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# Soil Properties and Multi-Pollution Affect Taxonomic and Functional Bacterial Diversity in a Range of French Soils Displaying an Anthropisation Gradient

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1 **Soil properties and multi-pollution affect taxonomic and functional bacterial**  
2 **diversity in a range of French soils displaying an anthropisation gradient**

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8

9 **Abstract**

10 The intensive industrial activities of the 20th century have left behind highly contaminated wasteland soils. It is  
11 well known that soil parameters and the presence of pollutants shape microbial communities. But in such  
12 industrial waste sites, the soil multi-contamination with organic (polycyclic aromatic hydrocarbons, PAH) and  
13 metallic (Zn, Pb, Cd) pollutants and long-term exposure may induce a selection pressure on microbial  
14 communities that may modify soil functioning. The aim of our study was to evaluate the impact of long-term  
15 multi-contamination and soil characteristics on bacterial taxonomic and functional diversity as related to the  
16 carbon cycle.

17 We worked on 10 soils from northeast of France distributed into 3 groups (low anthropised controls, slag  
18 heaps, and settling ponds) based on their physico-chemical properties (texture, C, N) and pollution level. We  
19 assessed bacterial taxonomic diversity by 16S rDNA Illumina sequencing, and functional diversity using Biolog®  
20 and MicroResp™ microtiter plate tools.

21 Although taxonomic diversity at the phylum level was not different among the soil groups, many OTUs were  
22 influenced by metal or PAH pollution, and by soil texture and total nitrogen content. Functional diversity was  
23 not influenced by PAH contamination while metal pollution selected microbial communities with reduced  
24 metabolic functional diversity but more tolerant to zinc. Limited microbial utilisation of carbon substrates in  
25 metal-polluted soils was mainly due to the nitrogen content. Based on these two observations, we  
26 hypothesised that reduced microbial activity and lower carbon-cycle-related functional diversity may have  
27 contributed to the accumulation of organic matter in the soils that exhibited the highest levels of metal  
28 pollution.

29

## 30 **Introduction**

31 The decline of the steel industry in the north east of France at the end of the 20th century left behind more  
32 than 6,000 ha of polluted wastelands [1]. On these sites, the soils are multi-contaminated with hydrocarbons  
33 and metallic trace elements (MTEs) [2, 3]. On sites of former coking plants or slag heaps, polycyclic aromatic  
34 hydrocarbon (PAH) pollution results from the use of coal tar during coke manufacturing. PAHs are ubiquitous  
35 contaminants produced from the incomplete combustion of organic matter [4], especially during industrial  
36 activities. In settling ponds resulting from the sewage sludge storage of blast furnace gases [5], high  
37 concentrations of MTEs (Cd, Cu, Zn, Pb, Ni) are also often encountered.

38 In soils, the fate of PAHs and MTEs depends on various abiotic and biotic processes. Over time PAHs adsorb  
39 onto organic matter and spread into the micropores of soil components. Thus is referred to as the pollutant  
40 ageing, and leads to a decrease of pollutant availability [6]. MTE availability depends mostly on soil properties  
41 (pH, organic matter, redox potential...) [7, 8]. PAH compounds can be degraded over time mainly through  
42 microbial processes [9] that can be limited by low PAH availability [10, 11]. MTEs, cannot be biodegraded, but  
43 microorganisms can modify their speciation through direct use or modifications of soil properties [12].

44 Numerous studies have focused on the short-term influence of PAHs or MTEs on microbial communities. The  
45 selective pressure exerted by PAH toxicity [13, 14] could modify microbial community composition [14, 15],  
46 reduce microbial taxonomic diversity [16], or inhibit microbial activity and the global activity of enzymes such  
47 as arylsulphatase, phosphatase, urease, dehydrogenase [13]. Similarly, various studies have highlighted the  
48 negative effects of MTE pollution. For example the effect of zinc addition has been studied on enzymatic  
49 activity and microbial community composition [17], as well as on the taxonomic diversity of bacterial  
50 communities [18].

51 In aged polluted soils, various adaptation processes enable the microbial community to cope with and even  
52 benefit from pollution. The selection of PAH degraders [15, 19] and MTE-resistant microbes [17, 20] in aged-  
53 polluted soils has been shown using acquired tolerance tests [21]. However, the long-term impact of chronic  
54 exposure to PAHs and MTEs on microbial community composition has been less addressed [22, 23]. The impact  
55 of pollutants has been mainly studied separately, although multi-contamination can have a synergistic effect  
56 [24, 25]. Studies considering aged pollution and the impact of multi-contamination on microbial taxonomic  
57 diversity (richness and evenness) are scarce [26] and therefore needed.

58 The carbon cycle is pivotal in soil functioning because it contributes to all biological processes, such as biomass  
59 production or respiration by organisms. It also affects other nutrient cycles through organic matter recycling  
60 and mineralisation. In a context of soil pollution, one can thus wonder how the different functions of the  
61 carbon cycle are affected, and study the microbial functions involved in key C-cycle processes. Metabolic  
62 functional diversity can be defined as the number (richness) and the evenness of the metabolic functions of the  
63 microbial community. One approach to estimating functional diversity is by comparing the degradation  
64 capacities of carbon substrates using Biolog® [27] and MicroResp™ [28] microtiter plate methods in the  
65 presence or absence of pollutants, as done with metals that decrease bacterial functional diversity [29].

66 Apart from this impact of pollutants, it is well known that soil parameters, such as the pH, texture, the C  
67 content or the C/N ratio, highly contribute to shape microbial community composition [30, 31] and functional

68 diversity [32] in soils of various origins (forest, grassland, agricultural...). In polluted soils, such as urban,  
69 industrial, traffic, mining and military areas [3], physico-chemical parameters have also been found to largely  
70 influence the composition of the bacterial and fungal communities [22].

71 We therefore hypothesised that microbial diversity in multi-contaminated soils may be influenced by both soil  
72 properties and pollutant concentrations and availability, but differently at the taxonomic and functional levels.  
73 In this context, the present study aimed to: i) assess taxonomic diversity and metabolic functional diversity of  
74 microbial communities in a context of aged multi-pollution, and ii) to determine which soil factors (physico-  
75 chemical parameters, pollutants...) affected these diversities. We studied microbial communities from a  
76 collection of 10 soils presenting gradients of PAH and metal pollution, and under different land uses, ranging  
77 from weakly to highly anthropised, i.e. from forest soil to industrial soil. We assessed bacterial taxonomic  
78 diversity through 16S rRNA gene tag-amplicon sequencing (Illumina MiSeq). We estimated metabolic functional  
79 diversity using the degradation functions of ecologically relevant carbon substrates through Biolog® and  
80 MicroResp™ microtiter plate methods.

## 81 **Materials and methods**

82

### 83 **Study sites and soil sampling**

84 Ten soils originating from industrial wastelands and waste sites, natural forests or ancient gravel pit, located in  
85 the “Grand Est” region (north-east of France), were sampled in November 2015. This soil collection was chosen  
86 to cover a wide range of anthropisation situations, i.e. from no or low contamination to high contamination,  
87 and represented an anthropisation gradient. All sampling sites are within 100km. Three soils were considered  
88 as the control soils (*ctrl*), i.e. two low-anthropised forest soils collected at Hémilly (*He*; 49°2’1”N/6°30’51”E;  
89 Moselle; Stagnic Luvisol) and Montiers-sur-Saulx (*Mo*; 48°31’55”N/5°16’8”E; Meuse; Calcaric Cambisol), and  
90 one anthropised but unpolluted former gravel pit soil collected at Dieulouard (*Di*; 48°49’44”N/6°5’2”E,  
91 Meurthe-et-Moselle; Fluvisol). Seven anthropised soils (Technosol) known to be polluted by MTEs and/or PAHs,  
92 where industrial activities stopped during 1980s, were collected from: i) former slag heaps (*sh*) at Homécourt  
93 (*Ho*; 49°12’79”N/5°59’72”E, Meurthe-et-Moselle), Terville (*Te*; 49°20’25”N/6°08’33”E; Moselle), Uckange (*Uc*;  
94 49°18’58”N/6°9’55”E; Moselle), and Neuves-Maisons (*NM*; 49°12’52”N/5°59’45”E; Meurthe-et-Moselle), and ii)  
95 former settling ponds (*sp*), dried since at least 30 years, at Pompey (*Po*; 48°46’8”N/6°8’8”E; Meurthe-et-  
96 Moselle), Mont-St-Martin (*MsM*; 49°32’9”N/5°46’46”E; Meurthe-et-Moselle), and Russange-Micheville (*RM*;  
97 49°28’58”N/5°55’51”E; Moselle).

98 All sites were colonized by trees (mainly birch and/or beech) and herbaceous plants, with a very heterogeneous  
99 colonization density from one site to another, except for the NM soil which was colonized only by some herbs.  
100 None of the soil samples were rhizosphere soils or directly under plant influence (sampling at least 2m from a  
101 tree). As plant colonization was sparse by location, we collected samples on bare soils. At each of the ten sites,  
102 samples were collected from three independent sub-sites 1 m apart. After removing the litter layer, if present,  
103 one soil block per sub-site (block of 20 cm on the side and 30 cm deep) was removed using shovel. The three  
104 sub-site soil blocks were mixed to get one composite sample *per* site. Back to the laboratory, the soil samples  
105 were air-dried at room temperature for one week, and then sieved at 2 mm. The dried and sieved soils were  
106 then stored at room temperature in the dark before chemical and microbiological analyses, and aliquots were  
107 stored at -80 °C before freeze-drying and PAH analyses.

108

### 109 **Soil physico-chemical characteristics**

110 The soil physico-chemical characteristics were determined from the dried sieved soils. The pH was measured  
111 (PHM210 Radiometer Analytical, equipped with a pH probe, Bioblock Scientific) in a soil suspension prepared in  
112 distilled water (1:5 w:v). The CaCO<sub>3</sub> content was estimated from measurements of CO<sub>2</sub> concentrations released  
113 after acid (4M HCl) decarbonation of 1 g of dw soil, using an infrared absorbance (Binos 1004 analyser), with  $\lambda$   
114 2325.6 cm<sup>-1</sup>. The water retention capacity was estimated from the difference in weight between dry and water-  
115 saturated soil, which corresponded to 100%. Measurements of the soil texture (clay fraction, 0-2  $\mu$ m; silt  
116 fraction, 2-63  $\mu$ m; sand fraction 63-2,000  $\mu$ m; ISO 11277), the cation exchange capacity (CEC; determined by  
117 Metson method, NF X 31-130), organic carbon, organic matter, nitrogen concentrations (C, OM, and N,

118 respectively; Dumas method, ISO 10694), the available phosphorus concentration (P; Olsen method, NF ISO  
119 11263), total (fluoridric acid extraction) and available (calcium chloride extraction) concentrations of metals  
120 (Cr, Cd, Cu, Ni, Pb, Zn for total and available concentrations, plus Tl, Mo, Al, Ca, Fe, K, Mg, Mn, and Na for total  
121 concentrations) were performed at the laboratoire d'analyse des sols (INRA, Arras, France).

122 We calculated a metal pollution index (Mi) in order to compare metallic pollution of soils using only one  
123 variable. Mi was the sum of the relative proportions ( $RP_{metal}$ ) of nine metal concentrations (Zn, Pb, Cd, Fe, Cr,  
124 Cu, Ni, Co, Tl).  $RP_{metal}$  in soils were calculated using the following formula:

$$RP_{metal} = \frac{MC_{soil} \times 100}{MC_{max}}$$

125 where  $MC_{soil}$  is the total metal concentration in the soil, and  $MC_{max}$  is the maximum total metal concentration  
126 found among all the soils of the collection. Total instead of available metal concentrations were used because  
127 some of the available metal values were lower than the detection limit. The nine metals were chosen because  
128 they commonly resulted from the steel industry, and when for at least one soil, the metal concentration was  
129 higher than the mud compost norm (NF 44 095) or the natural geochemical background of French soils [33].

130

### 131 **PAH extraction and analysis**

132 Soil samples stored at -80 °C were freeze-dried and ground to 500 µm (Mixed Mill MM 400, Retsch). Total PAHs  
133 were extracted from 1 g dw soil in triplicate with dichloromethane, at 130 °C and 100 bars, using accelerated  
134 solvent extraction (DIONEX® 200 ASE), as described in Cennerazzo et al. [34]. Available PAHs were extracted  
135 from 1 g dw soil in triplicate with 20 ml of hydroxypropyl-β-cyclodextrin (50 mM) in Teflon™ FEP Oak Ridge  
136 centrifuge tubes (Nalgene, USA) [35]. After mixing (16 h) at 24 °C and centrifugation, PAHs were extracted from  
137 the aqueous solution through liquid:liquid extraction using dichloromethane. Both solvent extracts of total and  
138 available PAHs were evaporated (nitrogen flow) and dissolved in acetonitrile for PAH analysis using a reverse-  
139 phase chromatography (UHPLC DIONEX® Ultimate 3000 system) equipped with a Diode Array Detector (UV  
140 detection, 254 nm) and a Zorbax Eclipse PAH column (2.1 x 100 mm, 1.8 µm, Agilent).

141

### 142 **Carbohydrate, organic acid, and dissolved organic carbon measurements**

143 Carbohydrates, organic acids, and dissolved organic carbon (DOC) were measured on triplicate soil aqueous  
144 extracts (1:5 w:v) using 6 g dw soil mixed with 30 ml of distilled water in Teflon™ FEP Oak Ridge centrifuge  
145 tubes (Nalgene, USA) for 2h at 20 °C [36, 37]. After centrifugation and filtration, aqueous extracts were  
146 recovered. Seven carbohydrates (inositol, trehalose, sucrose, glucose, xylose, mannose, and fructose) were  
147 quantified by an ICS 3000 ion-exchange chromatograph equipped with a Dionex CarboPac SA10 column  
148 (Thermo Scientific). Eleven organic acids (gluconic, lactic, acetic, propionic, formic, pyruvic, succinic, maleic,  
149 oxalic, fumaric, and citric acids) were quantified using an ICS 2100 ion chromatographer equipped with a  
150 Dionex IonPac AS11 HC column (Thermo Scientific). DOC was measured on a TOC-V analyser (Shimadzu).

151

### 152 **Quantification of culturable bacteria**

153 The most probable number (MPN) was measured on 1 g dw soil (in triplicate) moistened (to 60% of their water  
154 retention capacity) and pre-incubated for 2 days (24°C) for reactivation of microbial community. After soil  
155 aliquots were resuspended in 10 ml of NaCl (0.9%) and mixed for 1 h, ten-fold dilution series were prepared in  
156 NaCl (0.9%) and used to inoculate 96-well microtiter plates (16 wells *per* dilution) filled with nutrient Broth No  
157 1 (Fluka analytical). After incubation for 3 days (24 °C), absorbance at 600 nm was measured using  
158 spectrophotometer (Safas, Monaco) and MPN counts were calculated using Mac Grady's tables, and expressed  
159 as MPN *per* gram of soil.

160

#### 161 **DNA extraction, real-time quantitative PCR, and sequencing**

162 Genomic DNA (gDNA) was extracted in triplicate from *ca.* 0.5 g of moistened soil (as described above) stored at  
163 -20 °C, using Fast DNA Spin Kit for Soil (MP Biomedicals, France), following the manufacturer's instructions.  
164 Concentration and purity ( $A_{260}/A_{280}$  ratio) were measured using a spectrophotometer (UV1800, Shimadzu)  
165 equipped with a TrayCell™ adapter (Hellma®). gDNA was diluted to 5 ng  $\mu\text{l}^{-1}$  for real-time quantitative PCR  
166 (qPCR) and sequencing library preparation. The abundance of fungi and bacteria was estimated by qPCR using  
167 the primer sets Fung5F/FF390R [38], 968F/1401R [39], targeting 18S and 16S rRNA genes, respectively. The  
168 qPCR assays were performed as previously described [40, 41]. Briefly, the reaction mixture (20  $\mu\text{l}$ ) was  
169 composed of 10  $\mu\text{l}$  iQ SYBR green SuperMix (Bio-Rad), 0.8  $\mu\text{l}$  primers (10  $\mu\text{M}$ ), 0.4  $\mu\text{l}$  bovine serum albumin  
170 solution (3%), 0.2  $\mu\text{l}$  dimethyl sulfoxide, 0.08  $\mu\text{l}$  T4gp32 (MP Biomedicals, France), and 1  $\mu\text{l}$  DNA (diluted gDNA  
171 or 10-fold dilution series from  $10^8$  to  $10^1$  copies  $\mu\text{l}^{-1}$  of the standard plasmids). Quantification was performed  
172 using a CFX96 Real-Time PCR detection system (Bio-Rad), using 56 °C and 50 °C as annealing temperature for  
173 16S and 18S rDNA quantification, respectively.

174 The V3/V4 region of bacterial 16S rRNA genes (*ca.* 550 bp) was amplified using primers  
175 S-D-Bact-0341-a-S-17 and S-D-Bact-0787-b-A-20 [42, 43] and following a previously described dual-index  
176 strategy [44] using PCR primers with Illumina adaptor, pad and index sequences [37]. PCR reactions were  
177 performed on 2  $\mu\text{l}$  of diluted gDNA using Phusion high-fidelity polymerase (Thermo Scientific). PCR reactions  
178 consisted of 31 cycles with touchdown annealing temperature for 18 cycles (63 °C to 54°C with a decrease of  
179 0.5 °C/cycle) and 13 cycles at 54 °C. Amplification products were checked on 1% agarose gel electrophoresis,  
180 and purified using the UltraClean-htp 96 Well PCR Clean-Up kit (Qiagen) following the manufacturer's  
181 instructions. After Quant-iT Picogreen ds-DNA assay Kit (Invitrogen) quantification, an amplicon library was  
182 prepared (equimolar pool at 10 nM), purified on a QIAquick PCR purification kit column (Qiagen), and sent for  
183 sequencing to Genewiz platform (South Plainfield, NJ, USA) using an Illumina MiSeq V2 Kit for 2 x 250 bp  
184 paired-end sequencing. Illumina MiSeq paired-end reads have been deposited in the SRA database under  
185 Bioproject accession number PRJNA450766. Sequence data were analysed following the MiSeq SOP procedure  
186 available in March 2017 and described in Kozich et al. [44], using Mothur v.1.38.0 [45]. Paired-end reads were  
187 trimmed using the following criteria: QS>20, 404 bp < length < 454 bp, and no ambiguous bases. Chimeras  
188 detected using Uchime [46] and singletons (sequences appearing only once among all samples) were removed.  
189 Alignment of unique sequences and taxonomy was assigned using the Silva bacteria database (cutoff = 80).

190 Sequences affiliated to archaea, eukaryota, unknown, mitochondria, and chloroplasts were removed.  
191 Sequences were clustered in Operational Taxonomic Units (OTUs) at 97% similarity. Finally, datasets were  
192 rarefied to the lowest number of sequences *per* sample (34 191 reads/sample). Alpha diversity was expressed  
193 by calculating Chao1, Pielou's evenness  $J'$ , and Shannon  $H'$  indices [47] and Beta-diversity was calculated with  
194 Bray-Curtis index, using Mothur.

195

## 196 **Carbon substrate utilisation using Biolog® plates and metal tolerance test**

197 Two types of Biolog® microtiter plates were used to assess the metabolic functional diversity of bacterial  
198 communities through utilisation patterns of 62 carbon substrates (Table S1): i) EcoPlates™ containing 31  
199 substrates of ecological relevance (5 guilds: 9 carboxylic acids, 6 amino acids, 8 carbohydrates, 4 polymers, and  
200 4 miscellaneous), and ii) MT2 microplates™ allowing to test 31 chosen substrates (4 PAHs, 6 aromatic acids, 8  
201 carboxylic acids, 3 amino acids, 6 carbohydrates, 3 polymers, and 1 miscellaneous). MT2 microplates™ were  
202 prepared following the manufacturer's instructions (0.3 mg of carbon in each well) except for organic acids,  
203 cellulose, and lignin which were 10-fold diluted because the recommended concentrations were toxic or the  
204 presence of solid particles flawed absorbance measurements (data not shown). PAHs and phenolic acids were  
205 dissolved in n-hexane and added to empty plates, and the solvent was eliminated by drying for 12 h in a sterile  
206 hood [37]. The water-soluble substrates (20 µl) were added just before inoculation and 20 µl of sterile water  
207 were added in the wells containing PAHs and phenolic acids and in the EcoPlate™ wells.

208 Microbial inocula were prepared by diluting soil aliquots (1 g of moistened soil prepared as described above) in  
209 10 ml of NaCl (0.9%) and stirring with glass beads (1.5-mm diameter) for 1 h. Supernatant was decanted for 15  
210 min and diluted in NaCl (0.9%) differently for each soil, based on MPN data to obtain a similar microbial  
211 abundance (from  $10^2$  to  $10^3$  cultivable bacteria  $ml^{-1}$ ) for all soil. Biolog® plates were inoculated with 100 µl of  
212 the appropriate dilution, and incubated at 24 °C in a plastic bag containing wet cotton to avoid desiccation.

213 Zinc was used to test bacterial community tolerance to metals. Biolog® substrate utilisation was measured in  
214 the presence of zinc, added as  $ZnCl_2$  (10 mg  $l^{-1}$  of Zn) directly in the inoculum. This zinc concentration was  
215 chosen to obtain a zinc effect without total inhibition of bacteria (preliminary experiments not shown).

216 To summarise, our experimental design tested 62 carbon substrates on 10 soils in triplicate, with or without  
217 zinc addition. The absorbance at 595 nm was monitored in an spectrophotometer (SAFAS, Monaco) over 7 days  
218 at  $t = 0, 24, 48, 72,$  and 96 h. Absorbance values were corrected ( $Abs_{corr}$ ) after subtraction of the absorbance of  
219 mean blank wells at the corresponding times. Substrate utilisation was considered positive when the corrected  
220 absorbance was  $> 0.2$ . For each soil,  $Abs_{corr}$  at 96 h was used to calculate functional alpha diversity estimators,  
221 i.e. metabolic richness (number of carbon substrates significantly utilised), Pielou's evenness ( $J'$ ; based on the  
222 relative proportions of utilisation intensity for each substrate) and Shannon ( $H'$ ; based on the two previous  
223 indices) diversity indices using the vegan package in R [48]. For each soil, the average well color development  
224 (AWCD) was calculated over time using the formula:

$$AWCD = \frac{\sum bs_{corr}}{62}$$

225

226 where  $Abs_{corr}$  was the corrected absorbance of the substrate. Zinc tolerance was assessed for each soil by  
227 calculating a percentage of inhibition based on a comparison of areas below the AWCD curves between  
228 conditions with or without zinc. Percentages of inhibition were compared to soil total and available zinc  
229 concentrations (transformed to 1 when below the detection limit, i.e.  $10 \mu\text{g kg}^{-1}$ ) through linear regressions.

230

### 231 **Soil metabolic profiling using the MicroResp™ method**

232 The MicroResp™ technique was used to measure basal respiration and substrate-induced respiration (SIR) [28].  
233 Soils were loaded (four replicates) into the 96-deep-well plates, and the mean mass of each soil was measured.  
234 Soils were moistened to 45% water retention capacity using sterile distilled water, and plates were pre-  
235 incubated 3 days at 24 °C in a plastic bag containing wet cotton to avoid desiccation. After this step, six carbon  
236 sources (pyruvate, succinate, citrate, L-asparagine, D-ribose, and D-mannitol), selected according to the  
237 contrasting levels of substrate utilisation in Biolog® plates among soils, were added to reach 80% of soil water  
238 retention capacities. Substrates were added at 20 mg of C per well except for L-asparagine (3 mg) and citrate (5  
239 mg) having lower solubility. Water was added to plates for basal respiration. Finally, deep-well plates were  
240 sealed to the CO<sub>2</sub>-trap microtiter plates and incubated in the dark at 24 °C for 4 to 9 h depending on the carbon  
241 source (Table S1) and for 8 h for basal respiration. Absorbance was measured at 570 nm using an  
242 spectrophotometer (SAFAS, Monaco) just before sealing to measure blank values to subtract to the final  
243 absorbance. Mean basal respiration (n = 8) was measured on two independent series and 6 SIR were measured  
244 on 4 replicates for the 10 soils. Absorbance values for SIR were corrected by subtracting the absorbance mean  
245 (n = 4) of the corresponding soil basal respiration series, and transformed into a CO<sub>2</sub> concentration using the  
246 following formula:

247

$$ppm \text{ of } CO_2 = 138.72 \times \exp^{(6.7974 \times ODC)}$$

248

249 where  $ODc$  is the corrected absorbance. Then the CO<sub>2</sub> concentration was standardised by dividing it by the soil  
250 quantity and the incubation time. Based on MicroResp instruction manual, ppm values of CO<sub>2</sub> were then  
251 expressed as quantity of carbon released as CO<sub>2</sub> per gram of soil per hour. For each soil, these corrected and  
252 standardised absorbance values were used to calculate the same functional diversity estimators (richness,  
253 evenness, and Shannon diversity indices) as the ones described above for Biolog data.

254

### 255 **Statistical analyses**

256 All statistical analyses were performed using RStudio v1.0.136. Significant differences among soils or among soil  
257 groups were assessed using Kruskal-Wallis rank sum test followed by a multiple comparison test included in the  
258 Vegan R package [48]. For principal component analysis (PCA) nine soil variables among soil physico-chemical,  
259 pollution, and texture characteristics, were used, and soil groups were made with Monte Carlo test using 1,000  
260 iterations. For redundancy analysis (RDA), explanatory variables were chosen by using the *ordistep* R function.  
261 Permutation tests on the RDA axes and variables were performed. Multivariate analyses were carried out on

262 standardised data using the ade4 [49] and vegan R packages. Linear regression was assessed by fitting to a  
263 linear model using “lm” function. The partial least square regression (PLSr) algorithm [50, 51] in regression  
264 mode was used to identify possible relationships between the soil physico-chemical characteristics and 96  
265 bacterial OTUs (each representing at least 1% of the total). The two matrices were z-normalised. The PLSr was  
266 implemented using the mixOmics package [52, 53]. Once calibrated, the PLSr model was validated using the  
267 leave-one-out cross-validation method. The  $R^2$  and the mean squared error of prediction were then used (data  
268 not shown) to select the appropriate number of principal components used to implement the model. The  
269 correlation between bacterial OTUs and soil physico-chemical parameters was displayed in a heatmap (“cim”  
270 function) resulting from the similarity matrix obtained from the PLSr model.

## 271 Results

272

### 273 Grouping of soils according to their physico-chemical characteristics

274 The physico-chemical and microbial characteristics of the ten soils are summarised in Table 1 and 2. Many  
275 characteristics differed among soils and soil groups (settling pond, slag heap, control). Although one of the  
276 control forest soils (ctrl-He) had a pH of 5.4 while all other soils had a slightly basic pH (from 7.0 to 8.0), no  
277 statistical pH difference was observed among the soil groups. The slag heap soils differed by a high available  
278 PAH concentration and intermediate values of the metal pollution index, whereas the settling pond soils had a  
279 high metal pollution index with especially high Zn, Pb, Cd, and Tl concentrations and intermediate values of  
280 available PAH concentrations. These two groups also presented high total PAH concentrations and high Fe  
281 concentrations as compared to the control soils. The control soils were mainly characterised by the highest  
282 dissolved organic carbon concentration and the lowest total PAH concentration and metal pollution index. The  
283 control soils also contained higher total potassium and aluminium concentrations than the other soils, probably  
284 in part due to their proportion of clay. No difference among soil groups was observed for the C/N ratio or for  
285 concentrations of total organic carbon, organic matter, nitrogen, organic acids, carbohydrates, and available  
286 phosphorus. Our collection of soils also presented highly variable iron concentrations, ranging from 18 g kg<sup>-1</sup> in  
287 ctrl-Di to a particularly high concentration of 452 g kg<sup>-1</sup> in sh-Uc. Concerning microbial parameters such as MPN  
288 counts and 16S rRNA gene abundance, soil groups were not statistically different. Interestingly, the ratios of  
289 fungal to bacterial abundance (18S /16S rDNA ratios) were similar between the control and slag heap soils, and  
290 the lowest for settling pond soils, probably because the three soil groups had similar 18S rRNA gene copy  
291 numbers while the settling pond soils tended to have higher 16S rRNA gene copy numbers ( $p = 0.053$ ).

292 We performed a PCA on the basis of soil physico-chemical, pollution, and texture characteristics (Fig. 1), which  
293 confirmed the distribution of the 10 soils in 3 groups based on their use. The first three components of the PCA  
294 explained 76.75% of total variance, with the first (PC1), the second (PC2) and the third (PC3) components of the  
295 PCA accounting for 35.3%, 28.5%, and 12.8%, respectively. The main contributing variables were the metal  
296 pollution index Mi (24.8% of total variance on the considered axis) and the total potassium concentration  
297 (18.9%) on the first axis, soil texture (sand: 33.1%; clay: 24.9%) and the pH (13.8%) on the second axis, and PAH  
298 (46.9%) and organic carbon (30.9%) concentrations on the third axis (not shown). On PC1, control soils were  
299 mainly separated from slag heap and settling pond soils by the potassium and clay fraction parameters.  
300 Interestingly, settling pond soils were separated from the control and slag heap soils by the metal pollution,  
301 organic carbon, and nitrogen parameters. On PC2, slag heap soils were separated from the control and settling  
302 pond soils by the sand fraction and pH parameters. PAH pollution was not a discriminative parameter (10.6% of  
303 the weight on PC2) and did not separate the soil groups according to their use on the PC3 axis (46.86% of the  
304 weight on PC3). To test if the soil groups were robust, we performed a Monte-Carlo test, and confirmed that  
305 our grouping was statistically significant (1,000 iterations,  $p = 1.998 \times 10^{-3}$ ). We also performed a second PCA  
306 without the pollution characteristics (data not shown) and confirmed the separation of the soils in three groups  
307 corresponding to their use (Monte Carlo test, 1000 iterations,  $p = 0.016$ ).

308

### 309 **Bacterial taxonomic diversity**

310 We determined bacterial taxonomic diversity by sequencing 16S rRNA genes. After read treatment and  
311 rarefaction to 34,191 reads *per* sample, we classified OTUs with 97% similarity taxonomically. The taxonomic  
312 alpha diversity estimators based on OTU data are shown in Table 2. Interestingly, slag heap soils harboured  
313 more diversified bacterial communities with higher Shannon's diversity (mean value of 6.203) and Pielou's  
314 evenness (mean value of 0.791) indices than settling pond soils. Compared to the other two soil groups, control  
315 soils had similar bacterial taxonomic diversity indices due to the low and high bacterial taxonomic diversity  
316 indices of ctrl-Mo and ctrl-Di, respectively. We investigated bacterial taxonomic diversity at the phylum level  
317 (Alpha, Beta, Gamma, and Delta classes were found for Proteobacteria). Their relative proportions in each soils  
318 are presented in Fig. 2. Based on the dissimilarity distance matrix, the soils were all highly different: the  
319 minimum Bray-Curtis dissimilarity index between two different soils was 0.814, while it ranged from only 0.254  
320 to 0.519 for replicates of a same soil (data not shown). Phylum proportions varied among soils, with a majority  
321 of Acidobacteria, Actinobacteria, and Proteobacteria. Acidobacteria were dominant (10.8% to 31.4%), except in  
322 ctrl-Mo (7.1%) and sh-NM (4.3%) communities. Within the soil collection, Actinobacteria represented 10.1% to  
323 25.9% of bacterial communities. Proteobacteria represented 16.2% to 35.1% of soil bacterial communities and  
324 were mainly represented by Alpha-Proteobacteria (up to 18.5% in sh-Te), except in ctrl-Mo where Beta-  
325 Proteobacteria were the dominant proteobacterial class and represented 13.2%. We can note that ctrl-He, sp-  
326 MsM, and sp-RM bacterial soil communities harboured the lowest proportions of Proteobacteria but high  
327 proportions of Verrucomicrobia (22.1% in ctrl-He) and of unclassified OTUs (16.5% and 17.8% in sp-MsM and  
328 sp-RM, respectively). Bacterial communities from soils sharing the same use had very different compositions.  
329 For example, Firmicutes represented 13.8% of the sp-MsM soil community, while they represented a maximum  
330 of 0.4% of the other settling pond soil bacterial communities. By comparing soil groups, we noted that  
331 Verrucomicrobia were present in a significantly higher proportion in control soils than in polluted soils  
332 ( $p = 9.415 \times 10^{-5}$ ). On the contrary, polluted soils harboured a significantly higher proportion of Candidate  
333 division TM7 phyla than control soils ( $p = 0.001$ ). We investigated the relationship between physico-chemical  
334 soil parameters and bacterial phylum proportions by searching significant linear correlations. The proportion of  
335 Actinobacteria was positively correlated to the total nitrogen concentration (Pearson,  $p = 6.74 \times 10^{-4}$ ,  $r = 0.88$ )  
336 and the soil cation-exchange capacity (Pearson,  $p = 2.04 \times 10^{-3}$ ,  $r = 0.85$ ). The proportion of Proteobacteria was  
337 positively correlated to total and available PAH concentrations (Pearson,  $p = 1.59 \times 10^{-3}$ ,  $r = 0.86$ , and  
338  $p = 9.99 \times 10^{-4}$ ,  $r = 0.87$ , respectively), mostly due to a correlation with gamma-Proteobacteria (Pearson,  $p =$   
339  $0.05$ ,  $r = 0.63$ , and  $p = 0.02$ ,  $r = 0.71$ , respectively). Positive correlations between the proportion of Chloroflexi  
340 and total zinc and lead concentrations (Pearson,  $p = 2.43 \times 10^{-3}$ ,  $r = 0.84$ , and  $p = 0.03$ ,  $r = 0.70$ , respectively)  
341 were also found. The proportion of unclassified OTUs was correlated with the total zinc concentration  
342 (Pearson,  $p = 5.42 \times 10^{-3}$ ,  $r = 0.80$ ) and the metal pollution index (Pearson,  $p = 1.93 \times 10^{-2}$ ,  $r = 0.72$ ). Similarly,  
343 the proportion of Gemmatimonadetes was positively correlated with the metal pollution index (Pearson,  $p =$

344  $1.44 \times 10^{-2}$ ,  $r = 0.74$ ) and with various metals such as total nickel and cobalt concentrations (Pearson,  $p = 1.66 \times$   
345  $10^{-2}$ ,  $r = 0.73$ , and  $p = 1.52 \times 10^{-2}$ ,  $r = 0.74$ , respectively).

346 The analysis at the OTU level generated 32,920 OTUs, which ranged from 2,159 (sp-RM) to 4,434 (ctrl-Di) mean  
347 OTUs *per* sample. The PLSr was computed to gain insights into the soil physico-chemical parameters that drove  
348 bacterial taxonomic diversity. The heatmap in Fig. S1 shows that the distribution of correlations between  
349 bacterial diversity and soil characteristics was rather scattered, reflecting a great heterogeneity of the  
350 relationships linking bacterial diversity to soil properties. Each soil factor selectively correlated only to a small  
351 number of OTUs (10-17). In particular, as identified by the high correlation coefficients ( $> 0.9$ ), the strongest  
352 correlations between soil properties and OTUs concerned the pH, the clay content, and the PAH content.  
353 Whereas the pH and clay seemed to correlate mostly to OTUs affiliated to the Verrucomicrobia (negatively) and  
354 Firmicutes (positively), respectively, the PAH content appeared to affect a more diverse range of OTUs. The soil  
355 PAH content was indeed positively and strongly correlated to the abundance of 5 OTUs affiliated to  
356 Bacteroidetes (*Flexibacter* and *Flavobacterium* members) but also to that of 3 OTUs affiliated to Chloroflexi,  
357 and 4 affiliated to Alpha-Proteobacteria (2 OTUs belonging to *Sphingomonadaceae*), Nitrospirae, and  
358 Actinobacteria (one OTU affiliated to *Arthrobacter*). Interestingly, these groups were mostly unaffected by all  
359 other soil parameters and were the only taxa affected by PAHs. Moreover, no OTU was negatively affected by  
360 PAHs. With respect to the other soil characteristics such as total N, available P, and the metal concentration  
361 index, we observed weaker correlations with bacterial diversity. These factors were positively correlated to one  
362 group of OTUs dominated by members of Actinobacteria. The total organic carbon and sand contents were  
363 slightly correlated to 2 other groups of OTUs, but with opposite effects. Organic C was positively correlated to a  
364 highly diversified taxonomic group, while the sand content was positively correlated with a group of 8  
365 Acidobacteria.

366

### 367 **Metabolic functional diversity**

368 The soil basal respiration and the substrate-induced respiration (SIR) measured for six different carbon sources  
369 (L-asparagine, citrate, succinate, pyruvate, D-mannitol, D-ribose) using MicroResp microtiter plates are shown  
370 in Fig. 3. The functional alpha diversity estimators based on MicroResp data are shown in Table 2. No statistical  
371 difference among soil groups emerged for substrate richness, probably due to the low number of carbon  
372 substrates we tested. The control and slag heap soils had similar functional Shannon indices and higher ones  
373 than the settling pond soils. Pielou's functional evenness index was statistically different among the 3 soil  
374 groups, with the highest for slag heap soils and the lowest for settling pond soils. The sum of SIRs for the 6  
375 different carbon sources was used as a proxy of the soil metabolic activity (Fig. 3). Most of the carbon sources  
376 were utilised by every soil, i.e. the SIR level was higher than basal respiration, except with D-mannitol that was  
377 not utilised by sh-Te, and citrate that was not utilised by ctrl-Mo, ctrl-Di, sh-Ho, and sp-Mi. The highest  
378 measured SIR was with pyruvate and the lowest with citrate. sp-Po showed the highest metabolic activity,  
379 while sh-Ho showed the lowest, mostly due to the great differences in SIR levels with the pyruvate substrate  
380 (data not shown). Kruskal-Wallis tests showed that all SIR values except with D-ribose significantly differed

381 among the soils. Based on soil groups, Kruskal-Wallis tests revealed that the settling pond soils had a  
382 significantly higher soil metabolic activity than the slag heap and control soils ( $p = 1.93 \times 10^{-3}$ ), mostly due to  
383 the high metabolic activity of sp-Po. Basal respiration and SIR with pyruvate were also the highest in the  
384 settling pond soils and the lowest in the slag heap soils ( $p = 1.73 \times 10^{-7}$  and  $p = 9.54 \times 10^{-5}$ , respectively). Finally,  
385 a positive linear correlation (Pearson,  $p = 6.99 \times 10^{-5}$ ,  $r = 0.94$ ) was found between soil metabolic activity and  
386 soil basal respiration. Additionally, these last two parameters were positively correlated with the total nitrogen  
387 concentration (Pearson,  $p = 8.42 \times 10^{-3}$ ;  $r = 0.77$ , and  $p = 1.53 \times 10^{-2}$ ;  $r = 0.74$ , respectively).

388 We assessed carbon substrate utilisation using Biolog microtiter plates to calculate soil metabolic richness and  
389 average well colour development (AWCD) (Fig. 4). The functional alpha diversity estimators based on Biolog  
390 data are shown in Table 2. Kruskal-Wallis test showed that bacterial communities from the settling pond soils  
391 had a significantly lower metabolic richness and Shannon's diversity indices than bacterial communities from  
392 the slag heap and control soils, while no difference among the three soil groups was observed for Pielou's  
393 evenness index. The metabolic richness of the ten soils ranged from 5 (sp-RM) to 31 (ctrl-Mo) utilised carbon  
394 sources, out of a total of 62 carbon sources. None of the 10 soils significantly catabolised PAHs in the  
395 conditions of the experiment, probably due to a too short incubation time. Thus, except PAHs for all soils, and  
396 polymers and miscellaneous substrates for sp-RM, at least one substrate from each substrate guild was utilised  
397 by each soil. Among the 62 carbon substrates, protocatechuic acid, L-asparagine, Tween 40, D-mannitol,  
398 succinic acid, and putrescine were the best utilised carbon substrates in the aromatic acid, amino acid,  
399 polymer, carbohydrate, carboxylic acid, and miscellaneous guilds, respectively. On the contrary, for these same  
400 carbon guilds, the less utilised substrates were syringic acid, glycine, alpha-cyclodextrin, D-sucrose, propionic  
401 acid, and pyrocatechol, respectively. Kruskal-Wallis tests showed significant differences in metabolic richness  
402 among soils, especially between ctrl-Mo and sp-RM. ctrl-Mo indeed exhibited higher total metabolic richness  
403 and a wider range of utilised carboxylic acids, carbohydrates, and polymers than sp-RM. No significant  
404 difference was observed among soils for miscellaneous, amino acid, and aromatic acid metabolic richness.  
405 Besides, as regards the soil groups, the settling pond soils showed significantly lower total metabolic richness  
406 ( $p = 0.007$ ), carboxylic acid ( $p = 0.017$ ) and carbohydrate ( $p = 0.004$ ) utilisation richness than the slag heap and  
407 control soils. Regarding utilisation of substrates from the polymer guild, the settling pond soils showed the  
408 lowest metabolic richness, and the control soils the highest ( $p = 0.03$ ), respectively. Finally, a positive linear  
409 correlation (Pearson correlation,  $p = 1.182 \times 10^{-5}$ ,  $r = 0.959$ ) was found between total metabolic richness and  
410 AWCD.

411

#### 412 **Zinc tolerance**

413 Soil microbial zinc tolerance was assessed using Biolog microtiter plates by adding  $ZnCl_2$  solution, and then  
414 percentages of inhibition due to zinc addition were calculated. Fig. 5 shows the linear regression between the  
415 soil available zinc concentration and the percentage of inhibition of carbon substrate utilisation. A similar linear  
416 regression was found with the total zinc concentration ( $p = 3.975 \times 10^{-5}$ ,  $R^2 = 0.45$ ). The settling pond soils had  
417 the highest available zinc concentration, and their metabolic activity was the least inhibited by zinc addition.

418 The ctrl-He soil also had a high available zinc concentration ( $38.1 \text{ mg kg}^{-1}$ ), due to its low pH (5.4, Table 1)  
419 leading to a higher MTE availability and a lower zinc inhibition of metabolic activity (46.2%). In comparison, ctrl-  
420 Mo and ctrl-Di had neutral pH values (7.0 and 7.3, respectively), a low available zinc concentration ( $< 10$  and  $11$   
421  $\mu\text{g kg}^{-1}$ , respectively) and a higher zinc inhibition of metabolic activity (49.3 and 54.7%, respectively).

422

### 423 **Effects of the soil characteristics on taxonomic and metabolic functional diversity**

424 We investigated the effects of soil environmental characteristics on bacterial taxonomic diversity (Fig. 6A), and  
425 MicroResp (Fig. 6B) and Biolog (Fig. 6C) metabolic functional diversity through redundancy analyses. We  
426 performed three RDAs using soil environmental characteristics as explanatory variables, and the taxonomic and  
427 functional diversity indices of the soil microbial communities (Table 1 and 2) as response variables. The  
428 explanatory variables that best explained the variation of diversity indices were selected by backward selection  
429 to maximise the percentage of explained variability leading to at least 99.3% of total variation. The  
430 supplemental Table S2 provides p values and coefficients for axes and soil properties significance. Only the first  
431 axis ( $p < 0.005$ ) in Fig. 6A and 6B was significant, and so were the first two axes ( $p < 0.005$  and  $p < 0.02$ ) in Fig.  
432 6C. Among the explanatory variables (metal pollution index, PAH concentration, pH, and sand fraction), only  
433 the sand fraction had a significant effect (first axis; Fig. 6A) on bacterial taxonomic diversity ( $p < 0.01$ ,  $F =$   
434  $16.30$ ). This relationship was confirmed by significant positive linear correlations between the proportions of  
435 soil sand fractions and Chao1 ( $p = 5.97 \times 10^{-3}$ ,  $r = 0.80$ ), Shannon diversity ( $p = 3.50 \times 10^{-3}$ ,  $r = 0.82$ ) and Pielou's  
436 evenness ( $p = 0.01$ ,  $r = 0.76$ ) indices. Total organic carbon and nitrogen concentrations were the two  
437 significantly explanatory variables ( $p < 0.005$ ,  $F = 8.71$ , and  $p < 0.01$ ,  $F = 4.46$ , respectively) accounting for the  
438 variation of MicroResp soil metabolic diversity (Fig. 6B). The total nitrogen concentration showed negative  
439 relationships with Shannon diversity ( $H'$ ) and Pielou's evenness ( $J$ ) indices, confirmed by significant Pearson  
440 linear correlations ( $p = 1.50 \times 10^{-4}$ ,  $r = -0.92$ , and  $p = 3.08 \times 10^{-3}$ ,  $r = 0.83$ , respectively). The metal pollution  
441 index and the total nitrogen concentration were the strongest determinants of both axes (Fig. 6C) and had a  
442 significant effect on Biolog metabolic diversity indices ( $p < 0.01$ ,  $F = 7.77$ , and  $p < 0.05$ ,  $F = 4.71$ , respectively).  
443 The metal pollution index showed a negative relationship with metabolic richness and Shannon diversity index  
444 ( $H'$ ) confirmed by slightly significant negative linear correlations ( $p = 0.06$ ,  $r = -0.61$  for both of them). Total  
445 nitrogen exhibited a negative relationship with Pielou's evenness index ( $J$ ), confirmed by a significant linear  
446 correlation ( $p = 0.02$ ,  $r = -0.73$ ).

## 447 **Discussion**

448

449 We studied ten different soils presenting metal and/or PAH pollution gradients and exhibiting a wide range of  
450 anthropisation levels. Although the ten soils came from various sites with different types and levels of plant  
451 colonization, they mostly clustered in three groups based on their physico-chemical properties and pollution  
452 levels. These three groups reflected their use, whether industrial or low anthropised. Settling pond soils were  
453 highly polluted with MTEs, with higher contents of total organic carbon and nitrogen and a siltier texture than  
454 the soils from the other groups. Slag heap soils were the sandiest and the most PAH-polluted soils, and also  
455 exhibited middle-high metal pollution: mostly MTEs for sh-Te, sh-NM, and sh-Ho, while sh-Uc contained a very  
456 high iron concentration. The control soils were the least metal- and PAH-polluted ones; they were also the  
457 most clayey soils, and had the highest dissolved organic carbon and potassium contents. Apart from the  
458 identification of well known edaphic factors impacting the microbial diversity, we also aimed at identifying the  
459 impact of pollutants on the microbial community composition. The taxonomic and metabolic functional  
460 diversity of the microbial communities inhabiting the ten soils was characterized. We studied bacterial  
461 taxonomic diversity based on 16S rRNA gene sequencing, and functional diversity based on measurements of  
462 the utilisation of various carbon substrates involved in different functions to estimate the impact of land use on  
463 microbial community diversity and functions. We focused on functions related to the carbon cycle, one of the  
464 most important biogeochemical cycles for soil functioning [54, 55]. Although the taxonomic and functional  
465 diversity of soil microorganisms is extensively studied to better understand ecosystem functioning, the real link  
466 between taxon occurrence and functional richness remains poorly understood in most ecosystems [56],  
467 particularly for microbial communities. Various authors suggest that the functions related to carbon  
468 mineralisation are redundant [57, 58]. Besides, the loss of species or the modification of microbial community  
469 composition does not necessarily induce a loss of functions. In order to better understand the effect of  
470 pollution on microbial communities, it is thus important to study both taxonomic and functional diversity, using  
471 a combination of tools.

472 One of the objectives of this study was to identify soil parameters mostly shaping microbial community  
473 diversity in the ten soils. Although, some of the measured diversity indicators seemed constant in all soils, our  
474 data revealed that both pollutant and physico-chemical parameters strongly affected part of the taxonomic  
475 and functional structures.

476

### 477 **Non-impacted microbial parameters**

478 We noted few variations in bacterial community composition among our soils, with all the major phyla present  
479 in each soil, and no phylum significantly dominant or under-represented in highly anthropised soils. The  
480 taxonomic diversity indices of the polluted soils were not significantly different from those of the control soils.  
481 Previous studies reported similar trends with no difference among the bacterial taxonomic diversity indices of  
482 historically mining-impacted vs. unpolluted sediments [59]. Indeed, long-term adaptation and resilience [60] of  
483 the bacterial communities, being as diverse as in control soil [22] or over a metal polluted gradient [61], was

484 previously observed. Additionally, except for sp-RM that exhibited a very strong decrease of metabolic  
485 richness, at least one substrate from each carbon substrate guild from Biolog plates was utilised by each soil. In  
486 some cases, metabolic potentials were not affected by metal pollution as shown in three forest soils gradually  
487 polluted by Zn and Cd [62]. The differences of substrate-induced respiration observed among soils were less  
488 pronounced using the MicroResp than the Biolog method, and were essentially linked to the soil basal  
489 respiration rates as previously observed [63]. Contrarily to many studies [63, 64, 65], basal respiration rates and  
490 substrate-induced respiration were mostly not affected by pollution (metals and PAH) level. Additionally to the  
491 bacterial activity, MicroResp can also integrate fungal respiration. Although, fungi are major actors of the  
492 carbon cycle [66], their relative abundances (ratio between 18S and 16S rDNA copy numbers) were not  
493 correlated with the respiration rates because the three soil groups had similar fungal density (18S rDNA  
494 copies.g<sup>-1</sup> dw soil). Moreover, even if fungi harbor high metal tolerance [67] no link between fungal  
495 abundances, respiration rates and soil pollution was highlighted.

496

#### 497 **Impact of metals on microbial diversity**

498 The relative toxicity of a pollutant depends more on its available content than on its total content. The  
499 bioavailability of MTEs in these soils was relatively low since most of them had a pH value above 7. Only soil He  
500 with a pH of 5.4 had a relatively higher MTE availability than the other soils of the same group, although it was  
501 not contaminated. Except for this soil, using the relative metal pollution index or the available fraction of MTEs  
502 such as Zn showed the same tendency, that is why we mainly used the former to compare the soils.

503 The metal pollution index and/or the total zinc content were positively correlated with the relative proportion  
504 of unclassified OTUs and Gemmatimonadetes and Chloroflexi phyla (correlation with available Zinc content  
505 have p value of 0.077, 0.088 and 0.120, respectively). Unclassified OTUs may represent extensive unexplored  
506 bacterial diversity that may have original properties allowing them to survive and grow in strongly metal-  
507 contaminated environments. Gemmatimonadetes are commonly found in heavy-metal contaminated soils [68].  
508 A positive relationship between the relative proportion of Gemmatimonadetes or Chloroflexi and metal  
509 pollution was shown in lake sediment historically polluted with zinc and lead [69] and in the soil of an  
510 abandoned Pb-Zn mine [70]. The Chloroflexi group is known to have a high stress tolerance ability [71], which  
511 could explain its development in our metal-polluted soils. At the OTU level, a few OTUs were positively  
512 correlated to the metal pollution index, and these OTUs were also positively correlated to the P and N  
513 contents. These OTUs were affiliated to Actinobacteria members, closely related to the AKIW543 clone, the  
514 *Acidimicrobineae* and *Aeromicrobium* genera, and to Firmicutes affiliated to the *Bacillus* genus. Very little  
515 information is available about the two former but few strains belonging to *Aeromicrobium* and *Bacillus* genera  
516 have already been isolated from former mining sites [72] and from heavy-metal contaminated soil [73].

517 The metal pollution index and/or the total and available zinc contents were negatively correlated to the  
518 richness and diversity of the substrates utilised in Biolog plates, suggesting that functional diversity (number of  
519 metabolic functions) was reduced in metal-polluted soils [74]. Similarly, when comparing soils sampled at  
520 different distances from a site historically metal-polluted with As, Cu, and Pb, Boshoff et al. [29] found lower

521 functional diversity in the soils closest to the contamination source. It may result from the replacement of  
522 sensitive species by more resistant ones having different and less versatile metabolic properties [75] maybe  
523 due to an energy cost balance between metal stress survival mechanisms and substrate utilisation efficiency  
524 [76]. Functional diversity may indicate the capacity of the community to adapt its metabolism, relative  
525 composition, and size to various changes in environmental conditions [77]. In our study, the low metabolic  
526 richness of the bacterial community in the metal-polluted soils may have disturbed carbon recycling and soil  
527 functioning.

528 Additionally, using Biolog for tolerance tests, lower zinc sensitivity (or higher tolerance) of the bacterial  
529 community inhabiting high metal-contaminated soils was shown as compared to low-polluted or unpolluted  
530 soils, suggesting an adaptation of communities to metal stress, as previously observed [78]. It is well accepted  
531 that microorganisms coming from highly polluted environments are more tolerant to pollution than those  
532 coming from less polluted environments [21].

533 Among our soil collection, sh-Uc had the highest iron concentration and relatively low concentrations of other  
534 metals, resulting in a middle-high metal pollution index. This soil had similar taxonomic but slightly higher  
535 functional diversity indices as compared to other soils presenting a middle-high metal pollution index. Unlike  
536 other metals such as Cd or Pb, Fe is an essential element for almost all living organisms and is involved in  
537 numerous metabolic processes. Moreover, due to its extremely high total concentration in sh-Uc, iron was  
538 probably mostly present as insoluble oxides and unavailable forms. This finding suggests that although iron was  
539 present in a very high total concentration in sh-Uc, it had a lower effect on microbial diversity than other  
540 metals such as Zn, Cd, or Pb.

541

#### 542 **Impact of PAH on microbial diversity**

543 We highlighted various correlations between PAH pollution and the proportions of some phyla or OTU  
544 abundance, but found no relationship with functional metabolic diversity, suggesting a limited impact of PAH  
545 on microbial community carbon-cycle related functions in our soils. Total and available PAH contents were both  
546 positively correlated to the proportions of Proteobacteria (mostly Gamma-Proteobacteria) and Nitrospirae  
547 phyla, even if in the literature the Nitrospirae phylum seemed to be negatively impacted by PAH content and  
548 bioavailability [79]. Proteobacteria and Gamma-Proteobacteria were previously found in greater proportions in  
549 pyrene-amended soil [80] and can be involved in PAH degradation [81]. The abundance of some OTUs was also  
550 positively correlated to the total PAH pollution level, but was not affected by all other soil parameters,  
551 suggesting an advantage conferred in the presence of PAHs. Most of them were found predominant in PAH-  
552 polluted environments, i.e. bacteria belonging to *Flavobacterium* [82], *Flexibacter* [83], *Sphingomonadaceae*  
553 [84] and Chloroflexi [85] were detected or isolated from PAH-contaminated soils. Firstly, PAH pollution could  
554 have contributed to shape bacterial communities by favouring species able to metabolise PAHs. Secondly, in  
555 our soils, due to ageing, available PAH concentrations were low as compared to total concentrations and did  
556 not negatively affect taxonomic or functional diversity at the community level, indicating no major toxicity  
557 impact of PAHs on bacterial diversity.

558

## 559 **Impact of physico-chemical parameters on microbial diversity**

560 Apart from the soil pollutants, microbial communities can be affected by physico-chemical parameters. It is  
561 well known that the pH is one of the major parameters that shapes microbial community composition [30, 86].  
562 Except one control soil (ctrl-He), our soils were chosen with a similar slightly alkaline pH to avoid a too high  
563 influence of this parameter that could have hidden the structuring impact of other parameters. Thus, the pH  
564 has little impact on taxonomic and functional diversity, although the proportions of few OTUs mostly affiliated  
565 to Verrucomicrobial DA101 members and commonly found as dominant bacteria in soils [87] increased when  
566 the pH decreased.

567 The soil texture, and especially the sand fraction proportion, was the strongest determinant of bacterial  
568 taxonomic diversity in our soils, but no link with metabolic functional diversity was highlighted. The relative  
569 proportion of the soil sandy fraction was indeed positively correlated with taxonomic richness, evenness, and  
570 Shannon diversity indices, and the relative proportion of Alpha-Proteobacteria [88], whereas the relative  
571 proportions of silt and clay negatively influenced the richness and evenness indices. Fine-textured soil can  
572 protect microbes from predation by protozoans, reduce variations in water availability, and maintain higher  
573 microbial abundance [89]. Conversely, coarse-textured soils allow for a better aeration and water circulation  
574 providing various microhabitats and supporting growth of diverse microorganisms adapted to limited nutrient  
575 conditions or able to use a wider range of substrates [88]. Similarly to our results, Chau et al. [90] found a  
576 positive linear relationship between sand content and taxonomic richness, while Kandeler et al. [91] found that  
577 the small-size fraction promoted bacterial diversity. These contradictory results could be related to soil  
578 fractionation techniques [90], as we based textures on particle granulometry, not on mineralogy (i.e. the sandy  
579 fraction could contain sand but also other particles). Finally, the texture may affect pollutant availability, which  
580 could indirectly affect microbial diversity.

581 While the soil total organic carbon content was not the main parameter explaining taxonomic diversity, it was  
582 negatively correlated to the functional diversity indices, calculated from MicroResp and Biolog data, to the  
583 utilisation level of 6 substrates (phthalic, ferulic, succinic, and aconitic acids, Tween 40, and phenylethylamine),  
584 and to the number of polymer, carboxylic acid and miscellaneous substrates utilised in Biolog microtiter plates.  
585 We noticed that in the redundancy analysis, the total organic carbon content co-varied with the metal pollution  
586 index, as previously shown by Valsecchi et al. [92] on a collection of 16 soils. As explained above, metal  
587 pollution may have reduced the functional capacities of the microbial community. The resulting lower microbial  
588 functional diversity and activity may have reduced organic carbon recycling in the soils, resulting in an  
589 accumulation of total organic carbon and organic matter. Similarly, rates of organic matter (i.e. litter or plant  
590 polymer such as cellulose) degradation were lower in metal-contaminated than unpolluted soils [93, 94]. This  
591 finding could be explained by a decrease of enzymatic activities involved in the C cycle in metal-polluted soils  
592 [91]. Moreover, Lucisine et al. [95] also explained that accumulation of organic matter at the surface of  
593 brownfield soil could be partly due to the lack of endogenous metal-sensitive earthworm species limiting  
594 organic matter incorporation in the deeper soil layers.

595 We observed that the total nitrogen content was positively correlated with soil basal respiration, with ribose-,  
596 pyruvate-, and asparagine-induced respiration, and with 16S rRNA gene copy numbers. These relationships  
597 suggest that in some of our soils, nitrogen present in very low concentrations was one of the limiting factors of  
598 microbial growth and activity. N limitation can indeed occur in some cases depending on the quality of the  
599 available carbon substrate [96, 97]. Additionally, the relative proportions of the Actinobacteria phylum and of a  
600 few OTUs affiliated to Actinobacteria and Firmicutes were positively correlated to the total nitrogen content.  
601 As discussed above, these OTUs were also positively correlated to the metal pollution index indicating a  
602 simultaneous influence of various soil parameters on microbial community composition. As explained for total  
603 organic carbon, metal pollution partly inhibits soil enzyme activities and organic matter decomposition [98], so  
604 that the total nitrogen content potentially increases in the most polluted soils. Moreover, several strains of  
605 Bacilli isolated from soils are known as nitrogen-fixing bacteria [99] and could contribute to increase the  
606 nitrogen content. On the contrary, Bacteroidetes phyla and a few OTUs affiliated to Acidobacteria were  
607 negatively correlated with the total nitrogen content. Acidobacteria are often classified as oligotrophs [100],  
608 which are favoured in nutrient-poor environments. Moreover, a negative relationship between the total  
609 nitrogen content and the functional evenness index was mostly explained by a higher mineralising activity in a  
610 few substrates. Nitrogen may have impacted bacterial community composition, favouring the development of  
611 certain species with higher metabolic activity for certain specific substrates.

612

## 613 **Conclusions**

614

615 We studied a collection of 10 soils presenting different anthropisation levels. Based on their physico-chemical  
616 characteristics and on metal and PAH pollution levels, these soils were gathered in 3 groups corresponding to  
617 their use, i.e. either industrial (slag heap and settling pond) or low anthropised (control soil). Although  
618 taxonomic diversity largely varied among soils, the occurrence of a few bacterial phyla and OTUs was  
619 influenced by metal and PAH pollution as well as by the pH, texture, and the total N content. Furthermore,  
620 metal pollution, particularly zinc concentrations, seems to have selected more metal-tolerant communities but  
621 with reduced metabolic functional diversity and activity. Apart from metal pollution, the N content appeared to  
622 be one of the main factors limiting microbial activity in our soils. These reduced activity and metabolic  
623 functional diversity may have contributed to an accumulation of organic matter in the most metal-polluted  
624 soils. Considering all abiotic and biotic soil characteristics, especially metabolic functional diversity, we are now  
625 able to rank our soils based on their impact on microbial communities (i.e. control soils have the lowest effect,  
626 while settling pond soils have the highest). Although it would be very interesting to study a wider range of soil  
627 to draw more generalizable conclusions, the study of 10 soils already allowed us to identify the drivers of  
628 microbial diversity at both the taxonomic and functional level.

629

630

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638 **Figure captions**

639

640 **Table 1 | Physico-chemical characteristics of the ten soils.** *Parameters were measured one time on one mean*  
641 *sample. For soil groups, values are means (n=3 for ctrl and sp soils, and n=4 for sh soil) ± standard error of the*  
642 *mean. Significant results of Kruskal-Wallis tests ( $p < 0.05$ ), testing differences among the 3 soil groups, are*  
643 *indicated by different letters, and no letter appears when non-significant differences were found. For statistical*  
644 *analyses, values lower than the detection limits of the method were replaced by 1, except for available*  
645 *phosphorus for which the value was replaced by 0.05. The metals used for calculating the metal pollution index*  
646 *(Mi) are indicated by a star. Abbreviations of soil names and groups: Di: Dieulouard, Ho: Homécourt, He:*  
647 *Hémilly, MsM: Mont St Martin, Mo: Montiers, RM: Russange-Micheville, NM: Neuves Maisons, Po: Pompey, Te:*  
648 *Terville, Uc: Uckange; ctrl: control, sh: slag heap, sp: settling pond. Abbreviations of soil characteristics: PAH:*  
649 *polycyclic aromatic hydrocarbons, CEC: cation exchange capacity.*

650

651 **Table 2 | Microbial, taxonomic and functional characteristics of the ten soils.** *Values are means (n = 3 or 4) ±*  
652 *standard error of the mean. For soil groups, values are means (n=3 for ctrl and sp soils, and n=4 for sh soil) ±*  
653 *standard error of the mean. Significant results of Kruskal-Wallis tests ( $p < 0.05$ ), testing differences among the 3*  
654 *soil groups, are indicated by different letters, and no letter appears when non-significant differences were*  
655 *found.*

656

657 **Figure 1 | Principal Component Analysis (PCA) and correlation circle based on the physico-chemical,**  
658 **pollution, and texture characteristics of the ten soils.** *On the PCA: Soils (Di: Dieulouard, Ho: Homécourt, He:*  
659 *Hémilly, MsM: Mont St Martin, Mo: Montiers, RM: Russange-Micheville, NM: Neuves Maisons, Po: Pompey, Te:*  
660 *Terville, Uc: Uckange) were grouped in three types according to their industrial history (sp: settling pond; sh:*  
661 *slag heap; ctrl: control). On the correlation circle: N: total nitrogen; C<sub>org</sub>: total organic carbon; P<sub>Olsen</sub>:*  
662 *available phosphorus; Mi: metal pollution index; PAH: sum of 16 regulatory PAHs; K<sub>t</sub>: total potassium; sand*  
663 *and clay: proportions of the sand and clay fractions, respectively.*

664

665 **Figure 2 | Taxonomic composition of the bacterial communities of the ten soil samples.** *Values are means*  
666 *(n = 3), and error-bars represent standard errors of the mean for each soil. The group named Others included*  
667 *OTUs affiliated to the following phyla: BD1-5, Chlamydiae, Chlorobi, Cyanobacteria, Deinococcus-Thermus,*  
668 *Fusobacteria, Spirochaetes, WCHB1-60, Synergistetes, and Candidate divisions BRC1, OD1, OP10, SR1, TG-1,*  
669 *TM6, WS3, and WS6. Statistical differences among phylum proportions in the soil groups are indicated by*  
670 *different letters (Kruskal-Wallis test,  $p < 0.05$ ).*

671

672 **Figure 3 | Cumulated substrate-induced respiration (SIR) in the presence of six carbon sources, and basal soil**  
673 **respiration measured using the MicroResp method.** Values are means ( $n = 4$  for SIR and  $n = 8$  for basal  
674 respiration), and error bars represent standard errors of the mean for each soil. Statistical differences among  
675 the soil groups are indicated by different letters (Kruskal-Wallis test,  $p < 0.05$ ).

676

677 **Figure 4 | Catabolic richness and average well color development (AWCD) measured after 96h of incubation**  
678 **using Biolog microtiter plates.** Values are means ( $n = 3$ ), and error bars represent standard errors of the mean  
679 for each soil. Sixty-two carbon sources were tested: 4 PAHs, 6 aromatic acids, 9 amino acids, 7 polymers, 14  
680 carbohydrates, 17 carboxylic acids, and 5 miscellaneous (for details see Supplemental Table S1). Statistical  
681 differences among the soil groups are indicated by different letters (Kruskal-Wallis test,  $p > 0.05$ ). As none of the  
682 PAH compounds was significantly degraded, they were excluded from the statistical analysis.

683

684 **Figure 5 | Linear regression between the inhibition rate of carbon substrate utilisation measured using**  
685 **Biolog microtiter plates ( $n = 3$ ) with and without zinc addition, and available zinc concentrations in the ten**  
686 **soils.** The blue line represents the linear model of regression ( $y = -0.0006x + 60.98$ ; where  $y$  is the inhibition rate  
687 and  $x$  is the available zinc content of the soil). The dark grey area represents the confidence interval, and the  
688 light grey area represents the prediction interval (95%). Settling pond, slag heap, and control soils are  
689 represented by red, green, and blue dots, respectively.

690

691 **Figure 6 | Redundancy analysis (RDA) of soil bacterial community diversity (A), MicroResp soil catabolic**  
692 **diversity (B), and Biolog catabolic diversity (C), each of them constrained by soil environmental**  
693 **characteristics.**

694 For each RDA, the explanatory variables that best explained the variation of diversity indices (response  
695 variables) were selected by backward selection. Explanatory and response variables are in red and black,  
696 respectively. Abbreviations of explanatory variables: N: total nitrogen; C<sub>org</sub>: total organic carbon; Mi: metal  
697 pollution index; PAH: sum of 16 regulatory PAHs; K<sub>t</sub>: total potassium; sand: proportion of the sandy fraction.  
698 Abbreviations of response variables: S: richness; Chao1: extrapolated richness; H: Shannon's diversity index; J:  
699 Pielou's evenness index; 16S: index for bacterial taxonomic diversity. MR: index for MicroResp soil catabolic  
700 diversity. BLG: index for Biolog catabolic diversity. Abbreviations for soils: Di: Dieulouard, Ho: Homécourt, He:  
701 Hémilly, MsM: Mont St Martin, Mo: Montiers, RM: Russange-Micheville, NM: Neuves Maisons, Po: Pompey, Te:  
702 Terville, Uc: Uckange). Soils were grouped in three types according to their history (sp: settling pond; sh: slag  
703 heap; ctrl: control). The  $p$  values and  $F$  coefficients, for axes and soil properties, are provided in the  
704 supplemental Table S2.

705

706

707 **Supplemental material**

708 **Table S1 | Substrates used in the Biolog and MicroResp experiments.**

709

710 **Table S2 | The p values and F coefficients for axes and soil properties for the redundancy analyses presented**  
711 **on the Figure 6.**

712

713 **Figure S1 | Heatmap based on the similarity matrix obtained from the PLSr analysis (implemented using 5**  
714 **components).** *The heatmap depicts the positive (red) and negative (blue) correlations between OTU*  
715 *abundances (the predictor matrix) and the soil physico-chemical properties (the response variables) investigated*  
716 *in this study. Dendrograms indicate the level of similarity among the clusters present in the heatmap. The*  
717 *hierarchical clustering was based on the Euclidean distance and the complete linkage method.*

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1007 **Table 1.**

		Mo	He	Di	Uc	Te	NM	Ho	Po	MsM	RM	Means (± SE) per group Kruskal-Wallis multiple comparison test (95% confidence)				
Soil group		ctrl	ctrl	ctrl	sh	sh	sh	sh	sp	sp	sp	ctrl	sh	sp		
Total organic carbon (g kg <sup>-1</sup> )		81.5	72.1	24.3	29.9	88.2	75.2	159.0	109.0	119.0	149.0	59.3 ± 17.7	88.1 ± 26.7	125.7 ± 12.0		
Dissolved organic carbon (g kg <sup>-1</sup> )		1.34	1.69	1.18	0.12	0.99	0.23	0.33	0.76	0.66	0.24	1.4 ± 0.2 <sup>a</sup>	0.4 ± 0.2 <sup>b</sup>	0.6 ± 0.2 <sup>b</sup>		
Organic matter (g kg <sup>-1</sup> )		141	125	42	52	153	130	276	189	206	257	102.7 ± 30.7	152.7 ± 46.5	217.3 ± 20.4		
Nitrogen (g kg <sup>-1</sup> )		4.56	2.85	1.74	0.56	4.35	2.35	2.92	7.19	4.18	3.83	3.1 ± 0.8	2.5 ± 0.8	5.1 ± 1.1		
C/N		17.9	25.3	14.0	53.6	20.3	32.0	54.6	15.2	28.5	38.8	19.1 ± 3.3	40.1 ± 8.4	27.5 ± 6.8		
Available phosphorus (g kg <sup>-1</sup> )		0.04	0.02	0.04	< 0.01	0.21	0.08	0.02	0.12	0.21	0.07	0.03 ± 0.01	0.08 ± 0.05	0.13 ± 0.04		
CEC (cmol kg <sup>-1</sup> )		35.0	15.5	9.9	3.6	20.0	17.5	9.8	51.2	16.2	10.8	20.1 ± 7.6	12.7 ± 3.7	26.1 ± 12.7		
Carbonates (g kg <sup>-1</sup> )		5.5	0.8	14.3	8.4	181.8	14.2	114.0	89.6	152.0	219.3	6.9 ± 3.9	79.6 ± 41.8	153.7 ± 37.4		
pH (H <sub>2</sub> O)		7.0	5.4	7.3	8.0	7.7	7.4	7.5	7.8	7.2	7.6	6.6 ± 0.6	7.6 ± 0.1	7.6 ± 0.2		
Organic acids (mg kg <sup>-1</sup> )		222.9	140.0	120.7	43.2	179.0	11.5	238.1	279.3	154.0	114.2	161.2 ± 31.4	118.0 ± 54.1	182.5 ± 49.7		
Carbohydrates (mg kg <sup>-1</sup> )		0.28	0.29	0.10	0.04	0.20	0.02	0.05	0.08	0.05	0.07	0.22 ± 0.06	0.08 ± 0.04	0.07 ± 0.01		
Soil texture (%)		Clay	48.9	25.5	11.5	5.5	10.0	13.3	7.0	17.5	10.9	10.9	28.6 ± 10.9	9.0 ± 1.7	13.1 ± 2.2	
		Silt	46.0	69.0	14.3	17.6	20.7	27.9	12.0	53.3	72.7	74.6	43.1 ± 15.9	19.6 ± 3.3	66.9 ± 6.8	
		Sand	5.1	5.5	74.2	76.9	69.3	58.8	81.0	29.2	16.4	14.5	28.3 ± 23.0	71.5 ± 4.9	20.0 ± 4.6	
Total concentration (hydrofluoric acid extraction)		Major elements (g kg <sup>-1</sup> )	Al	67.2	51.9	45.0	19.9	25.5	39.9	40.0	39.6	21.0	22.0	5.5 ± 0.7 <sup>a</sup>	3.1 ± 0.5 <sup>b</sup>	2.8 ± 0.6 <sup>b</sup>
			Ca	16.2	3.8	11.6	46.7	125.0	99.2	151.0	71.9	88.1	129.0	1.1 ± 0.4	10.5 ± 2.2	9.6 ± 1.7
			K	14.6	18.6	28.6	0.6	5.4	9.2	5.8	5.4	1.5	3.4	2.1 ± 0.4 <sup>a</sup>	0.5 ± 0.2 <sup>b</sup>	0.3 ± 0.1 <sup>b</sup>
			Mg	5.8	4.8	3.2	5.9	23.5	7.4	9.9	11.3	4.3	3.9	0.5 ± 0.1	1.2 ± 0.4	0.6 ± 0.2
			Na	2.52	5.87	8.87	0.40	1.61	2.73	1.49	0.76	0.40	0.28	0.58 ± 0.18	0.16 ± 0.05	0.05 ± 0.01
			Fe *	44.1	27.3	18.0	452.0	108.0	193.0	65.8	93.0	304.0	53.6	3.0 ± 0.8 <sup>b</sup>	20.5 ± 8.7 <sup>a</sup>	15.0 ± 7.8 <sup>a</sup>
		Metallic trace elements (mg kg <sup>-1</sup> )	Zn *	144	248	60.5	74	1 650	2 540	314	29 600	55 400	119 000	151 ± 54 <sup>b</sup>	1 144 ± 580 <sup>b</sup>	68 000 ± 26 565 <sup>a</sup>
			Pb *	44	93	31	23	475	653	303	34 700	14 400	39 500	56 ± 19 <sup>b</sup>	363 ± 134 <sup>b</sup>	29 533 ± 7 692 <sup>a</sup>
			Cd *	0.7	0.3	0.1	0.1	4.5	3.3	1.1	152.0	17.6	22.0	0.4 ± 0.2 <sup>b</sup>	2.2 ± 1.0 <sup>b</sup>	63.9 ± 44.1 <sup>a</sup>
			Cr *	75.1	82.1	18.7	22.5	345.0	1 220.0	177.0	150.0	166.0	61.9	58.6 ± 20.1	441.1 ± 267.8	126.0 ± 32.4
Available concentration (calcium chloride extraction; µg kg <sup>-1</sup> )	Cu *	16.4	12.9	10.2	9.4	146.0	144.0	93.6	148.0	186.0	42.3	13.2 ± 1.8	98.3 ± 32.0	125.4 ± 43.0		
	Ni *	42.5	21.7	13.2	7.3	55.3	113.0	29.9	73.1	246.0	34.3	25.8 ± 8.7	51.4 ± 22.8	117.8 ± 65.1		
	Co *	14.2	19.0	4.8	17.4	20.2	28.8	11.3	10.6	51.4	11.3	12.7 ± 4.2	19.4 ± 3.6	24.4 ± 13.5		
	Ti *	0.77	0.59	1.13	0.09	0.52	0.83	0.26	88.30	4.79	8.63	0.83 ± 0.16 <sup>b</sup>	0.43 ± 0.16 <sup>b</sup>	33.91 ± 27.22 <sup>a</sup>		
	Mo	1.5	0.9	0.5	0.6	4.6	12.3	10.0	7.9	21.0	1.5	1.0 ± 0.3	6.9 ± 2.6	10.1 ± 5.7		
	Mn	932	1 570	285	740	3 480	9 890	2 520	68 100	3 830	771	929 ± 371	4 158 ± 1 993	24 234 ± 21 951		
Metal pollution index		Zn	< 10	38 100	11	< 10	36	128	38	2 310	49 200	64 000	12 704 ± 12 698	51 ± 27	38 503 ± 18 594	
		Pb	< 3	94	< 3	< 3	< 3	< 3	< 3	32	397	1 440	32 ± 31 <sup>b</sup>	1 ± 0 <sup>b</sup>	623 ± 422 <sup>a</sup>	
		Cd	2.3	82.8	1.3	< 1.0	1.6	2.4	1.7	48.7	111.0	25.3	28.8 ± 27.0	1.7 ± 0.3	61.7 ± 25.6	
		Cr	< 10	< 10	< 10	< 10	< 10	20	< 10	29	< 10	< 10	1 ± 0	5.7 ± 4.7	10.5 ± 9.5	
		Cu	41	30	27	24	129	84	237	98	187	29	33 ± 4	118 ± 45	105 ± 46	
Sum of 16 regulatory PAH (n = 3; mg kg <sup>-1</sup> )		Total	3.5	0.1	0.9	0.9	114.7	1 095.9	937.7	21.2	49.6	6.6	1.5 ± 0.6 <sup>b</sup>	537.3 ± 146.2 <sup>a</sup>	25.8 ± 6.4 <sup>a</sup>	
		Available	0.0	0.0	0.0	0.0	6.3	83.0	54.1	0.2	4.6	0.0	0.0 ± 0.0 <sup>c</sup>	35.8 ± 10.7 <sup>a</sup>	1.6 ± 0.8 <sup>b</sup>	

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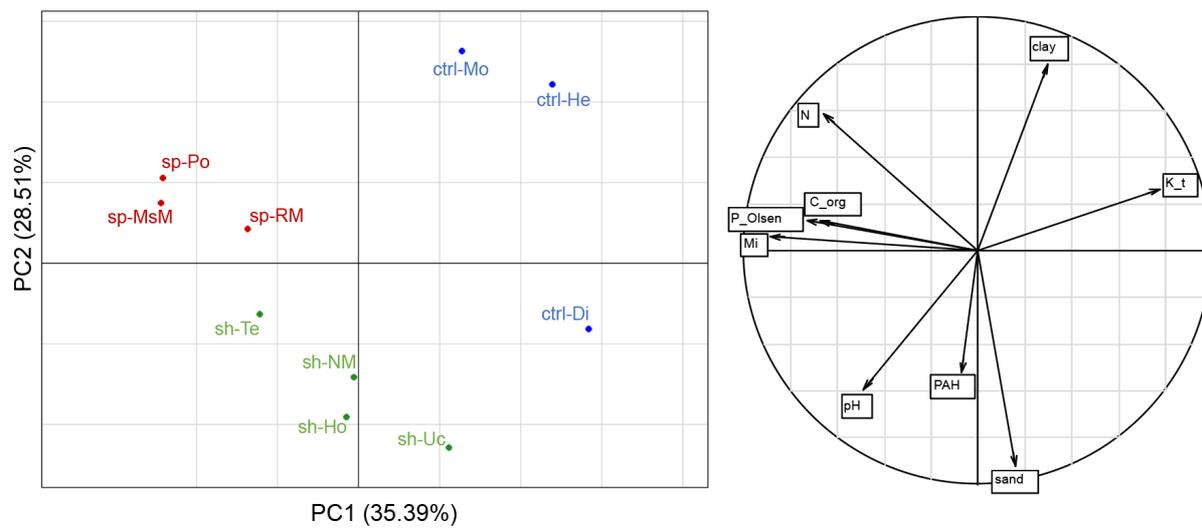
1011 **Table 2.**

	Soil group	Mo	He	Di	Uc	Te	NM	Ho	Po	MmM	RM	Means ( $\pm$ SE) per group Kruskall-Wallis multiple comparison test (95% confidence)		
		ctrl	ctrl	ctrl	sh	sh	sh	sh	sp	sp	sp	ctrl	sh	sp
Microbial characteristics (n = 3)	MPN ( $\times 10^3$ g <sup>-1</sup> )	1 434.5 $\pm$ 327.7	40.2 $\pm$ 8.1	488.6 $\pm$ 126.1	161.2 $\pm$ 53.9	586.1 $\pm$ 170.0	496.9 $\pm$ 214.8	2 071.4 $\pm$ 952.1	80.2 $\pm$ 19.1	38.5 $\pm$ 4.1	0.1 $\pm$ 0.1	654.5 $\pm$ 410.9	828.9 $\pm$ 424.1	39.6 $\pm$ 23.1
	16S rRNA gene copy number ( $\times 10^{10}$ g <sup>-1</sup> )	17.8 $\pm$ 2.4	25.0 $\pm$ 2.2	30.1 $\pm$ 1.1	6.9 $\pm$ 0.5	41.5 $\pm$ 1.2	4.4 $\pm$ 0.3	14.8 $\pm$ 0.7	41.5 $\pm$ 0.4	26.4 $\pm$ 1.2	17.8 $\pm$ 0.4	24.3 $\pm$ 2.1	16.9 $\pm$ 4.5	28.6 $\pm$ 3.5
	18S/16S rRNA gene copy ratio	0.03 $\pm$ 0.00	0.18 $\pm$ 0.01	0.07 $\pm$ 0.00	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01	0.08 $\pm$ 0.00	0.04 $\pm$ 0.00	0.04 $\pm$ 0.00	0.05 $\pm$ 0.00	0.09 <sup>a</sup> $\pm$ 0.02	0.08 <sup>a</sup> $\pm$ 0.00	0.04 <sup>b</sup> $\pm$ 0.00
Bacterial taxonomic diversity (n = 3)	Chao1 index	2 621 $\pm$ 205	3,161 $\pm$ 254	4 966 $\pm$ 32	4 293 $\pm$ 291	4 380 $\pm$ 38	2 541 $\pm$ 100	4 966 $\pm$ 100	3 422 $\pm$ 223	3 151 $\pm$ 2	2 478 $\pm$ 15	3,583 $\pm$ 367	4 045 $\pm$ 282	3 017 $\pm$ 154
	Shannon's index (H')	5.40 $\pm$ 0.03	6.38 $\pm$ 0.05	7.12 $\pm$ 0.02	6.68 $\pm$ 0.03	6.97 $\pm$ 0.01	6.37 $\pm$ 0.01	7.06 $\pm$ 0.04	6.65 $\pm$ 0.02	6.06 $\pm$ 0.02	5.99 $\pm$ 0.02	6.30 <sup>ab</sup> $\pm$ 0.25	6.77 <sup>a</sup> $\pm$ 0.08	6.23 <sup>b</sup> $\pm$ 0.11
	Pielou's evenness index (J')	0.70 $\pm$ 0.01	0.80 $\pm$ 0.01	0.85 $\pm$ 0.01	0.81 $\pm$ 0.00	0.84 $\pm$ 0.00	0.82 $\pm$ 0.00	0.84 $\pm$ 0.00	0.83 $\pm$ 0.00	0.77 $\pm$ 0.00	0.78 $\pm$ 0.00	0.78 <sup>ab</sup> $\pm$ 0.02	0.83 <sup>a</sup> $\pm$ 0.00	0.79 <sup>b</sup> $\pm$ 0.01
MicroResp catabolic diversity (n = 4)	Catabolic richness (n = 6)	5.0 $\pm$ 0.0	5.8 $\pm$ 0.3	5.0 $\pm$ 0.0	5.8 $\pm$ 0.3	4.5 $\pm$ 0.3	5.0 $\pm$ 0.4	4.5 $\pm$ 0.3	5.8 $\pm$ 0.3	5.0 $\pm$ 0.4	4.5 $\pm$ 0.3	5.3 $\pm$ 0.2	4.9 $\pm$ 0.2	5.1 $\pm$ 0.3
	Shannon's index (H')	1.53 $\pm$ 0.03	1.62 $\pm$ 0.06	1.57 $\pm$ 0.01	1.72 $\pm$ 0.05	1.41 $\pm$ 0.07	1.57 $\pm$ 0.08	1.49 $\pm$ 0.07	1.13 $\pm$ 0.02	1.41 $\pm$ 0.09	1.39 $\pm$ 0.07	1.57 <sup>a</sup> $\pm$ 0.03	1.55 <sup>a</sup> $\pm$ 0.05	1.31 <sup>b</sup> $\pm$ 0.06
	Pielou's evenness index (J')	0.95 $\pm$ 0.02	0.93 $\pm$ 0.02	0.97 $\pm$ 0.01	0.98 $\pm$ 0.00	0.94 $\pm$ 0.02	0.98 $\pm$ 0.01	0.99 $\pm$ 0.00	0.65 $\pm$ 0.02	0.88 $\pm$ 0.03	0.93 $\pm$ 0.01	0.95 <sup>b</sup> $\pm$ 0.01	0.97 <sup>a</sup> $\pm$ 0.01	0.82 <sup>c</sup> $\pm$ 0.04
Biolog catabolic diversity (n = 3)	Catabolic richness (n = 62)	30.7 $\pm$ 1.3	17.7 $\pm$ 2.2	21.3 $\pm$ 1.45	25.7 $\pm$ 2.6	22.0 $\pm$ 9.5	12.7 $\pm$ 3.5	15.7 $\pm$ 5.8	15.0 $\pm$ 1.5	12.0 $\pm$ 4.4	5.0 $\pm$ 1.15	23.2 <sup>a</sup> $\pm$ 2.1	19.0 <sup>a</sup> $\pm$ 3.0	10.7 <sup>b</sup> $\pm$ 2.0
	Shannon index (H')	3.27 $\pm$ 0.04	2.77 $\pm$ 0.13	2.97 $\pm$ 0.07	3.15 $\pm$ 0.10	2.78 $\pm$ 0.38	2.35 $\pm$ 0.36	2.43 $\pm$ 0.41	2.47 $\pm$ 0.09	2.22 $\pm$ 0.39	1.52 $\pm$ 0.25	3.00 <sup>a</sup> $\pm$ 0.08	2.68 <sup>a</sup> $\pm$ 0.17	2.07 <sup>b</sup> $\pm$ 0.20
	Pielou's evenness index (J')	0.96 $\pm$ 0.00	0.97 $\pm$ 0.01	0.97 $\pm$ 0.00	0.97 $\pm$ 0.01	0.95 $\pm$ 0.01	0.96 $\pm$ 0.02	0.94 $\pm$ 0.01	0.92 $\pm$ 0.02	0.95 $\pm$ 0.01	0.98 $\pm$ 0.01	0.97 $\pm$ 0.00	0.95 $\pm$ 0.01	0.95 $\pm$ 0.01

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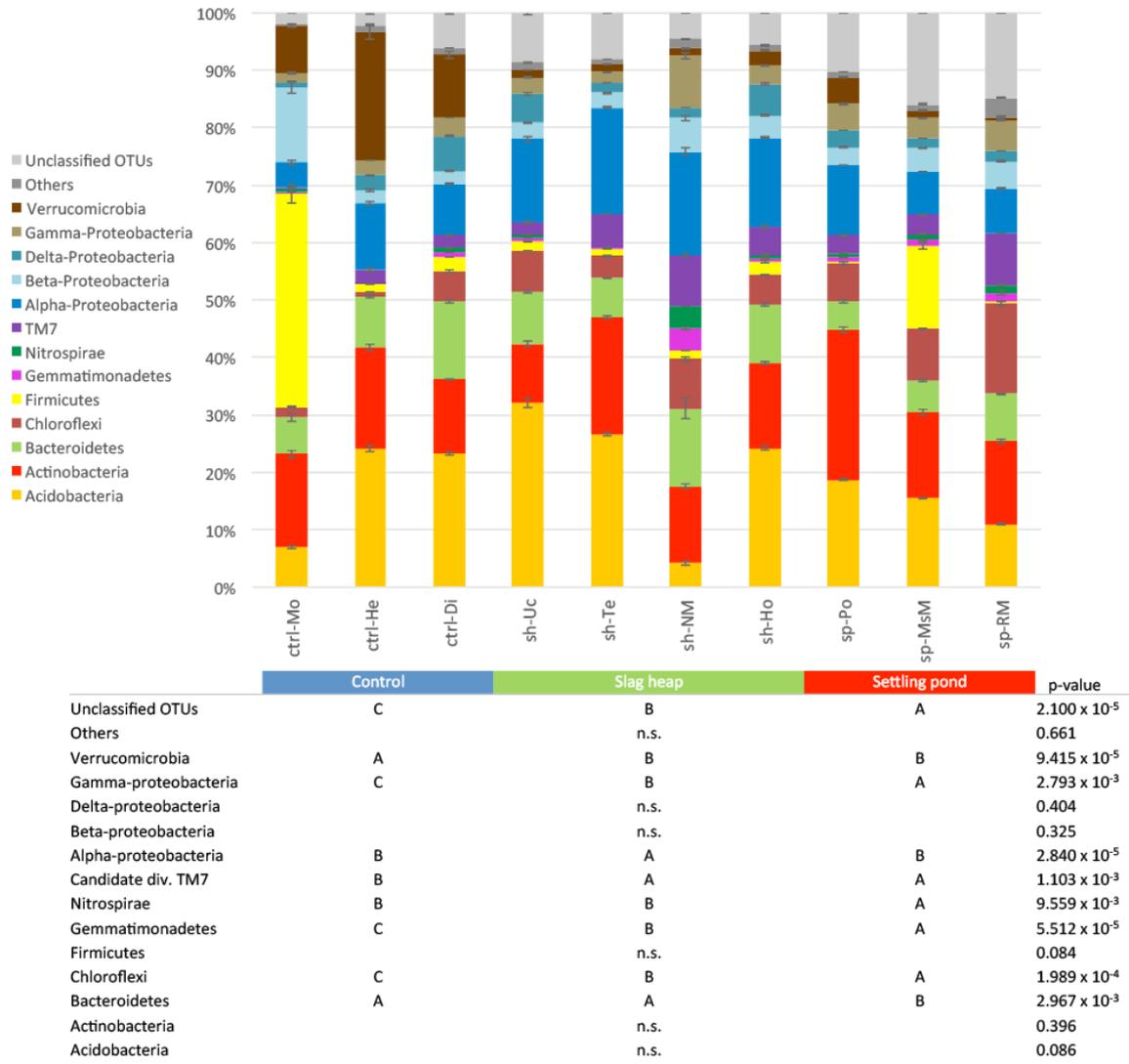
1014 Figure 1.



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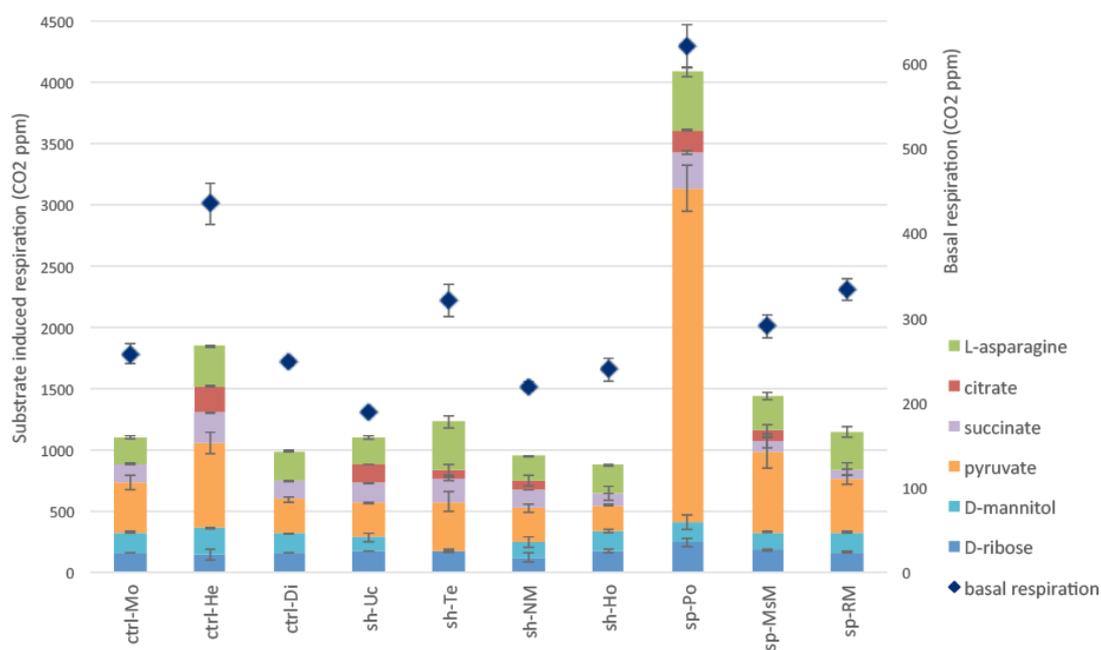
1017 **Figure 2.**



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1020 **Figure 3.**

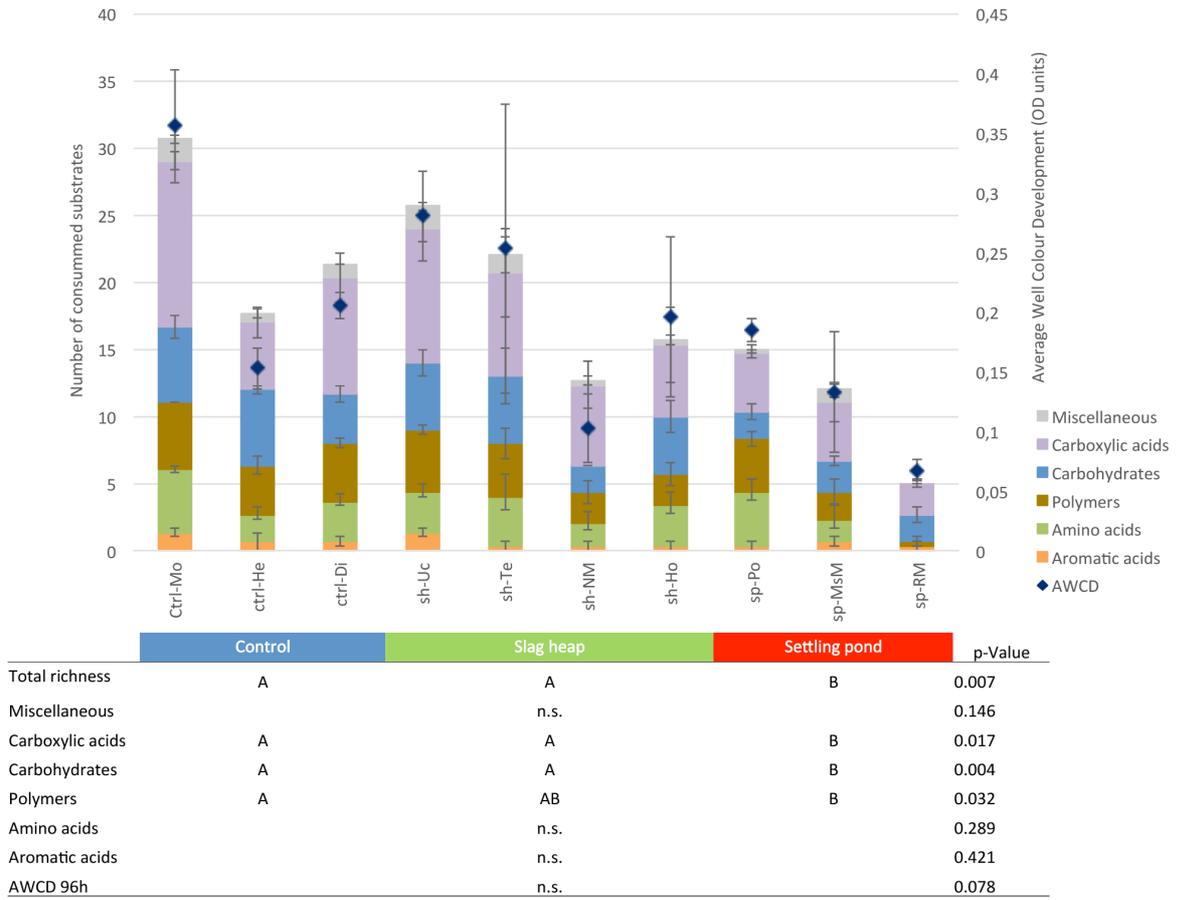


	Control	Slag heap	Settling pond	p-Value
Sum of SIRs	B	B	A	1.983 x 10 <sup>-3</sup>
L-asparagine	AB	B	A	0.037
Citrate		n.s.		0.754
Succinate		n.s.		0.753
Pyruvate	B	C	A	9.542 x 10 <sup>-5</sup>
D-mannitol	A	AB	C	0.021
D-ribose		n.s.		0.163
Basal respiration	B	C	A	1.733 x 10 <sup>-7</sup>

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1023 **Figure 4.**

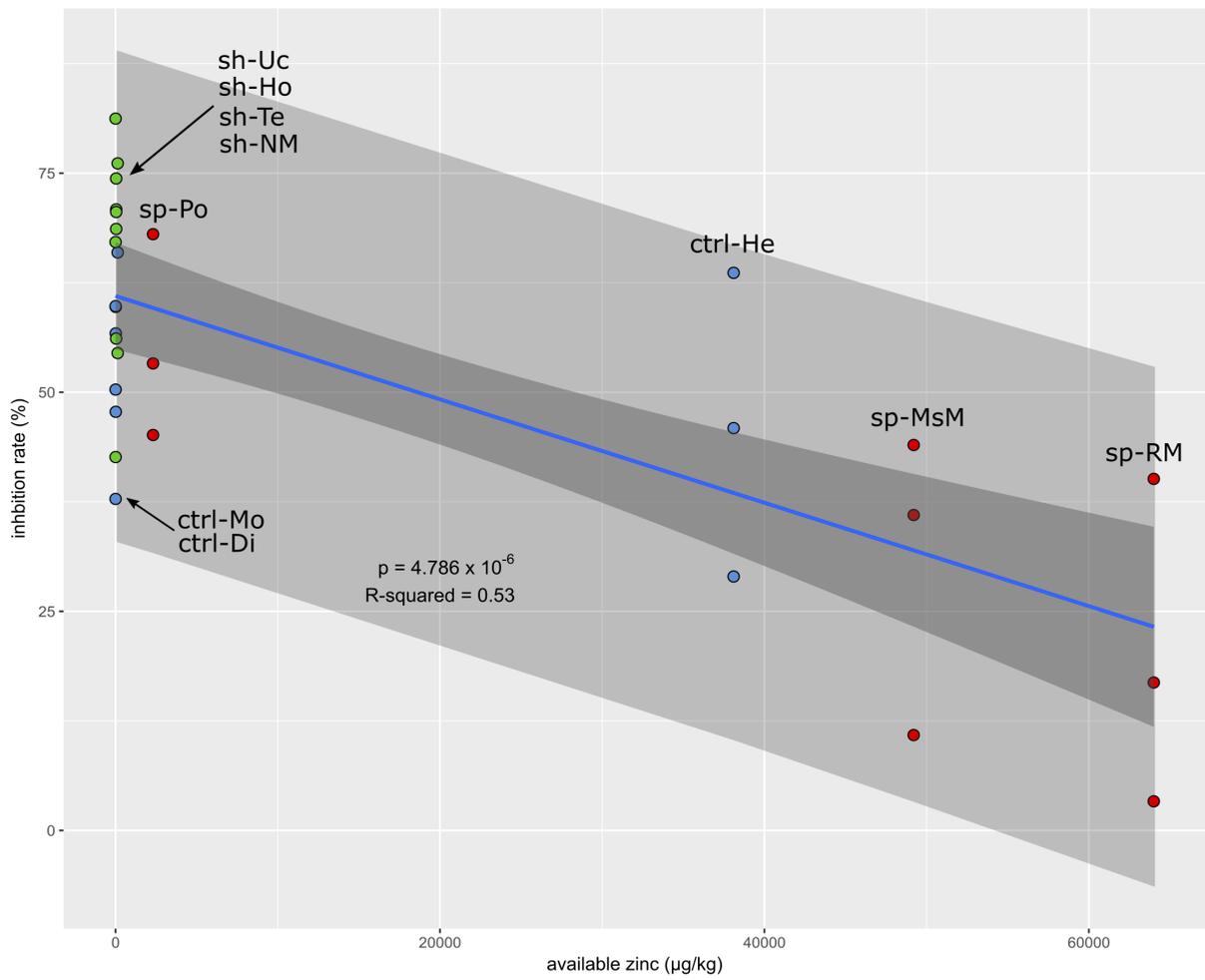


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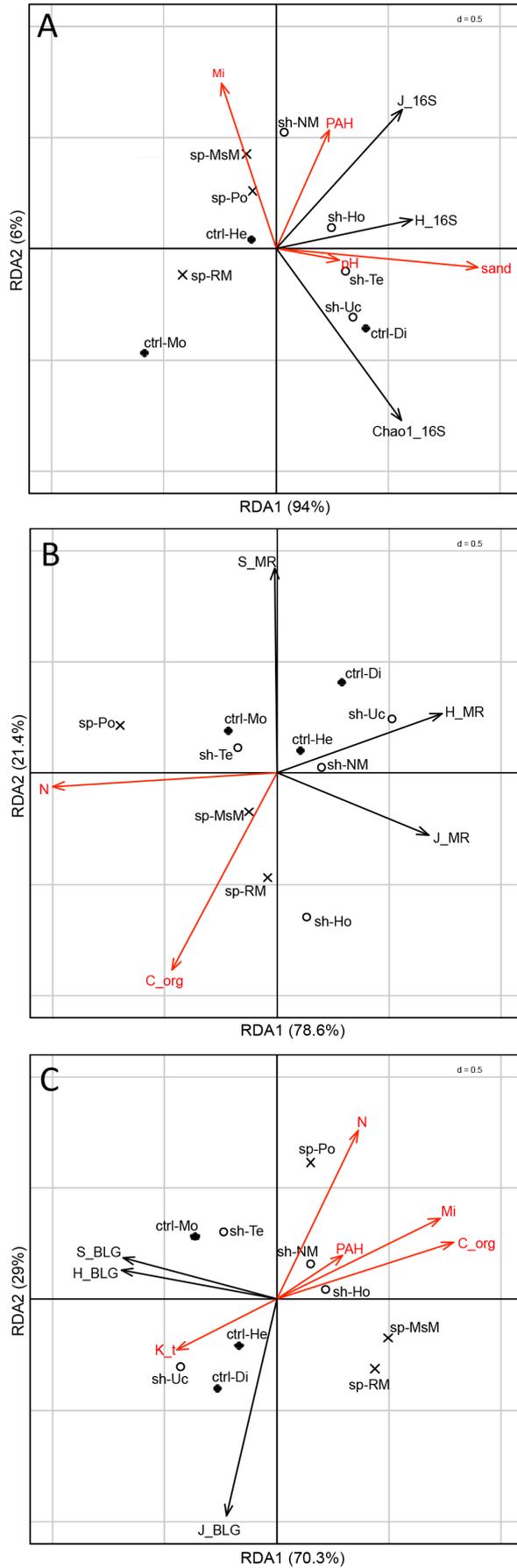
1026 **Figure 5.**

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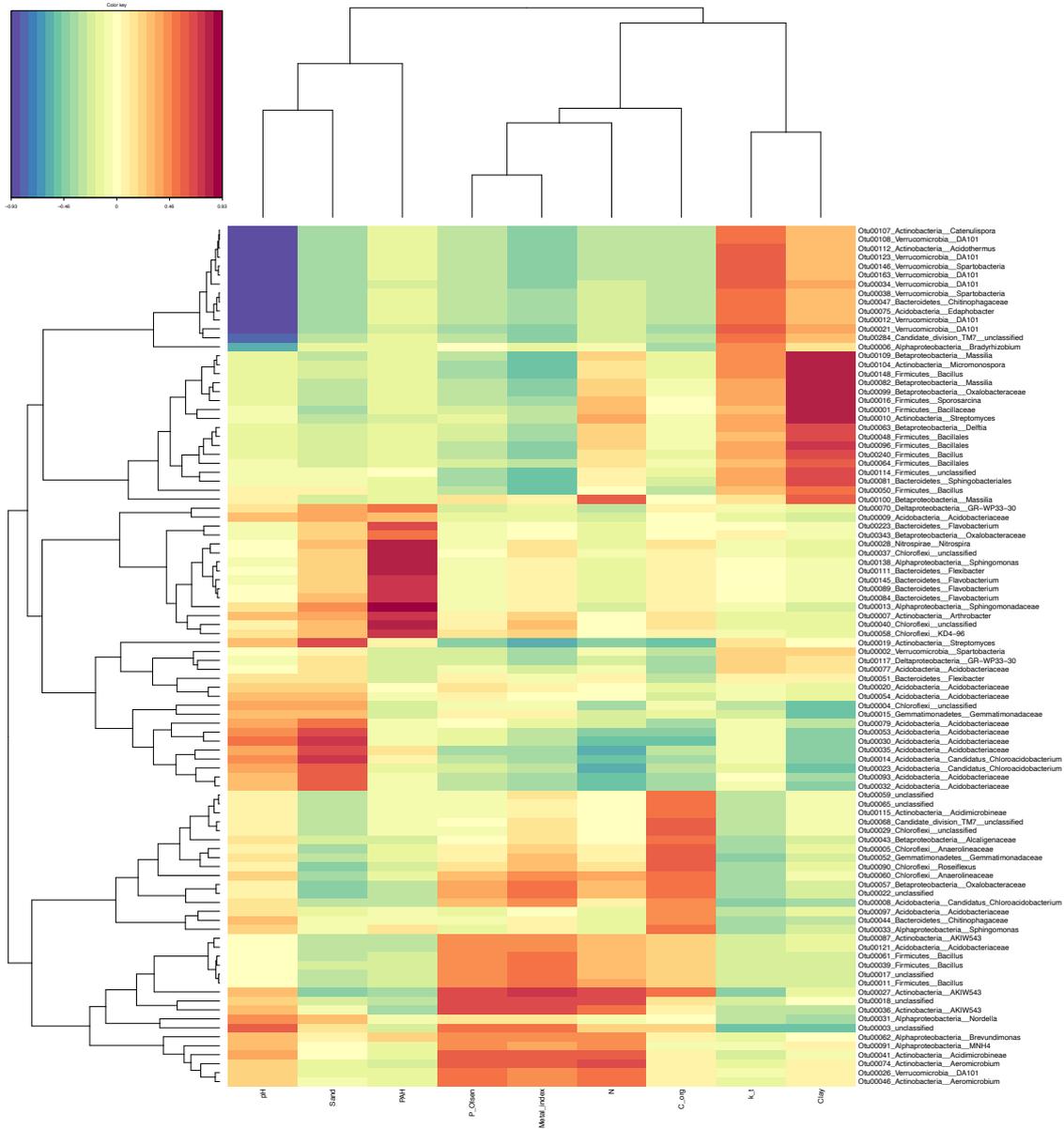




1033 Supplementary material

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1035 Figure S1.



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1038 **Table S1.**

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Chemical guild	Substrate	Chemical formula	Biolog		MicroResp	
			Eco	MT2	Substrate	Time (h)
PAHs	Phenanthrene	C <sub>14</sub> H <sub>10</sub>		✓		
	Pyrene	C <sub>16</sub> H <sub>10</sub>		✓		
	Fluoranthene	C <sub>16</sub> H <sub>10</sub>		✓		
	Fluorene	C <sub>13</sub> H <sub>10</sub>		✓		
Aromatic acids	Benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>		✓		
	Protocatechuic acid	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>		✓		
	Phtalic acid	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>		✓		
	P-Coumaric acid	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>		✓		
	Ferulic acid	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>		✓		
	Syringic acid	C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>		✓		
Carboxylic acids	Pyruvic acid	C <sub>3</sub> H <sub>4</sub> O <sub>3</sub>		✓	✓	4.67
	Propionic acid	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>		✓		
	D-Gluconic acid	C <sub>6</sub> H <sub>12</sub> O <sub>7</sub>		✓		
	Oxalic acid	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>		✓		
	Succinic acid	C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>		✓	✓	6.67
	Citric acid	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>		✓	✓	6.17
	Trans-Aconitic acid	C <sub>6</sub> H <sub>6</sub> O <sub>6</sub>		✓		
	L-Tartaric acid	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>		✓		
	D-Galactonic acid γ-lactone	C <sub>6</sub> H <sub>10</sub> O <sub>6</sub>		✓		
	D-Galacturonic acid	C <sub>6</sub> H <sub>10</sub> O <sub>7</sub>		✓		
	γ-Hydroxybutyric acid	C <sub>4</sub> H <sub>8</sub> O <sub>3</sub>		✓		
	D-Glucosaminic acid	C <sub>6</sub> H <sub>13</sub> NO <sub>6</sub>		✓		
	Itaconic acid	C <sub>5</sub> H <sub>6</sub> O <sub>4</sub>		✓		
	α-Ketobutyric acid	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>		✓		
	D-Malic acid	C <sub>4</sub> H <sub>6</sub> O <sub>5</sub>		✓		
	2-Hydroxy benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>		✓		
	4-Hydroxy benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>		✓		
	Amino acids	L-Proline	C <sub>5</sub> H <sub>9</sub> NO <sub>2</sub>		✓	
Glycine		C <sub>2</sub> H <sub>5</sub> NO <sub>2</sub>		✓		
L-Alanine		C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>		✓		
L-Arginine		C <sub>6</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>		✓		
L-Asparagine		C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub>		✓	✓	5.33
L-Phenylalanine		C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>		✓		
L-Serine		C <sub>3</sub> H <sub>7</sub> NO <sub>3</sub>		✓		
L-Threonine		C <sub>4</sub> H <sub>9</sub> NO <sub>3</sub>		✓		
Glycyl-L-Glutamic Acid		C <sub>7</sub> H <sub>12</sub> N <sub>2</sub> O <sub>5</sub>		✓		
Carbohydrates	D-Ribose	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>		✓	✓	8.00
	D-Glucose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>		✓		
	D-Fructose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>		✓		
	D-Sucrose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>		✓		
	D-Trehalose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>		✓		
	D-Mannose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>		✓		
	β-Methyl-D-glucoside	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>		✓		
	D-Xylose	C <sub>5</sub> H <sub>10</sub> O <sub>5</sub>		✓		
	<i>i</i> -Erythritol	C <sub>4</sub> H <sub>10</sub> O <sub>4</sub>		✓		
	D-Mannitol	C <sub>6</sub> H <sub>14</sub> O <sub>6</sub>		✓	✓	8.17
	N-Acetyl-D-glucosamine	C <sub>8</sub> H <sub>15</sub> NO <sub>6</sub>		✓		
	D-Cellobiose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>		✓		
	α-D-Lactose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>		✓		
	Pyruvic acid methyl ester	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>		✓		
	Polymers	Lignin (alkali)	(C <sub>14</sub> H <sub>20</sub> O <sub>3</sub> S) <sub>n</sub>		✓	
Cellulose (from Spruce)		(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ) <sub>n</sub>		✓		
Xylan		(C <sub>5</sub> H <sub>8</sub> O <sub>5</sub> ) <sub>n</sub>		✓		
Glycogen		(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ) <sub>n</sub>		✓		
α-Cyclodextrine		C <sub>36</sub> H <sub>60</sub> O <sub>30</sub>		✓		
Tween 40				✓		
Miscellaneous	Tween 80		✓			
	Glucose-1-phosphate	C <sub>6</sub> H <sub>13</sub> O <sub>9</sub> P		✓		
	D,L-α-Glycerol phosphate	C <sub>3</sub> H <sub>9</sub> O <sub>6</sub> P		✓		
	Phenylethyl-amine	C <sub>8</sub> H <sub>11</sub> N		✓		
	Putrescine	C <sub>4</sub> H <sub>12</sub> N <sub>2</sub>		✓		
	Catechol	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>			✓	

classement d'après Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles - a critique (Preston-Mafham et al. 2002) avec changement de groupes (\*) ou ajout (#)

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1043 Table S2.

		F-value	P-value		
RDA-A	Axis 1	26.338	0.001 ***	p < 0.05	*
	Axis 2	1.683	0.216	p < 0.01	**
	Ti_metals	2.072	0.198	p < 0.001	***
	PAH	1.729	0.238		
	sand	16.304	0.002 **		
	pH	3.251	0.108		
RDA-B	Axis 1	10.353	0.002 **		
	Axis 2	2.815	0.111		
	C_org	4.458	0.009 **		
	N	8.709	0.002 **		
RDA-C	Axis 1	19.903	0.002 **		
	Axis 2	8.222	0.012 *		
	Ti_metals	7.767	0.010 **		
	PAH	1.047	0.412		
	C_org	2.963	0.110		
	N	4.708	0.032 *		
	K_t	2.396	0.139		

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