporters (more than 80,000 reads), with inner membrane RND pumps (such as *czcA* or *cusA*), membrane fusion proteins (such as *czcB* or *cusB*), and outer membrane proteins (such as *czcC* and *cusC*). Cation diffusion facilitators (czcD) were also found but the number of reads was low (6770 reads), and it was the same for ATPase transporters (1002 reads for a Cd-transporting ATPase) (Table S9).

The two most abundant gene categories in the the Copper_homeostasis group (35,859 reads) were Cu-translocating P-type ATPases (17,667 reads) and multicopper oxidases (11,108 reads). We also find other genes, with low number of reads, such as *copG* (1070 reads), *copB* (901 reads), and *copD* (768 reads) (Table S10). Finally, the Zinc_resistance category (14,807 reads) featured the elements of a two-component system for Zn detection (a gene for a sensor protein with 1252 reads, and a gene for a response regulator with 13,555 reads).

For the MG-RAST-independent analysis a more stringent protocol was used (homologies with >80% or >90% identities to the BacMet database were retained, with an Evalue < 1.00e-05). This analysis showed that the most abundant resistance systems for Cd, Zn and Pb (the dominant metals in Auby) were P-type ATPases (Table 4). For instance,

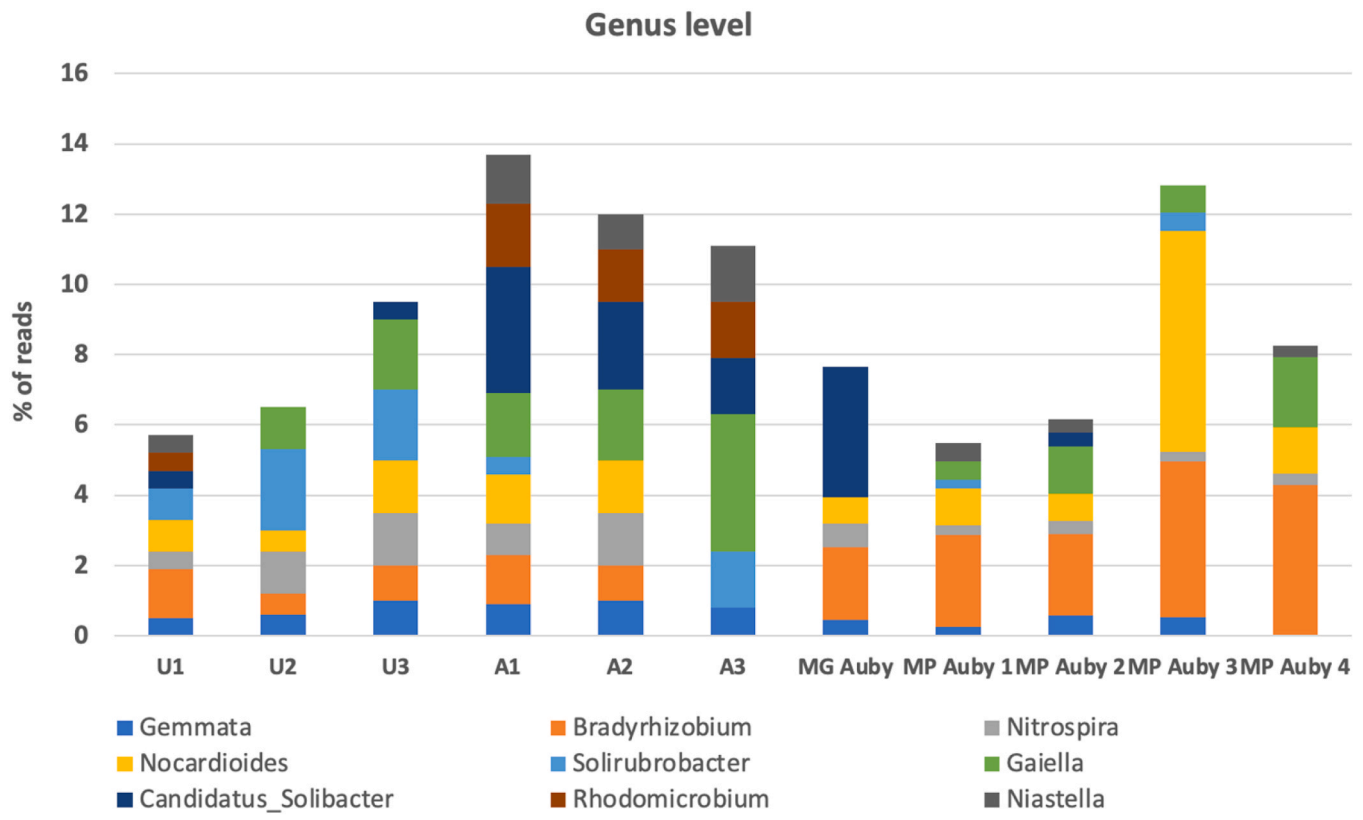

Fig. 3. Results of MT (controls, U1–3; Auby soil, A1–3), MG and MP (MP Auby 1–4) at the genus level. The taxa not found in MP analysis are not shown in the figure for MT and MG.

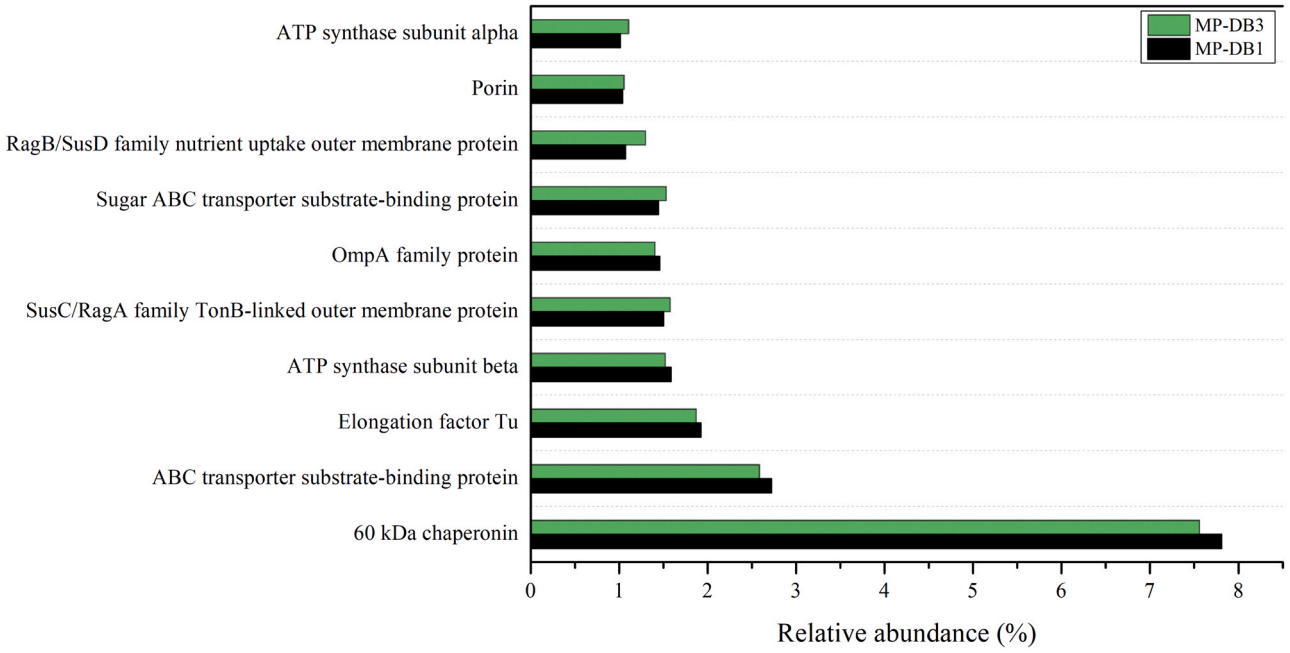

Fig. 4. Results of MP in Auby. Identified proteins (mean relative abundance > 1%) in the four soil samples using databases DB1 and DB3. The relative abundance of proteins was expressed as mean values of four replicates.

over the 875 sequences that had a significant homology with the BacMet database (id>90%), 80 sequences were identified as the P-type ATPase *czcP* (i.e., 9.1%). The situation was similar for Cu and Ag, with P-type ATPases representing the dominant systems.

For proteins involved in metal resistance (MP analysis), when DB1 was used only a tellurite resistance protein (TerB) was found in one of the four replicate samples (with 2 peptides), and a CusA/CzcA family heavy metal efflux RND transporter in another replicate sample (with one peptide). Using DB2, a total of 15 metal-resistance proteins were found, but not in every replicate sample (Table 5). This illustrates the

Table 4
MG-RAST independent analysis with the BacMet database. Nb Id> 90%, number of reads identified in the database by DIAMOND with an identity > 90% and an Evalue of 1.00e-05. Nb Id> 80%, number of reads identified in the database by DIAMOND with an identity > 80% and an Evalue of 1.00e-05.

Metal	Gene	Nb Id> 90%	%	Nb Id> 80%	%
Cd/ Zn	actR (regulator)	31	3.5	82	0.7
	czcA (RND efflux)	8	0.9	195	1.6
	czcP (P-type ATPase)	80	9.1	883	7.2
	czrA (RND efflux)	11	1.3	251	2.1
	nccA (RND efflux)	10	1.1	107	0.9
Pb	pbrA (P-type ATPase)	32	3.7	344	2.8
	pbrT (uptake)	9	1.0	16	0.1
Cu	actP (P-type ATPase)	27	3.1	725	5.9
	copA (P-type ATPase)	25	2.9	708	5.8
	copA (Multi-Cu oxidase)	24	2.7	124	1.0
	copB (Cu sequestration)	30	3.4	143	1.2
	copF (P-type ATPase)	29	3.3	285	2.3
	pcoA (Multi-Cu oxidase)	23	2.6	187	1.5
	cusA (RND efflux)	7	0.8	233	1.9
Ag	silP (P-type ATPase)	10	1.1	283	2.3
	silA (RND efflux)	8	0.9	137	1.1
Co/Ni	cnrA (RND efflux)	12	1.4	232	1.9
Hg	merA (reductase)	47	5.4	272	2.2
Cr	chrA (chromate efflux)	3	0.3	189	1.5
As	arsM (methyltransferase)	17	1.9	361	3.0
	SUM	**320**	36.5	**4501**	36.8
	TOTAL	875	100.0	12216	100.0

Table 5
Proteins associated with metal resistance identified in the four soil samples by MP using protein database 2 (DB2). All of the proteins were identified with one peptide.

Protein name	Taxonomy	Sample
Arsenate reductase	*Bradyrhizobiaceae, Enterococcus*	S2, S3
Arsenical pump-driving ATPase	*Planctomycetes*	S3
Cation diffusion facilitator family transporter	*Sphingobacterium*	S3
Cation efflux system protein CusA	*Alteromonas*	S3
Cobalt-zinc-cadmium resistance protein	*Solirubrobacteraceae*	S3
Cobalt-zinc-cadmium resistance protein CzcA	*Chitinophagia*	S2
Copper chaperone	*Candidimonas*	S2
Copper chaperone CopZ	*Ekhidna*	S3
Copper resistance protein CopB	*Rhodanobacter*	S3
Copper-exporting P-type ATPase	*Ruminococcus*	S1
Mercuric reductase	*Nostocaceae, Filimonas*	S1, S3
MerR family transcriptional regulator	*Streptomyces, Pseudonocardiaceae, Nocardiopsis, Micromonospora, Paenibacillus, Amycolatopsis, Lachnospiraceae, Clostridium, Azospirillum, Knoellia*	S1, S2, S3, S4
Multicopper oxidase domain-containing protein	*Methylococcaceae*	S1
Periplasmic heavy metal sensor	*Deltaproteobacteria*	S3
Probable Co/Zn/Cd efflux system membrane fusion protein	*Agarivorans*	S3

importance of the database in metaproteomics. Most of these proteins were detected in sample 3 (S3), with one peptide and a wide taxonomic distribution. As the abundance of these peptides was very low, it was not possible to judge on the most abundant metal resistance system at the protein level (i.e., RND-based, multicopper-oxidase-based or P-type ATPase-based).

When the combined database DB3 was used, only 6 proteins involved in metal resistance were identified, but not in all the replicates and with only one peptide (Table 6). Among these proteins we find an RND transporter of the CusA/CzcA family, a metal translocating P-type ATPase, two transcriptional regulators of the MerR and ArsR family, and two tellurite resistance proteins.

3.7.2. Degradation of carbohydrates

An important soil function for an efficient carbon cycle is the degradation of major polysaccharides such as cellulose, hemicellulose, chitin, lignin and starch. According to MG-RAST, the most abundant MG reads in the carbohydrate category (Table S11) were involved in the central carbohydrate metabolism (376,756 reads), with genes of the glycolysis, Entner-Doudoroff pathway, or the Krebs cycle. Although these genes are not directly involved in the degradation of polysaccharides, a total of 15,973 reads were classified in the chitin and N-acetylglucosamine utilization category (Table S12). For cellulose, 7405 reads were β-glucosidases, meaning that this polymer might also be degraded in the soil. In the monosaccharides category, the most abundant reads were L-rhamnose (32,035 reads), D-galacturonate/D-glucuronate (26,336 reads), and xylose utilization genes (21,336 reads), meaning that hemicellulose might be degraded. A total of 26,724 reads belonged to the glycogen metabolism category, with glycogen-branching and debranching enzymes, particularly of archaeal type (TIGR01561; 3945 reads). In the cellulosome category, most of the reads were identified as SusC, an outer membrane protein involved in starch binding (2790 reads) and SusD, an outer membrane protein involved in starch degradation was also detected (685 reads).

At the protein level (shotgun MP) the proteins involved in starch binding and degradation were confirmed: SusC (10–29 peptides; 1.1–2.3%) and Sus D (17–24 peptides; 1.3–1.9%) were found in 3 of the 4 replicates with DB1. Sugar ABC transporters were also found (14–22 peptides, 1.8%) in all replicates with DB1. Finally, one chitinase was found in one replicate sample but with one peptide (DB1).

4. Discussion

4.1. Metals, diversity and structure of the community

Total levels of Zn, Cd and Pb of the soil in the P é ru Parc were in the range of what was observed for other calaminiferous soils. Metal levels were ca. 4 times less elevated than those observed in a wood located 250 m in the south (Bert et al., 2000). Metal levels were also less elevated than in the previously reported calaminiferous soils (Orłowska et al., 2002; Epelde et al., 2010; Olenska et al., 2020). However, metal concentrations of the P é ru Parc were much more elevated than those observed in a calaminiferous soil in Poland (6–16x for Zn, 18x for Cd, 1–8x for Pb; Gołębiewski et al., 2014).

Our results suggest that high metal levels have an impact on the

Table 6
Proteins involved in metal resistance identified by metaproteomics using the combined protein database (DB3) (S: sample number). All proteins were found with only one peptide.

Protein name	**Taxonomy**	**Sample source**
MerR family transcriptional regulator	*Nocardia*	S1
Tellurite resistance protein	*Flavisolibacter*	S1
Tellurite resistance protein TerB	*Flavisolibacter*	S1
ArsR family transcriptional regulator	*Betaproteobacteria*	S2
CusA/CzcA family heavy metal efflux RND transporter	*Micrococcaceae*	S2, S3
Heavy metal translocating P-type ATPase	*Proteobacteria*	S3

diversity of the active members of the bacterial community (i.e., species visualized with the MT approach) as shown by the diversity indexes that were all significantly reduced in Auby (e.g., by a factor 3 for Chao1, and a factor 2 for Simpson), when compared to a control soil. This is not surprising as other studies have shown that metals may significantly impact bacterial diversity, even after long periods of metal exposure (Gans et al., 2005; Sheik et al., 2012; Singh et al., 2014; Abdu et al., 2017; Gillan and Van Houdt, 2019). It must be noted here that most of these previous studies focused on total DNA extracts and were not focused on active bacterial populations.

At the taxonomical point of view, and at the genus level, it must first be emphasized that most of the active bacterial genera could not be identified (Ca. 57% of the MT reads in Auby, and 36–77% of the MP reads). Such a situation may be explained by the well-known biases and limitations of the MP and MT approaches, particularly during identification of the reads by comparison to gene databases (e.g., Werner et al., 2019, Salvato et al., 2021, Dubey et al., 2020, Shakya et al., 2019). Although the "black box" of active bacteria in the calaminiferous soil is still important, we will below combine results from MT and MP as both methods have the potential to detect active members in a microbial community (we will focus on bacterial genera with an abundance >1% and that were significantly enriched in Auby).

Among the active members of the Auby soil we first find Acidobacteria and the genus *Candidatus_Solibacter* (subgroup 3 of the Barns classification), with levels increased 7.7 times in comparison to the controls (2.6% in Auby, MT analysis). Acidobacteria are mainly aerobic heterotrophs that have been very difficult to isolate and culture in the laboratory (Ward et al., 2009). For *Candidatus_Solibacter*, genomic and culture traits have indicated the use of carbon sources that span simple sugars to more complex substrates such as hemicellulose, cellulose, and chitin (Ward et al., 2009). This bacterium is thus well equipped to degrade organic matter in soils and might represent and important community member for the carbon cycle of calaminiferous soils (Ward et al., 2009; Challacombe et al., 2011). *Candidatus_Solibacter* was also found in a calaminiferous soil in Poland (Gołębiewski et al., 2014). Acidobacteria are probably well equipped to deal with high metal levels as they have been detected in many metal-contaminated environments, such as uranium-contaminated subsurface sediments (Barns et al., 2007), stream sediments (Vishnivetskaya et al., 2011), farmlands (Guo et al., 2017; Hu et al., 2021), mines (Liu et al., 2022) and soils of Zn/Pb smelters (Li et al., 2020). *Bryobacter* (subgroup 3; 3.5% in Auby) and *Aridibacter* (subgroup 4; 0.8% in Auby) are two other acidobacterial genera that were enriched in Auby. These bacteria are also aerobic heterotrophs and able to use several sugars, a few amino acids, organic acids and different complex protein substrates (Huber et al., 2017).

For Alphaproteobacteria, MT analysis identified the genus *Rhodomicrobium*, a Gram-negative bacterium widely distributed in various ecosystems, that form buds and long prosthecae of several micrometers (Duchow and Douglas, 1949). For that reason, it was placed in the Hyphomicrobiaceae. Various cell morphologies may be produced during the cell cycle: multicellular arrays (cells linked by filaments), unattached motile cells (swarmers) and nonmotile angular cells (exospores) (Whittenbury and Dow, 1977). These bacteria are facultative phototrophs able to grow in anaerobic environments on a wide range of aromatic hydrocarbons (Wright and Madigan, 1991; Ramana et al., 2013). This is probably important for soils because a diverse array of aromatic compounds is present, primarily as substances released from dead plant material. However, the ability of the genus to grow in metal-contaminated environments has never been reported and the genus was not found in the previously investigated calaminiferous soils (Epelde et al., 2010; Gołębiewski et al., 2014; Beattie et al., 2018; Olenska et al., 2020).

MP analysis also indicated that three genera of Alphaproteobacteria might represent important community members in Auby: *Mesorhizobium* (8.0 ± 4.0%), *Bradyrhizobium* (3.7 ± 1.5%), and *Azospirillum* (1.4 ± 0.4%). These organisms are generally described as plant growth promoting bacteria (PGPB) that promote nitrogen fixation and therefore may improve nitrogen availability to plants (Bai et al., 2003; Wani et al., 2007; Bashan and de-Bashan, 2010). The finding of such organisms is not surprising as the Auby soil is covered by metallophytes. Some *Mesorhizobium* species are even known to resist high levels of Cu, Zn, Cr and Cd in soils and help plants to grow with metals (Wani et al., 2007; Vidal et al., 2009; Hao et al., 2015). High abundances of *Bradyrhizobium* were observed in mercury mines and calamine tailings featuring Zn, Pb, Cd and Hg (Bianucci et al., 2011; Sujkowska-Rybkowska et al., 2020; Salmi and Boulila, 2021). Additionally, the intracellular thiol glutathione pool (GSH) in *Bradyrhizobium* may acts as a detoxifying agent and was attributed to its Cd tolerance (Bianucci et al., 2011). We must note here that the glutathione pool is also involved in tellurium resistance (see below). Finally, high abundance of the alphaproteobacterium *Azospirillum* in soils contaminated with metals was also reported in previous studies (e.g., Vezza et al., 2020).

Among the Betaproteobacteria, MP analysis suggested that the genus *Variovorax* (2.4 ± 0.6%) may represent an important community member. Organisms in this genus are aerobic and chemoorganotrophic. *Variovorax* species have diverse metabolic capabilities, including the biodegradation of both biogenic compounds and anthropogenic contaminants. Interestingly, *V. paradoxus* is known to engages in mutually beneficial interactions with both bacteria and plants and has been classified in the plant growth-promoting rhizobacteria (Han et al., 2011). Variovorax species are known to be resistant to various metals, including Cd and Hg, and are known to oxidize arsenite to arsenate and to use phenol as a single carbon and energy source (Hupert-Kocurek et al., 2013).

In the phylum Bacteroidetes, MT analysis suggested that the genus *Niastella* is an active member in the Auby soil. This genus belongs to the family Chitinophagaceae (Zhang et al., 2010) and features heterotrophic, Gram-negative, strictly aerobic, and form long filamentous rods (up to 15 μm) that may be found in soils (Kim et al., 2015). The ability of the genus to grow in metal-contaminated environments has never been reported and the genus was not found in the previously investigated calaminiferous soils.

Finally, we have the phylum Actinobacteria mostly represented in Auby by the genus *Nocardioides* found by MP analysis (1.0–6.2% of the MP reads). It is a Gram-positive aerobic soil bacterium that develops a mycelium that fragments into irregular rod- to coccus-like elements (Yoon and Park, 2006). The genus has been found in many metal-contaminated soils (e.g., Liu et al., 2021, Siebielec et al., 2020, Fang et al., 2019). These bacteria are aerobic heterotrophs able to use many simple sugars but also able to degrade complex organic materials like chitin. *Nocardioides* is known to be able to reduce As(V) in As(III) using arsenate reductase. Members of the genus can tolerance high levels of As(III), up to 5 mM, and high levels of As(V) up to 100 mM (Bagade et al., 2016). Notably, *Nocardioides* was found associated to the rhizosphere of *Dahlia pinnata* and *Coreopsis grandiflora*, two Cd hyperaccumulators, together with other bacteria such as *Gemmatimonas, Bryobacter* and *Bradyrhizobium*. All these bacteria probably facilitate Cd tolerance and accumulation in the plant (Li et al., 2022a, 2022b). It is therefore interesting to find it in Auby where it might be associated to the rhizosphere of metallophytes. In the Actinobacteria, the genus *Gaiella* was retrieved by both MT and MP analysis. This genus belongs to the Gaiellales, a deep-branching actinobacterial lineage described in 2011 (Albuquerque et al., 2011) with two isolates recovered from a deep mineral water aquifer in Portugal. These bacteria are aerobic and assimilate carbohydrates, organic and amino acids. They play important roles in nitrogen cycling by reducing nitrate to nitrite and are proposed as potential sulfadiazine degraders in agricultural soils (Albuquerque et al., 2011; Chen et al., 2019). This genus was not previously found in calaminiferous soils.

4.2. Functional profiling

Although MP identified few proteins involved in the carbon cycle, the MG-RAST approach detected many genes that might be used to degrade various types of organic materials, among which chitin, N-acetylglucosamine, cellulose and glycogen. This suggests that the important carbon cycle functions of the heterotrophic community are maintained in the contaminated soil. As for uncontaminated soils, plant and animal debris are probably decomposed efficiently, although the activity of the various enzymes must still be determined. The idea of a relatively unimpaired heterotrophic activity is also supported by the abundance of genes of the central carbohydrate metabolism (i.e. glycolysis and Krebs cycle). In aerobic conditions the Krebs cycle enzymes are expected to be dominant, contrary to the glycolysis genes that would dominate under anaerobic conditions (Dijkstra et al., 2011). Further studies are therefore needed in Auby to determine oxygen levels but also the rate of organic matter mineralization that might be affected by metals as shown in other studies (Berg et al., 1991; Gillan and Van Houdt, 2019). Finally, this heterotrophic activity is made possible using several metal-resistance systems. On this point, our MG approach, particularly the MG-RAST independent analysis, concluded that P-type ATPases represented the dominant metal-resistance systems, at least for the major metals in Auby (Cd, Zn and Pb). The activity of such efflux pumps probably consumes a lot of ATP, and this might explain the abundance of genes involved in the central carbohydrate metabolism.

Few metal-resistance proteins were found by MP. The proteins identified by our MP approach, using DB1 and the combined database (DB3), are mainly housekeeping proteins used for the maintenance of homeostasis and that can be found in nearly all bacteria (Fajardo et al., 2019). These proteins were also found in seawater by another MP analysis (Géron et al., 2019). A 60 kDa chaperonin was the most abundant protein in all our soil samples. The detection of such a chaperonin may suggest that protein refolding processes are important in the investigated soil, where some proteins may be damaged by the exposure to excessive levels of metals (Sowell et al., 2009). The next dominant proteins were substrate-binding transporters which belong to the ABC superfamily. ABC transporters play a key role in the import of essential nutrients such as sugars and amino acids but also the import of toxic molecules. Some bacterial ABC transporters are also involved in the regulation of several physiological processes (Sowell et al., 2009; Fajardo et al., 2019). Microbial ABC transporters have been reported to improve the tolerance of plants to metals through uptake, chelation, translocation and sequestration of metals (Singh et al., 2016).

Although metal-resistance proteins were not detected in every replicate sample, RND transporters of the CusA/CzcA family as well as efflux proteins of the P-type ATPase group were found. Efflux is thus the major mechanism of metal resistance in the soil community of the calaminiferous soil in Auby, as found in other studies (Gillan, 2016; Jia et al., 2013; Roosa et al., 2014; Gillan et al., 2015; Kaci et al., 2014; Chen et al., 2015). According to the soil type and levels of metals, a predominance of RND efflux proteins is found is some studies while a predominance of P-type ATPases is found in other studies (Gillan, 2016).

Surprisingly we also detected tellurite resistance proteins as abundant proteins in the Auby soil, suggesting a contamination by tellurium. This may be explained by the presence of the industrial zone and the fact that tellurium is a known additive of lead alloys (Guo et al., 2009). The tellurium oxyanion, tellurite (TeO_3^{2-}), is extremely toxic to most living organisms. Its toxicity has been mainly related to the generation of reactive oxygen species (ROS) as well as to an unbalancing of the thiol: redox buffering system (Arenas et al., 2014). The reduced cellular thiol pool, especially that of glutathione (GSH), is an important tellurite target. This pool is oxidized in the presence of the toxicant (Turner et al., 1999, 2001) and this process is accompanied by the generation of superoxide that may damage the cell. As we did not detect any proteins involved in the fight against ROS we can assume that the tellurite resistance proteins are efficient. Previous studies have shown that some bacteria were able to live at high tellurite concentrations. One mechanism of resistance is the enzymatic and non-enzymatic reduction of tellurite to the less toxic elemental tellurium. This reduction may produce nano- to micrometric tellurium crystals that display different shapes and sizes (Arenas et al., 2014). Tellurite resistance genes were frequently found to be grouped in a large cluster of genes (*terZABCDEF*) referred to as the *ter* determinant (Walter and Taylor, 1992; Whelan et al., 1995). Although the *ter* operon is widely distributed in gram-negative bacteria (Nguyen et al., 2021) *ter* genes were not found with our MG approach.

5. Conclusions

On the taxonomic point of view, the present study is another example of the fact that studying complex soil communities by only one 'omics approach such as shotgun MG is highly misleading. For instance, *Mesorhizobium* was not found to be an important community member in Auby by shotgun MG although the organism was the first in the list of identified organisms by shotgun MP. A second important point is that half of our peptides (ca. 50%) could not be identified, probably because many microorganisms are still not isolated or studied in pure culture and are therefore absent from the genomic databases. We may conclude from these two points that most of the microbial communities in soils are still far from being known in terms of taxonomic composition, particularly for metal-contaminated soils.

On the functional point of view, we may conclude that the microbial communities of the calaminiferous soil investigated are well adapted to their metal-enriched environment. Many of them are slow growers like Acidobacteria and may therefore participate in the carbon cycle despite the large Cd and Zn levels. Using their membrane efflux systems, mainly HME-RND and P-type ATPase pumps, these microorganisms are probably able to maintain important ecological functions. This is demonstrated by the abundance of peptides from the Alphaproteobacteria *Mesorhizobium* and *Bradyrhizobium*, two genera that are free-living but that can also establish a N_2-fixing symbiosis on some plant roots.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: David C Gillan reports financial support was provided by FRS-FNRS.

Data Availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.microb.2023.100002.

References

Abdu, N., Abdullahi, A.A., Abdulkadir, A., 2017. Heavy metals and soil microbes. Environ. Chem. Lett. 15, 65–84.

Albuquerque, L., Franc, L., Rainey, F.A., Schumann, P., Fernanda Nobre, M., da Costad, M.S., 2011. *Gaiella* occulta gen. nov., sp. nov., a novel representative of a deep branching phylogenetic lineage within the class Actinobacteria and proposal of Gaiellaceae fam. nov. and Gaiellales ord. nov. Syst. Appl. Microbiol 34, 595–599.

Arenas, F.A., Pugin, B., Henriquez, N.A., Arenas-Salinas, M.A., Diaz-Vasquez, W.A., Pozo, M.F., et al., 2014. Isolation, identification and characterization of highly tellurite-resistant, tellurite-reducing bacteria from Antarctica. Polar Sci. 8, 40–52. https://doi.org/10.1016/j.polar.2014.01.001.

Bagade, A.V., et al., 2016. Characterization of roseomonas and nocardioides spp. for arsenic transformation. J. Hazard Mater. 318, 742–750. https://doi.org/10.1016/j.jhazmat.2016.07.062.

Bai, Y., Zhou, X., Smith, D.L., 2003. Enhanced soybean plant growth resulting from coinoculation of bacillus strains with bradyrhizobium japonicum. Crop Sci. 43, 1774–1781. https://doi.org/10.2135/cropsci2003.1774.

Baker, A.J.M., Ernst, W.H.O., Van Der Ent, A., Malaisse, F., Ginocchio, R., 2010. Metallophytes: the unique biological resource, its ecology and conservational status in Europe, central Africa and Latin America. In: Batty, L.C., Hallberg, K.B. (Eds.), Ecology of Industrial Pollution. Cambridge University Press, Cambridge, pp. 7–40. https://doi.org/10.1017/CBO9780511805561.003.

Barns, S.M., Cain, E.C., Sommerville, L., Kuske, C.R., 2007. Acidobacteria Phylum Sequences in Uranium-Contaminated Subsurface Sediments Greatly Expand the Known Diversity within the Phylum. Appl. Environ. Microbiol 73, 3113–3116.

Bashan, Y., de-Bashan, L.E., 2010. How the Plant Growth-Promoting Bacterium Azospirillum Promotes Plant Growth—A Critical Assessment. In: Advances in Agronomy, Vol 108. Elsevier,, pp. 77–136. https://doi.org/10.1016/S0065-2113(10)08002-8.

Beattie, R.E., Henke, W., Campa, M.F., Hazen, T.C., McAliley, L.R., Campbell, J.H., 2018. Variation in microbial community structure correlates with heavy-metal contamination in soils decades after mining ceased. Soil Biol. Biochem 126, 57–63. https://doi.org/10.1016/j.soilbio.2018.08.011.

Berg, B., Ekbohm, G., Soderstrom, B., Staaf, H., 1991. Reduction of decomposition rates of scots pine needle litter due to heavy-metal pollution. Water Air Soil Pollut. 59, 165–177.

Bert, V., Macnair, M.R., De Laguerie, P., Saumitou-Laprade, P., Petit, D., 2000. Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* (Brassicaceae). N. Phytol. 146, 225–233.

Bertin, P.N., Heinrich-Salmeron, A., Pelletier, E., Goulhen-Chollet, F., Arsène-Ploetze, F., Gallien, S., et al., 2011. Metabolic diversity among main microorganisms inside an arsenic-rich ecosystem revealed by meta- and proteo-genomics. ISME J. 5, 1735–1747. https://doi.org/10.1038/ismej.2011.51.

Bianucci, E., Fabra, A., Castro, S., 2011. Cadmium Accumulation and Tolerance in Bradyrhizobium spp. (Peanut Microsymbionts). Curr. Microbiol 62, 96–100. https://doi.org/10.1007/s00284-010-9675-5.

Buchfink, B., Reuter, K., Drost, H.G., 2021. Sensitive protein alignments at tree-of-life scale using DIAMOND. Nat. Methods 18, 366–368.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Challacombe, J.F., Eichorst, S.A., Hauser, L., Land, M., Xie, G., Kuske, C.R., 2011. Biological consequences of ancient gene acquisition and duplication in the large genome of *Candidatus Solibacter* usitatus Ellin6076. PLoS ONE 6 (9), e24882. https://doi.org/10.1371/journal.pone.0024882.

Chen, J., Jiang, X., Tong, T., Miao, S., Huang, J., Xie, S., 2019. Sulfadiazine degradation in soils: dynamics, functional gene, antibiotic resistance genes and microbial community. Sci. Tot Environ. 691, 1072–1081. https://doi.org/10.1016/j.scitotenv.2019.07.230.

Chen, M., Li, Y., Zhang, L., Wang, J., Zheng, C., Zhang, X., 2015. Analysis of gene expression provides insights into the mechanism of cadmium tolerance in acidithiobacillus ferrooxidans. Curr. Microbiol 70, 290–297. https://doi.org/10.1007/s00284-014-0710-9.

Dubey, R.K., Tripathi, V., Prabha, R., Chaurasia, R., Pratap Singh, D., Rao, C.S., et al., 2020. Metatranscriptomics and metaproteomics for microbial Communities Profiling. Springer Briefs in Environmental Science, Unravelling the Soil Microbiome. Springer,, pp. 51–68.

Duchow, E., Douglas, H.C., 1949. *Rhodomicrobium vannielii*, a new photoheterotrophic bacterium. J. Bacteriol. 58, 409–416.

Edgar R.C. (2016) UCHIME2: improved chimera prediction for amplicon sequencing. bioRxiv. doi: https://doi.org/10.1101/074252.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27 (16), 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Epelde, L., Becerril, J.M., Barrutia, O., Gonzalez-Oreja, J.A., Garbisu, 5, 2010. Interactions between plant and rhizosphere microbial communities in a metalliferous soil. Environ. Pollut. 158, 1576–1583.

Fajardo, C., Costa, G., Nande, M., Botias, P., Garcia-Cantalejo, J., Martin, M., 2019. Pb, Cd, and Zn soil contamination: Monitoring functional and structural impacts on the microbiome. Appl. Soil Ecol. 135, 56–64. https://doi.org/10.1016/j.apsoil.2018.10.022.

Fang, L., Ju, W., Yang, C., Duan, C., Cui, Y., Han, F., Shen, G., Zhang, C., 2019. Application of signaling molecules in reducing metal accumulation in alfalfa and alleviating metal-induced phytotoxicity in Pb/Cd-contaminated soil. Ecotox Environ. Saf. 182, 109459.

Fortin, N., Beaumier, D., Lee, K., Greer, C.W., 2004. Soil washing improves the recovery of total community DNA from polluted and high organic content sediments. J. Microbiol. Methods 56 (2), 181–191. https://doi.org/10.1016/j.mimet.2003.10.006.

Gans, J., Wolinsky, M., Dunbar, J., 2005. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309, 1387–1390.

Géron, A., Werner, J., Wattiez, R., Lebaron, P., Matallana-Surget, S., 2019. Deciphering the Functioning of Microbial Communities: Shedding Light on the Critical Steps in Metaproteomics. Front. Microbiol. 10, 2395. https://doi.org/10.3389/fmicb.2019.02395.

Gillan, D., Van Houdt, R., 2019. The impact of metal contamination on soil microbial community dynamics. In: van Elsas, J.D., Trevors, J.T., Soares Rosado, A., Nannipieri, P. (Eds.), Modern Soil Microbiology, Third edition.,. CRC Press, Boca Raton, pp. 403–419.

Gillan, D.C., 2016. Metal resistance systems in cultivated bacteria: are they found in complex communities? Curr. Opin. Biotechnol. 38, 123–130. https://doi.org/10.1016/j.copbio.2016.01.012.

Gillan, D.C., Roosa, S., Kunath, B., Billon, G., Wattiez, R., 2015. The long-term adaptation of bacterial communities in metal-contaminated sediments: a metaproteogenomic study: bacteria in metal-contaminated sediments. Environ. Microbiol 17, 1991–2005. https://doi.org/10.1111/1462-2920.12627.

Gołębiewski, M., Deja-Sikora, E., Cichosz, M., Tretyn, A., Wrobel, B., 2014. 16S rDNA pyrosequencing analysis of bacterial community in heavy metals polluted soils. Microb. Ecol. 67, 635–647. https://doi.org/10.1007/s00248-013-0344-7.

Guo, H., Nasira, M., Lva, J., Daia, Y., Gao, J., 2017. Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing. Ecotox Environ. Saf. 144, 300–306.

Guo, W.X., Shu, D., Chen, H.Y., Li, A.J., Wang, H., Xiao, G.M., et al., 2009. Study on the structure and property of lead tellurium alloy as the positive grid of lead-acid batteries. J. Alloy. Compd. Volume 475 (Issues 1–2), 102–109.

Han, J.I., Choi, H.K., Lee, S.W., Orwin, P.M., Kim, J., LaRoe, S.L., et al., 2011. Complete Genome Sequence of the Metabolically Versatile Plant Growth-Promoting Endophyte Variovorax paradoxus S110. J. Bacteriol. 193, 1183–1190. https://doi.org/10.1128/JB.00925-10.

Hao, X., Xie, P., Zhu, Y.G., Taghavi, S., Wei, G., Rensing, C., 2015. Copper tolerance mechanisms of mesorhizobium amorphae and its role in aiding phytostabilization by robinia pseudoacacia in copper contaminated soil. Environ. Sci. Technol. 49, 2328–2340. https://doi.org/10.1021/es504956a.

Hu, X., Wang, J., Lv, Y., Liu, X., Zhong, J., Cui, X., et al., 2021. Effects of heavy metals/metalloids and soil properties on microbial communities in farmland in the vicinity of a metals smelter. Front. Microbiol. 12, 707786 https://doi.org/10.3389/fmicb.2021.707786.

Huber, K.J., Geppert, A.M., Groß, U., Luckner, M., Wanner, G., Cooper, P., et al., 2017. Aridibacter nitratireducens sp. nov., a member of the family Blastocatellaceae, class Blastocatellia, isolated from an African soil. Int J. Syst. Evol. Microbiol 67, 4487–4493. https://doi.org/10.1099/ijsem.0.002318.

Hupert-Kocurek, K., Saczyńska, A., Piotrowska-Seget, Z., 2013. Cadmium increases catechol 2,3-dioxygenase activity in Variovorax sp. 12S, a metal-tolerant and phenol-degrading strain. Antonie Van. Leeuwenhoek 104, 845–853. https://doi.org/10.1007/s10482-013-9997-y.

Jansson, J.K., Hofmockel, K.S., 2018. The soil microbiome — from metagenomics to metaphenomics. Curr. Opin. Microbiol 43, 162–168. https://doi.org/10.1016/j.mib.2018.01.013.

Jia, S., Wang, Z., Zhang, X.X., Liu, B., Li, W., Cheng, S., 2013. Metagenomic analysis of cadmium and copper resistance genes in activated sludge of a tannery wastewater treatment plant. J. Environ. Biol. 34, 375–380.

Kaci, A., Petit, F., Lesueur, P., Boust, D., Vrel, A., Berthe, T., 2014. Distinct diversity of the czcA gene in two sedimentary horizons from a contaminated estuarine core. Environ. Sci. Pollut. Res 21, 10787–10802. https://doi.org/10.1007/s11356-014-3029-y.

Kim, B.R., Shin, J., Guevarra, R.B., Hyung Lee, J., Kim, D.W., Seol, K.H., Lee, J.H., Kim, H.B., Isaacson, R.E., 2017. Deciphering diversity indices for a better understanding of microbial communities. J. Microbiol Biotechnol. 27, 2089–2093.

Kim, S.J., Ahn, J.H., Weon, H.Y., Hong, S.B., Seok, S.J., Kim, J.S., Kwon, S.W., 2015. Niastella gongjuensis sp. nov., isolated from greenhouse soil. Int J. Syst. Evol. Microbiol 65, 3115–3118.

Lemoine, G., 2012. De l'importance des pelouses calaminaires d'Auby et notamment du Parc Peru. Bull. Soc. Bot. N. Fr. 65, 51–58.

Li, S., Zhao, B., Jin, M., Hu, L., Zhong, H., He, Z., 2020. A comprehensive survey on the horizontal and vertical distribution of heavy metals and microorganisms in soils of a Pb/Zn smelter. J. Hazard Mater. 400, 123255 https://doi.org/10.1016/j.jhazmat.2020.123255.

Li, X., Li, B., Jin, T., Chen, H., Zhao, G., Qin, X., Yang, Y., Xu, J., 2022a. Rhizospheric microbiomics integrated with plant transcriptomics provides insight into the Cd response mechanisms of the newly identified Cd accumulator Dahlia pinnata. Front. Plant Sci. 13, 1091056. https://doi.org/10.3389/fpls.2022.1091056.

Li, X., Li, B., Zheng, Y., Luo, L., Qin, X., Yang, Y., Xu, J., 2022b. Physiological and rhizospheric response characteristics to cadmium of a newly identified cadmium accumulator Coreopsis grandiflora Hogg. (Asteraceae). Ecotox Environ. Saf. 241, 113739.

Liu, J.L., Yao, J., Zhu, X., Zhou, D.L., Duran, R., Mihucz, V.G., Bashir, S., Hudson-Edwards, K.A., 2021. Metagenomic exploration of multi-resistance genes linked to microbial attributes in active nonferrous metal(loid) tailings. Environ. Pollut. 273, 115667.

Liu, Y., Gao, T., Wang, X., Fu, J., Zuo, M., Yang, Y., et al., 2022. Effects of heavy metals on bacterial community surrounding Bijiashan mining area located in northwest China. Open Life Sci. 17, 40–54.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., et al., 2008. The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinforma. 9, 386. https://doi.org/10.1186/1471-2105-9-386.

Nguyen, T.T.H., Kikuchi, T., Tokunaga, T., Iyoda, S., Iguchi, A., 2021. Diversity of the tellurite resistance gene operon in Escherichia coli. Front. Microbiol. 12, 681175 https://doi.org/10.3389/fmicb.2021.681175.

Olenska, E., Imperato, V., Małek, W., Włostowski, T., Wójcik, M., Swiecicka, I., Vangronsveld, J., Thijs, S., 2020. Trifolium repens-Associated Bacteria as a Potential Tool to Facilitate Phytostabilization of Zinc and Lead Polluted Waste Heaps. Plants 9, 1002. https://doi.org/10.3390/plants9081002.

Orłowska, E., Zubek, Sz, Jurkiewicz, A., Szarek-Łukaszewska, G., Turnau, K., 2002. Influence of restoration on arbuscular mycorrhiza of Biscutella laevigata L. (Brassicaceae) and Plantago lanceolata L. (Plantaginaceae) from calamine spoil mounds. Mycorrhiza 12, 153–160.

Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E., Larsson, D.G.J., 2014. BacMet: antibacterial biocide and metal resistance genes database. Nucleic Acids Res. 42, D737–D743.

Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18, 1403–1414. https://doi.org/10.1111/1462-2920.13023.

Ramana, V.V., Raj, P.S., Tushar, L., Sasikala, C., Ramana, C.V., 2013. *Rhodomicrobium udaipurense* sp. nov., a psychrotolerant, phototrophic alphaproteobacterium isolated from a freshwater stream. Int J. Syst. Evol. Micro 63, 2684–2689.

Roosa, S., Wattiez, R., Prygiel, E., Lesven, L., Billon, G., Gillan, D.C., 2014. Bacterial metal resistance genes and metal bioavailability in contaminated sediments. Environ. Pollut. 189, 143–151. https://doi.org/10.1016/j.envpol.2014.02.031.

Salmi, A., Boulila, F., 2021. Heavy metals multi-tolerant Bradyrhizobium isolated from mercury mining region in Algeria. J. Environ. Manag. 289, 112547 https://doi.org/10.1016/j.jenvman.2021.112547.

Salvato, F., Hettich, R.L., Kleiner, M., 2021. Five key aspects of metaproteomics as a tool to understand functional interactions in host- associated microbiomes. PLoS Pathog. 17 (2), e1009245.

Shakya, M., Lo, C.C., Chain, P.S.G., 2019. Advances and challenges in metatranscriptomic analysis. Front. Genet. 10, 904. https://doi.org/10.3389/fgene.2019.00904.

Sheik, C.S., Mitchel, T.W., Rizvi, F.Z., Rehman, Y., Faisal, M., Hasnain, S., et al., 2012. Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. PLoS One 7, e40059.

Siebielec, S., Siebielec, G., Sugier, P., Wozniak, M., Grzadziel, J., Galazka, A., Stuczynski, T., 2020. Activity and diversity of microorganisms in root zone of plant species spontaneously inhabiting smelter waste piles. Molecules 25, 5638. https://doi.org/10.3390/molecules25235638.

Singh, B.K., Quince, C., Macdonald, C.A., Khachane, A., Thomas, N., Al-Soud, W.A., et al., 2014. Loss of microbial diversity in soils is coincident with reductions in some specialized functions. Environ. Microbiol 16, 2408–2420.

Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M., 2016. Heavy Metal Tolerance in Plants: Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics. Front. Plant Sci. 6. https://doi.org/10.3389/fpls.2015.01143.

Sowell, S.M., Wilhelm, L.J., Norbeck, A.D., Lipton, M.S., Nicora, C.D., Barofsky, D.F., et al., 2009. Transport functions dominate the SAR11 metaproteome at low-nutrient extremes in the Sargasso Sea. ISME J. 3, 93–105. https://doi.org/10.1038/ismej.2008.83.

Sujkowska-Rybkowska, M., Banasiewicz, J., Rekosz-Burlaga, H., Stepkowski, T., 2020. Anthyllis vulneraria and Lotus corniculatus on calamine heaps form nodules with Bradyrhizobium liaoningense-related strains harboring novel in Europe symbiotic nifD haplotypes. Appl. Soil Ecol. 151, 103539 https://doi.org/10.1016/j.apsoil.2020.103539.

Turner, R.J., Weiner, J.H., Taylor, D.E., 1999. Tellurite-mediated thiol oxidation in Escherichia coli. Microbiology 145, 2549–2557. https://doi.org/10.1099/00221287-145-9-2549.

Turner, R.J., Aharonowitz, Y., Weiner, J.H., Taylor, D.E., 2001. Glutathione is a target in tellurite toxicity and is protected by tellurite resistance determinants in Escherichia coli. Can. J. Microbiol. 47, 33–40. https://doi.org/10.1139/cjm-47-1-33.

Vezza, M.E., Olmos Nicotra, M.F., Agostini, E., Talano, M.A., 2020. Biochemical and molecular characterization of arsenic response from Azospirillum brasilense Cd, a bacterial strain used as plant inoculant. Environ. Sci. Pollut. Res 27, 2287–2300. https://doi.org/10.1007/s11356-019-06959-1.

Vidal, C., Chantreuil, C., Berge, O., Mauré, L., Escarré, J., Béna, G., et al., 2009. Mesorhizobium metallidurans sp. nov., a metal-resistant symbiont of Anthyllis vulneraria growing on metallicolous soil in Languedoc, France. Int J. Syst. Evol. Microbiol 59, 850–855. https://doi.org/10.1099/ijs.0.003327-0.

Vishnivetskaya, T.A., Mosher, J.J., Palumbo, A.V., Yang, Z.K., Podar, M., Brown, S.D., et al., 2011. Mercury and other heavy metals influence bacterial community structure in contaminated Tennessee streams. Appl. Environ. Microbiol 77, 302–311.

Walter, E.G., Taylor, D.E., 1992. Plasmid-mediated resistance to tellurite: Expressed and cryptic. Plasmid 27, 52–64. https://doi.org/10.1016/0147-619X(92)90006-V.

Wani, P.A., Khan, S., Zaidi, A., 2007. Chromium-reducing and plant growth-promoting Mesorhizobium improves chickpea growth in chromium-amended soil. Biotechnol. Lett. 30, 159–163.

Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., et al., 2009. Three genomes from the phylum acidobacteria provide insight into the lifestyles of these microorganisms in soils. Appl. Environ. Microbiol 75, 2046–2056.

Werner, J., Géron, A., Kerssemakers, J., Matallana-Surget, S., 2019. mPies: a novel metaproteomics tool for the creation of relevant protein databases and automatized protein annotation. Biol. Direct 14, 21. https://doi.org/10.1186/s13062-019-0253-x.

Whelan, K.F., Colleran, E., Taylor, D.E., 1995. Phage inhibition, colicin resistance, and tellurite resistance are encoded by a single cluster of genes on the IncHI2 plasmid R478. J. Bacteriol. 177, 5016–5027. https://doi.org/10.1128/jb.177.17.5016-5027.1995.

Whittenbury, R., Dow, C.S., 1977. Morphogenesis and differentiation in *Rhodomicrobium vannielii* and other budding and prosthecate bacteria. Bacteriol. Rev. 41, 754–808.

Wright, G.E., Madigan, M.T., 1991. Photocatabolism of aromatic compounds by the phototrophic purple bacterium Rhodomicrobium vannielii. Appl. Environ. Microbiol 57, 2069–2073.

Yoon J.H., Park Y.H. , 2006, Chapter 1.1.29. The genus Nocardioides. Prokaryotes 3: 1099–1113. DOI: 10.1007/0–387-30743–5_44.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30 (5), 614–620.

Zhang, K., Wang, Y., Tang, Y., Dai, J., Zhang, L., An, H., et al., 2010. Niastella populi sp. nov., isolated from soil of Euphrates poplar (Populus euphratica) forest, and emended description of the genus Niastella. Int J. Syst. Evol. Microbiol 60, 542–545.