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# 1 **Microcosm-scale biogeochemical stabilization of Pb, As, Ba and Zn in mine** 2 **tailings amended with manure and ochre**

3

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12

## 13 **Abstract:**

14 Mine tailings are major sources of metals and metalloids in the environment, making the  
15 physical and geochemical stabilization of tailings a serious environmental challenge. With a  
16 view to facilitate the development of covering vegetation and of decreasing the mobility of Pb  
17 in the acid tailings of a former Ag-Pb mine, laboratory microcosm experiments were  
18 performed to enable comparison of the effectiveness of several treatments. Tailings were  
19 mixed with 5 % by weight of ochre, an iron-rich material produced during the treatment of a  
20 coal mine water, and with cow manure (0, 0.15, 1 and 2 % by weight), either solely or in  
21 combination. They were then submitted to weekly watering over 84 days. All treatments  
22 raised the pH values from 4 to values between 7 and 8 and induced a strong decrease in the  
23 total dissolved Pb concentration in the percolating water (from 13–15 mg.L<sup>-1</sup> to less than 0.5  
24 mg.L<sup>-1</sup>). Several processes seemed to be involved in the immobilization of Pb by the  
25 amendments: precipitation as hydroxide, sulfate, carbonate and phosphate, and adsorption on  
26 iron hydroxides. A transient increase was observed in both Pb mobility and functional  
27 microbial diversity with 1% and 2% manure, with a peak after 28 days of incubation. This  
28 peak corresponded to an Average Well Color Development (AWCD) in Biolog<sup>TM</sup> Ecoplates  
29 increase from 0.5 to 0.8 with 1% manure and from 0.6 to 1.5 with 2% manure. However, at  
30 the end of experiment, Pb immobilization was strengthened by 2% manure and microbial

31 functional biodiversity fell back, with AWCD values of 0.5 and 0.8 for 1 % and 2% manure,  
32 respectively. Other toxic elements present in the tailings, namely As, Zn and Ba, were not  
33 strongly mobilized by the treatments, although cow manure slightly increased the leaching of  
34 Ba and As, which maximum concentrations in the leaching water reached  $65 \mu\text{g.L}^{-1}$  Ba and  $9$   
35  $\mu\text{g.L}^{-1}$  As. All amendments improved the growth of ryegrass, which maximum dry biomass  
36 ranged from 38 mg/microcosm without amendment to 155 mg/microcosm with 0.15%  
37 manure. The results provide key information about the biogeochemical processes driving the  
38 mobility of Pb, As, Zn and Ba in acid mine tailings during the first 84 days following their  
39 amendment with iron-rich ochre and manure.

40 **Key words:** mine tailings, metals, arsenic, amendments, iron oxide-hydroxide, cow manure,  
41 microbial processes

42

## 43 **Introduction**

44 Mining activities have generated massive amounts of solid wastes throughout the world and  
45 every year the sector continues to mobilize some  $50 \times 10^9$  tons of rocks. Huge amounts of  
46 solid waste, known as tailings, are produced when the valuable fractions of these materials are  
47 processed to extract the desired mining resources (Douglas and Lawson, 2000). For example,  
48 the extraction of metals such as Cu, Ni or Au can produce up to 1,000 tons of tailings for one  
49 kilogram of pure element. These wastes are usually stored behind tailings dams close to the  
50 extraction sites, often with inadequate management, especially on older mining sites. Tailings  
51 may contain large fractions of sulfide materials that are subject to oxidation when exposed to  
52 air and water. Their leaching by rainwater often induces the mobilization of metals and  
53 metalloids towards the surrounding environmental compartments, including groundwater,  
54 surface water, soils and sediments (Fuge et al., 1993; Paulson, 1997). Moreover, runoff water  
55 can carry downstream metal-rich solid particles that may become soluble if the water  
56 characteristics or redox conditions change.

57 Stabilization of mine tailings is facilitated by amendments that adsorb, complex or (co)-  
58 precipitate inorganic pollutants (Kumpiene et al., 2008) and metals and metalloids can be  
59 rendered less mobile by amendment of tailings with organic or inorganic materials. In general,  
60 amendments not only decrease the leachability of pollutants but also stimulate plant growth,  
61 and the resulting covering vegetation contributes to stabilizing tailing particles. Amendments  
62 have the additional advantage of being inexpensive and readily available in large quantities,  
63 since they derive from agricultural or industrial by-products (Guo et al., 2006). Chemical and  
64 inorganic amendments that have been used widely include alkaline and phosphate materials  
65 (Hooda and Alloway, 1996; Derome, 2000; Le Forestier et al., 2017). Alkaline materials have  
66 been shown to reduce the solubility of divalent metals such as Pb and Cu by increasing pH  
67 and phosphate materials to efficiently stabilize Pb by ionic exchange and precipitation of  
68 pyromorphite-type minerals  $[\text{Pb}_5(\text{PO}_4)_3\text{X}]$ ;  $\text{X} = \text{F}, \text{Cl}, \text{B}$  or  $\text{OH}$ ] (Mc Gowen et al., 2001;  
69 Kumpiene et al., 2008). Organic amendments improve soil agronomic properties and can  
70 contribute to reduced mobility of metals by pH buffering (Zeng et al., 2011). A number of  
71 organic materials have been tested as amendments for polluted soils: compost, municipal  
72 biosolids, peat, chipped wood, composted sewage sludge, manure (Basta and Sloan, 1999; Li  
73 et al., 2000; Brown et al., 2003; Hattab et al., 2015), and biochars (Oustrière et al., 2016;  
74 Lebrun et al., 2016; Lahori et al., 2017; Norini et al., 2019). However, while alcalinization  
75 reduces the mobility of divalent metals, it may also induce the mobilization of As, a toxic

76 metalloid frequently present in mine tailings. As pH increases, the efficiency of the adsorption  
77 of arsenate (As(V)) by iron oxides is reduced (Dixit and Hering, 2003). The difference in  
78 chemical behavior between metal cations and oxi-anions such as As(V) must therefore be  
79 taken into account in the initial choice of amendments to stabilize mine tailings. An  
80 alternative is amorphous iron hydroxide (ferrihydrite), which can be an effective sorbent for  
81 both cation and anion pollutants (Kumpiene et al., 2008). In fact, Martin and Ruby (2003)  
82 have observed efficient stabilization of both Pb and As in a smelter-contaminated soil by 5 %  
83 ferrihydrite together with 0.5 % of calcium phosphate. Iron oxides are produced as waste  
84 materials, sometimes in huge amounts, during the treatment of mine waters such as those  
85 from coal mines. Doi et al. (2005) have shown that iron-oxide rich sludge materials from  
86 mining sites, containing 24 % to 62 % of total iron, could be effective in stabilizing As in  
87 polluted soils. Treatments not only decreased soil As leachability but also reduced the uptake  
88 of As by radishes planted in amended soils. Nielsen et al. (2011) showed a significant, long-  
89 term decrease of As concentration in the pore water of a soil polluted by As and Cr and  
90 amended with 5 % of an iron-rich water-treatment residue, mainly composed of ferrihydrite.  
91 More recently, Olimah et al. (2015) tested four different iron-rich wastes produced in mine  
92 water treatment plants for the remediation of As-polluted soils. Their results suggest that this  
93 treatment is efficient in decreasing As mobility in water. However, the benefits in terms of  
94 uptake of As by plants and plant growth were not significant. The prime focus of these  
95 previous studies was As as the main target pollutant of the remediation strategy. In addition,  
96 chemical data alone do not take account of the effects of pollutants on the soil's habitat  
97 function. The microbiological status of a soil, considered as an indicator of its potential to  
98 sustain microbiological activity, can be used to assess the effectiveness of chemical  
99 stabilization of metals and metalloids by amendment (Pérez de Mora et al., 2006).

100 The work reported here investigated the potential for and mechanisms of biogeochemical  
101 stabilization of mine tailings of which the principal pollutant was Pb, associated mainly with  
102 As, Zn, and Ba. The tailings were amended with iron oxides from a coal mine water treatment  
103 plant, combined or not with different doses of cow (*Bos taurus*) manure to improve the  
104 agronomic properties of the material. Laboratory microcosm leaching experiments simulating  
105 percolation of rainwater were designed to evaluate the efficiency of stabilization of the main  
106 pollutants in relation to the biogeochemical evolution of the amended tailings.

107

## 108 **Material and methods**

### 109 **Characteristics of the mine tailings**

110 Tailings samples were taken from the site of a former Ag and Pb mine at Pontgibaud (Puy de  
111 Dôme, France; 45°47'29"N 2°49'18"E) operating since antiquity but with a peak of activity  
112 in the second half of the XIX<sup>th</sup> century (Cottard, 2010). The mine is located in a medium-  
113 altitude mountainous area (altitude 750 m). Mine residues (3 m<sup>3</sup>) were recovered over a depth  
114 of 0–60 cm from a mine waste dump, using a power shovel. Residues were taken from seven  
115 discrete zones within a 25 m<sup>2</sup> area. The material was coarsely mixed with the shovel and  
116 transported to the laboratory for the stabilization experiments.

117 The details on all characterization and analytical methods (detection limits, analytical quality  
118 controls) are given in the chemical analysis section below and in the SM1. The grain size  
119 distribution of the tailings was determined by wet processing sieving. Only 7 % (by weight)  
120 of the material was less than 60 µm, and that 76 % was in the 315 µm to 2 mm range. The  
121 > 2 mm fraction represented 2 %, and the remaining fraction (15 %) was in the 60–315 µm  
122 range. The pH of the tailings – consisting mainly of quartz, orthoclase and phyllosilicate –  
123 was 4.9 (measured in water according to ISO 10390). Pb bearing phases were detected by X-  
124 ray diffraction (XRD): anglesite (PbSO<sub>4</sub>) and beudantite ((PbFe<sub>3</sub>(AsO<sub>4</sub>)(SO<sub>4</sub>)(OH)<sub>6</sub>); a solid  
125 solution between plumbojarosite, pure SO<sub>4</sub> pole, and segnitite, pure AsO<sub>4</sub> pole). Anglesite  
126 and beudantite had already been detected in tailings from the same mine district (Pascaud et  
127 al., 2014). The concentrations of the main constituents were: 26,432 mg.kg<sup>-1</sup> Pb, 265 mg.kg<sup>-1</sup>  
128 Zn, 1,063 mg.kg<sup>-1</sup> Ba, 1,134 mg.kg<sup>-1</sup> As, 820 mg kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 1.5 % Fe<sub>2</sub>O<sub>3</sub>, 81 % SiO<sub>2</sub> and 1.54  
129 wt% SO<sub>4</sub>. CaO and MgO concentrations were less than 1 %. Characteristics of organic matter  
130 are given in Table 1. Standard leaching testing in demineralized water (ISO/TS 21268-2)  
131 resulted in 19.6 mg.L<sup>-1</sup> Pb, 2.3.µg.L<sup>-1</sup> As, and 0.17 mg.L<sup>-1</sup> Zn (Fe and Ba concentrations, were  
132 below the detection limits, respectively 0.1 mg.L<sup>-1</sup> and 10 µg.L<sup>-1</sup>).

### 133 **Amendments**

134 The ochre – iron oxide-hydroxide, mainly composed of goethite – was produced in a coal  
135 mine water treatment plant in France, by cascade aeration and sedimentation of the iron  
136 precipitates generated in a settling pond. Samples of concentrated sludge taken from the  
137 settling pond were dried gently at 30 °C and then ground in a mortar and sieved at 100 µm.  
138 The initial water content was 92 %. Size particle distribution before drying was analyzed with  
139 a laser granulometer (Malvern); it showed 86.5 % of particles smaller than 10 µm, 98 %

140 smaller than 89  $\mu\text{m}$  and 100% smaller than 200  $\mu\text{m}$ . The particle fraction most present was  
141 the 2–6  $\mu\text{m}$  group. The pH in water (measured according to ISO 10390) of the dried ochre  
142 was 8.1. The concentrations of the main constituents were: 1.99 % C, 1.81 % H, 0.69 % S  
143 0.66 % TOC, 1.39 % MinC, 261,232  $\text{mg.kg}^{-1}$  Fe, 10.1  $\text{mg.kg}^{-1}$  Pb, 505.7  $\text{mg.kg}^{-1}$  As, 14,717  
144  $\text{mg.kg}^{-1}$  Zn and 69.8  $\text{mg.kg}^{-1}$  Ba. N was not detected. Standard leaching testing in  
145 demineralized water (ISO/TS 21268-2) resulted in 0.4  $\text{mg.L}^{-1}$  Fe, 0.23  $\text{mg.L}^{-1}$  Pb, 2.5  $\mu\text{g.L}^{-1}$   
146 As, 44.1  $\text{mg.L}^{-1}$  dissolved organic carbon (DOC) and 4.4  $\text{mg.L}^{-1}$  dissolved organic nitrogen  
147 (DON) in the leachate. Zn was below the quantification limit, i.e. 0.01  $\text{mg.L}^{-1}$ .

148 Cow manure was collected from a farm near Orléans (France); its pH in water (measured  
149 according to ISO 10390) was 9.9. The manure was dried gently at 30°C, ground in a mortar  
150 and sieved at 2 mm. The concentrations of the main constituents were: 35.40 % C, 4.51 % H,  
151 2.48 % N, 0.18 % S, 28.95 % TOC, 2.17% MinC, 2.82  $\text{mg.kg}^{-1}$  Pb, 0.75  $\text{mg.kg}^{-1}$  As,  
152 53.8  $\text{mg.kg}^{-1}$  Zn and 78.2  $\text{mg.kg}^{-1}$  Ba. Standard leaching testing (ISO/TS 21268-2) gave  
153 0.33  $\text{mg.L}^{-1}$  Pb, 7  $\mu\text{g.L}^{-1}$  As, 0.33  $\text{mg.L}^{-1}$  Zn, 0.27  $\text{mg.L}^{-1}$  Ba, 1,130  $\text{mg.L}^{-1}$  DOC and  
154 131  $\text{mg.L}^{-1}$  DON in the leachate.

#### 155 **Microcosm leaching experiments**

156 Microcosms were prepared in 200 mL polystyrene pots (50 mm diameter) of which the  
157 bottoms were perforated with a 0.9 mm needle, 13 holes for each pot. To retain soil particles  
158 in the pots, a fine layer of glass wool was laid at the bottom and covered with 10  $\text{cm}^3$  of clean  
159 Fontainebleau sand. Both glass wool and sand were cleaned prior to use in 10 %  $\text{HNO}_3$ ,  
160 rinsed with demineralized water and dried before use.

161 The pots were watered to simulate rainwater, all watering being performed with Mont  
162 Roucous mineral water (pH 5.85, 3.1  $\text{mg.L}^{-1}$   $\text{Na}^+$ , 2.4  $\text{mg.L}^{-1}$   $\text{Ca}^{2+}$ , 0.5  $\text{mg.L}^{-1}$   $\text{Mg}^{2+}$ , 2.0  $\text{mg.L}^{-1}$   
163  $\text{SO}_4^{2-}$ , 6.3  $\text{mg.L}^{-1}$   $\text{HCO}_3^-$ , 3.0  $\text{mg.L}^{-1}$   $\text{NO}_3^-$ ).

164 Each microcosm contained 150 g of air-dried material sieved at 2 mm. The different  
165 amendment conditions tested were as follows: tailings without amendment (T); tailings +  
166 5 wt% ochre (TO); tailings +5 wt% ochre + 0.15 wt% manure (TOM 0.15%); tailings +5 wt%  
167 ochre + 1 wt% manure (TOM 1%); tailings +5 wt% ochre + 2 wt% manure (TOM 2%). These  
168 proportions were chosen based on the results of preliminary tests in slurries, that were  
169 performed to determine the minimum amendments concentrations allowing immobilization of  
170 both Pb and As (data not shown). The material for each condition was prepared by mixing the



171 dry amendments with the tailings in a rotating agitator for 2 hours. Nine microcosms of each  
172 condition were then prepared.

173 For the first watering, 30 mL of water were sprinkled carefully onto the soil surface. Then,  
174 72 h later, the microcosms were watered again with 25 mL of water (solid/liquid ratio of 6).  
175 The quantity of water added and draining out was recorded by weighing. Global aerobic non-  
176 saturated conditions were maintained. Microcosms were incubated in a Plant Growth  
177 Chamber (MEMMERT HPP750 IPP PLUS) at 25°C, in the dark, with 80 % of air-water  
178 saturation. The soils were never dried during incubation.

179 Watering with 25 mL (solid/liquid ratio of 6) water was then performed once a week. The  
180 percolated water was filtered at 0.45 µm. A sample was acidified with a drop of concentrated  
181 HNO<sub>3</sub> for total As and Pb analyses. The remaining filtered solution was stored in a  
182 refrigerator for complementary analyses.

183 Three microcosms of each of the amendment test conditions were sacrificed for analysis after  
184 incubation periods of 7, 28, and 84 days. When the microcosms were sacrificed, their contents  
185 were carefully mixed in sterile bags with a spatula and samples were taken to determine the  
186 moisture and for biological and molecular analyses (0.5 g of soil in sterile tubes stored at -20  
187 °C). The remaining soil was stored at 5°C. Each time microcosms were sacrificed, As, Ba, Pb  
188 and Zn were analyzed in the percolated water.

### 189 **Chemical analyses**

190 The details on all analytical methods (detection limits, analytical quality controls) are given in  
191 SM1.

192 Metals and metalloids in the tailings were determined by ICP/AES (Ameteck Spectro  
193 apparatus, Arcos model) after dissolution according to NF X31-147. Sulfate concentration in  
194 the tailings was determined as per NF ISO 11048. Metals and metalloids in the ochre were  
195 determined by ICP-MS (Agilent 8900-Triple Quad apparatus) after complete dissolution by  
196 hydrofluoric acid attack (HNO<sub>3</sub> 65 %, HCl 37 % and HF 40 %) (US-EPA 3052 method  
197 modified). Metals and metalloids in the manure were determined by ICP-MS after complete  
198 mineralization (heating at 600 °C for 3 h, followed by mineralization in 65 % HNO<sub>3</sub> and 37 %  
199 HCl) (US-EPA 3015A method).

200 Different chemical analyses were carried out on the solutions from leaching tests and on the  
201 filtered percolated water (0.45 µm) from microcosms. As, Pb and Ba were analyzed by oven

202 AAS (Varian AA220Z), and Zn by flame AAS (Varian AA22FS). pHs of percolated water  
203 were measured. Ion chromatography (IC), using a 940 Professional IC Vario instrument  
204 (Metrohm) equipped with conductivity detectors, was used to quantify major ions ( $\text{Li}^+$ ,  $\text{Na}^+$ ,  
205  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{2-}$ ). Anions were separated with a  
206 Metrosep A Supp 16 ionic resin column (150 mm  $\times$  4 mm) and cations with a Metrosep C6  
207 (150 mm  $\times$  4 mm). Only certain ions were detected:  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  
208  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ . DOC and DON concentrations were determined using a TOC 5050/SSM 5000-A  
209 elemental analyzer (Shimadzu).

210 Measurements of total carbon (C), nitrogen (N), hydrogen (H) and sulfur (S) were obtained  
211 using an elemental flash pyrolyser analyzer (Flash 2000, Thermo Fischer Scientific). Total  
212 organic carbon (TOC) and mineral carbon (MinC) were determined by Rock-Eval pyrolysis  
213 (Rock-Eval 6 Turbo, Vinci Technologies). The moisture content of the samples was evaluated  
214 according to NF ISO 11465.

### 215 **Biological analyses**

216 The functional diversity of soil microbial communities was assessed using Biolog™  
217 Ecoplates community-level substrate utilization assay. This method enables study of the  
218 metabolic capability of soil microbial communities to utilize a variety of individual carbon  
219 sources, as well as of the communities' physiological properties (Insam and Goberna, 2004).  
220 Each Biolog™ Ecoplates contained three replicate wells of 31 different carbon sources,  
221 including carbohydrates, carboxylic acids, amino acids, amines, polymers, phenolic acids, and  
222 a control. Soil samples were suspended in a (1:4 ratio) sterile saline solution (0.85 % w/v  
223 NaCl), agitated (60 rpm) for 30 min at 25 °C, sonicated at 45 kHz twice for 20 s, and agitated  
224 (60 rpm) again at 25 °C overnight. The soil suspensions were then centrifuged at 3,000 x g for  
225 10 min.

226 Triplicates of each treatment were pooled taking 200  $\mu\text{L}$  of each triplicate, and resuspended in  
227 17.4 mL of the sterile saline solution (0.85 % w/v NaCl). Each Biolog™ Ecoplates well was  
228 filled with 150  $\mu\text{L}$  of the suspension. The Biolog™ Ecoplates were incubated at 25 °C and  
229 color development in each well was recorded as optical density (OD) at 590 nm, according to  
230 the protocols described by Garland (1997). The absorbance values of the samples were  
231 monitored using an Omega SPECTROstar (BMG Labtech) microplate spectrophotometer at  
232  $t=0$  and 168 h (7 days). The well absorbance values were adjusted by subtracting the average  
233 absorbance of the control well (water only) from the absorbance measured at  $t=0$ . A well was

234 considered as positive when  $OD_{590nm}$  in well –  $OD_{590nm}$  in the control well  $> 0.25$ .  
235 Negative readings ( $OD < 0$ ) were set to zero for all subsequent analyses. The potential  
236 metabolic diversity from the Biolog™ Ecoplates was evaluated by functional richness  $S'$ ,  
237 expressed as the number of substrates used on each plate (i.e. number of positive well  
238 numbers); microbial activity was expressed according to the Garland and Mills method (1991)  
239 as Average Well Color Development (AWCD), as follows:  $AWCD = \sum ODi/31$ , where  $ODi$  is  
240 the optical density value for each well. The Shannon-Weaver index ( $H'$ ) was calculated as  
241 follows:  $H' = -\sum Pi \times \ln Pi$ , where  $Pi$  is the ratio of activity for a particular carbon source  
242 ( $ODi=OD_{590nm}$  in each well- $OD_{590nm}$  in the control well) to the sum of activities on all  
243 substrates ( $\sum ODi$ ).

244 As-transforming microorganisms were enumerated by the Most Probable Number (MPN)  
245 method, detailed in Thouin et al. (2016) for As(III)-oxidizing and in Thouin et al. (2018) for  
246 As (V)-reducing bacteria, modified as follows: the weight of soil dispersed in 10 mL of sterile  
247 saline solution was 2.5 g instead of 0.25 g.

248 Total soil DNA was extracted in triplicate from 0.5 g of wet weight of each soil sample, using  
249 the FastDNA® SPIN Kit for soil (MP Biomedicals) according to the manufacturer's protocol.  
250 Extracted dsDNA was quantified by fluorimetry using a Quantus Fluorometer (Promega) with  
251 the Promega Quantifluor®, per the manufacturer's recommendation.

252 Bacterial communities were estimated by real-time quantitative PCR using universal primer  
253 sets 341F (5'-CCTACGGGAGGCAGCAG-3') and 515R (5'-  
254 ATTACCGCGGCTGCTGGCA-3'). Real-time quantitative PCR was run in a CFX Connect  
255 (BioRad) and was performed in 20  $\mu$ L reaction volumes containing 10  $\mu$ L of 2 $\times$  iQSYBR  
256 Green SuperMix (Bio-Rad), supplemented with 0.16  $\mu$ L of each primer (50  $\mu$ mol. $\mu$ L<sup>-1</sup>),  
257 0.2  $\mu$ L of T4 bacteriophage gene 32 Product (500 ng. $\mu$ L<sup>-1</sup>) (MP Biomedicals), and 2  $\mu$ L of  
258 template DNA (10 times dilution series of plasmid standard of *Pseudomonas putida*  
259 (KT2440) and environmental samples DNA). Positive control *Pseudomonas putida* (KT2440)  
260 quantified gene copy or water served as positive and negative controls, respectively. The  
261 amplifications were carried out with the following temperature profiles: step one heated to  
262 95°C (3 min), followed by 35 cycles (30 s of denaturation at 95 °C, 30 s at the primers  
263 specific annealing temperature (60 °C), 30 s of post-elongation at 72 °C and 30 s at 80 °C for  
264 plate read). Finally, at the end of amplification, a melting curve analysis was performed by

265 measurement of the SYBR Green signal intensities during a 0.5 °C temperature increment  
266 every 10 s from 65 °C to 95 °C.

### 267 **SEM observations**

268 The structure and elemental composition material samples were analyzed by scanning  
269 electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) using a  
270 TM 3000 (Hitachi) unit, operating at 15 kV accelerating voltage, coupled to a Swift ED 3000  
271 X-Stream module (Hitachi). The samples were air dried, deposited on a carbon adhesive and  
272 directly analyzed. The acquisition time for EDS point analyses was 300 s and between 15 and  
273 30 minutes for maps with a resolution of 512 x 384 pixels. The AZtecEnergy Analyser  
274 Software displays and interprets X-ray data to provide accurate and reliable analysis without  
275 standard (Burgess et al., 2007).

### 276 **Plant growth test**

277 Plant growth tests were performed with rye-grass (*Lolium perenne* L.). The content of the  
278 three replicates of microcosms sacrificed after 28 days of experiment was pooled and then re-  
279 distributed in three 200 mL polystyrene pots. Twelve *Lolium perenne* L. seeds were placed  
280 onto the soil surface of each pot and the soils were watered with 20 mL of Mont Roucoux  
281 mineral water. The pots were incubated in a phytotron of which the parameters were adjusted  
282 as follows: 60 % air-water saturation, 16 h of light (white fluorescent light 500–600  $\mu\text{m m}^{-2} \text{s}^{-1}$ )  
283 <sup>1</sup>) at 25 °C alternating with 8 h of darkness at 18 °C). The pots were watered each time surface  
284 drying was observed. After 51 days of incubation, the number of surviving plants was counted  
285 for each microcosm and plant biomass was evaluated after plant drying (roots and shoots  
286 separately).

### 287 **Statistics**

288 All statistical tests were conducted with R 3.2.3 (R Development Core Team, 2014). Inter-  
289 species averages were used to analyze these data. ANOVA and a post-hoc test for  
290 homogeneity of variance were performed. Multiple comparisons were performed by a post-  
291 hoc Tukey-HSD test at different levels, to compare the five test microcosm conditions  
292 between samples for a given duration (7, 28 or 84 days) with 95 % confidence level. Different  
293 letters in tables and in figures indicate the significant differences between samples according  
294 to the test.

295 To facilitate interpretation of the large dataset, a multivariate statistical analysis was  
296 performed by principal component analysis (PCA) applied to the data matrix formed by 23  
297 variables, namely total metal and metalloid concentrations and major ions in the percolation  
298 water, constituents of organic matter, pH, and measured biological parameters of the five  
299 amendment conditions tested, at 7, 28 and 84 days.

300

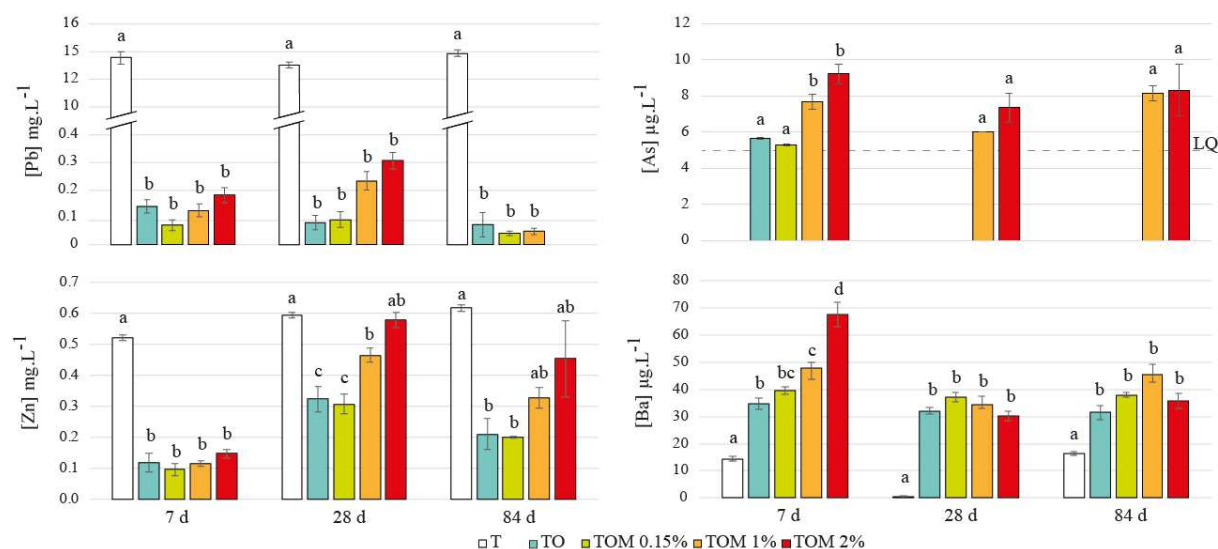
## 301 **Results**

### 302 **Percolated water composition**

303 In the non-amended tailings, the pH value was low and stable after 28 days ( $4.26 \pm 0.08$ ;  
304  $n=13$ ; SM.2). In the amended conditions, pH remained close to neutral over the 84 days of the  
305 experiment. pH values in TO, TOM 0.15%, TOM 1% and TOM 2% were respectively  $7.49 \pm$   
306  $0.12$ ,  $7.67 \pm 0.04$ ,  $7.46 \pm 0.04$  and  $7.37 \pm 0.06$  ( $n=39$ ; SM.2).

307 The ion composition of tailings percolation water was dominated by sulfate, potassium,  
308 nitrate and sodium (SM.3). Microcosms with ochre (TO, TOM 0.15%, TOM 1%, and TOM  
309 2%) were characterized by high concentrations of sulfate, nitrate, sodium, calcium and  
310 magnesium, with respective values above  $2 \text{ g.L}^{-1}$ ,  $40 \text{ mg.L}^{-1}$ ,  $400 \text{ mg.L}^{-1}$ ,  $450 \text{ mg.L}^{-1}$ , and  
311  $80 \text{ mg.L}^{-1}$ . Percolation water from the pots containing manure was also characterized by  
312 higher chloride, ammonium and potassium concentrations. After 7 days,  $[\text{Cl}^-]$ ,  $[\text{NH}_4^+]$ , and  
313  $[\text{K}^+]$  in TOM 2% reached, respectively,  $581.6 \pm 38.2 \text{ mg.L}^{-1}$ ,  $12.5 \pm 1.1 \text{ mg.L}^{-1}$ , and  $894.8 \pm$   
314  $46.5 \text{ mg.L}^{-1}$ .

315 Total dissolved concentrations of As, Zn and Ba were determined respectively at 7, 28 and 84  
316 days and every week for Pb for the five tested conditions. Pb concentrations decreased  
317 significantly, from  $15 \text{ mg.L}^{-1}$  in the tailings to  $< 0.4 \text{ mg.L}^{-1}$  in the amended samples (Fig. 1  
318 and Fig. 2). While, for TO and TOM 0.15%, Pb concentration always remained in the 50–  
319  $100 \text{ } \mu\text{g.L}^{-1}$  range, in the presence of 1% and 2% manure, Pb concentration increased to 300–  
320  $40 \text{ } \mu\text{g.L}^{-1}$  during the first 28 days of the experiment. It then decreased and even dropped  
321 below the detection limit with 2% manure (Fig. 2).

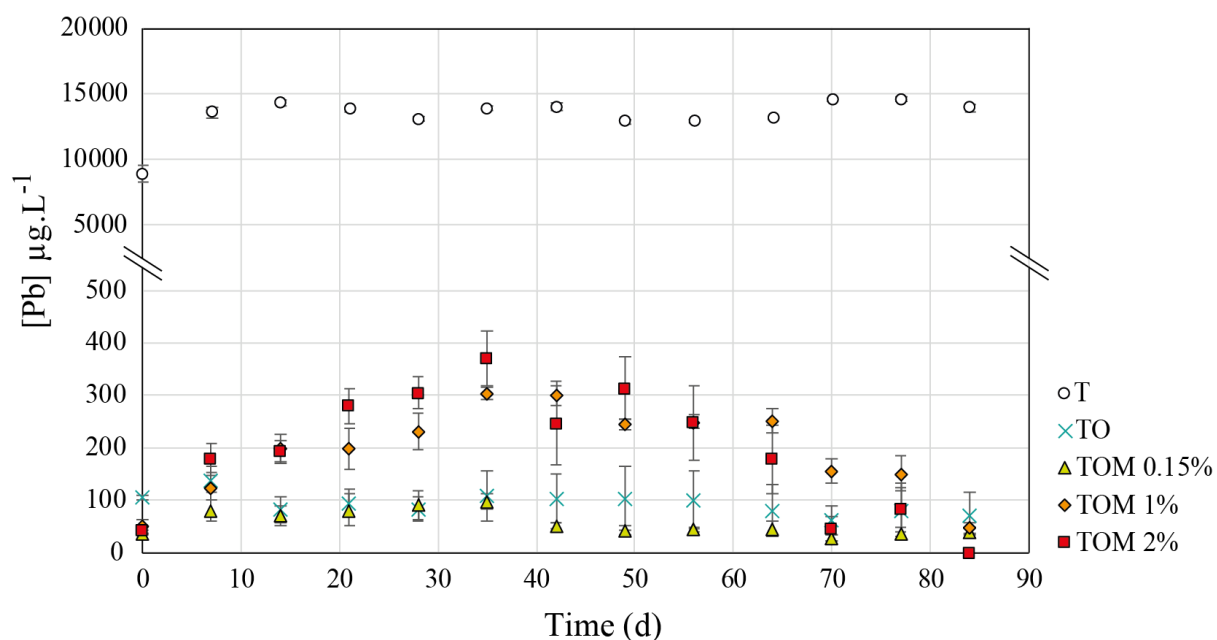


322

323 **Figure 1:** Total dissolved concentrations of Pb, As, Zn and Ba in the percolation water of the  
 324 five test-condition microcosms after 7, 28 and 84 days of experiment.. Multiple comparisons  
 325 were performed by a post-hoc Tukey-HSD test at different levels  $P < 0.05$  ( $n=3$ ), to compare  
 326 the five test microcosm conditions between samples for a given duration (7, 28 or 84 days)  
 327 with 95% confidence level. Different letters indicate significant differences between samples  
 328 according to the test.

329 T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure.  
 330 TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure. Error bars indicate  
 331 the standard error ( $n=3$ ).

332 Zn concentrations decreased significantly in the percolated water from amended tailings after  
 333 7 days (Fig. 1). However, later in the experiment, and particularly for the TOM 1% and TOM  
 334 2% conditions, Zn concentrations tended to increase. As was not detectable in the percolation  
 335 water from the non-amended tailings ( $<0.5 \mu\text{g.L}^{-1}$ ), whereas with 1% and 2% of manure As  
 336 concentration was in all cases  $>0.5 \mu\text{g.L}^{-1}$ . All the amendments induced a significant increase  
 337 in concentration of Ba in percolation water. Maximum Ba concentration,  $67.6 \pm 4.39 \mu\text{g.L}^{-1}$ ,  
 338 was observed in TOM 2% after 7 days, whereas after 28 and 84 days of incubation, no  
 339 difference was observed between amended conditions.



340

341 **Figure 2:** Temporal evolution of Pb total dissolved concentration in the percolation water of  
 342 the five test-condition microcosms during experiment (in days, d). Error bars indicate the  
 343 standard error with  $n=3$ .

344 T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure.  
 345 TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

### 346 Soil chemical and morpho-chemical characterization

347 Total C, TOC, MinC, total N, H and S in the soil of the five test microcosm conditions at 7,  
 348 28 and 84 days were measured by elemental flash pyrolyser analyzer and Rock-Eval pyrolysis  
 349 (Table 1). C and N contents were very low in the tailings throughout the experiment. S  
 350 content did not increase with the addition of ochre or manure. C, H, and N levels were higher  
 351 in the samples amended with manure, and increased proportionally with the proportion of  
 352 manure. These parameters did not evolve during the experiment.

353 **Table 1 :** Concentrations of the main constituents of organic matter analyzed in the five test-  
 354 condition microcosms at days 7, 28 and 84 (d) by <sup>a</sup>Flash pyrolyser analyzer and <sup>b</sup> Rock-Eval  
 355 6.

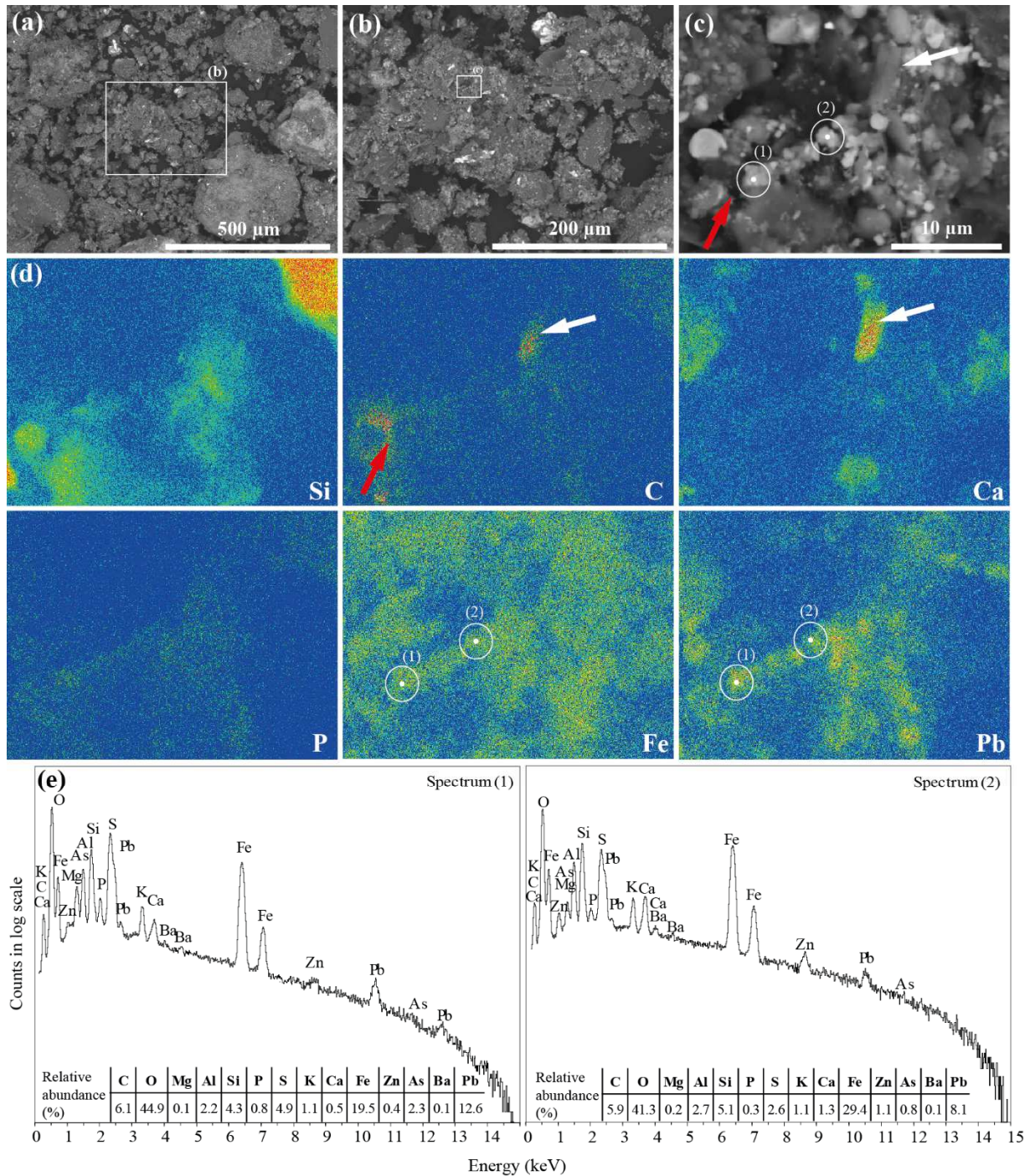
356 TOC: total organic carbon. MinC: mineral carbon. T: tailings without amendment. TO: tailings + 5% ochre.  
 357 TOM 0.15%: tailings + 5% ochre + 0.15% manure. TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%:  
 358 tailings + 5% ochre + 2% manure.

	%	T	TO	TOM 0.15%	TOM 1%	TOM 2%
<b>0 d</b>	<b>C<sup>a</sup></b>	0.05	0.16	0.20	0.53	1.22
	<b>H<sup>a</sup></b>	0.18	0.17	0.21	0.29	0.34
	<b>N<sup>a</sup></b>	0.01	0.02	0.01	0.03	0.08
	<b>S<sup>a</sup></b>	0.56	0.59	0.56	0.76	0.76

	<b>TOC<sup>b</sup></b>	0.09	0.05	0.07	0.20	0.62
	<b>MinC<sup>b</sup></b>	0.04	0.13	0.17	0.44	0.44
<b>7d</b>	<b>C<sup>a</sup></b>	0.05	0.20	0.53	0.66	0.69
	<b>H<sup>a</sup></b>	0.15	0.23	0.29	0.26	0.31
	<b>N<sup>a</sup></b>	0.62	0.41	0.76	0.48	0.60
	<b>S<sup>a</sup></b>	0.01	0.01	0.03	0.04	0.04
	<b>TOC<sup>b</sup></b>	0.07	0.13	0.17	0.37	0.45
	<b>MinC<sup>b</sup></b>	0.04	0.12	0.13	0.29	0.32
<b>28d</b>	<b>C<sup>a</sup></b>	0.15	0.32	0.46	0.46	0.74
	<b>H<sup>a</sup></b>	0.22	0.30	0.25	0.25	0.30
	<b>N<sup>a</sup></b>	0.01	0.02	0.03	0.03	0.05
	<b>S<sup>a</sup></b>	0.53	0.78	0.56	0.56	0.51
	<b>TOC<sup>b</sup></b>	0.09	0.16	0.17	0.27	0.44
	<b>MinC<sup>b</sup></b>	0.04	0.12	0.13	0.20	0.26
<b>84d</b>	<b>C<sup>a</sup></b>	0.03	0.22	0.34	0.60	0.66
	<b>H<sup>a</sup></b>	0.15	0.22	0.25	0.25	0.25
	<b>N<sup>a</sup></b>	0.01	0.01	0.02	0.03	0.04
	<b>S<sup>a</sup></b>	0.54	0.41	0.51	0.43	0.33
	<b>TOC<sup>b</sup></b>	0.05	0.14	0.18	0.34	0.64
	<b>MinC<sup>b</sup></b>	0.03	0.12	0.16	0.22	0.21

359 Ochre and manure were first observed with SEM, to identify their micro-morphological  
360 characteristics and chemistry. A selection of samples from the microcosms was studied in  
361 detail: tailings +5% iron hydroxide (TO), tailings +5% iron hydroxide +2% manure (TOM  
362 2%) at 7 days (Fig. 3), 28 days and 84 days of incubation. For these samples, the most  
363 outstanding result was the high amount of Pb tied to iron hydroxides brought by the ochre, in  
364 all TO and TOM conditions.





365

366 **Figure 3:** SEM observations from TOM 2% at 7 days (a, b and c). (d) Elemental maps from  
 367 backscattered electron image (c). Maps show small iron-rich particles, which were Pb carriers  
 368 (targets (1) and (2)). White arrow show  $\text{CaCO}_3$  without Pb and red arrow show carbon  
 369 fragments. (e) EDS point analyses of two Pb-rich particles represented by the targets (1) and  
 370 (2). (The acquisition time for EDS point analyses was 300 s and 15 s for maps).

371 In the tailings amended with ochre only (TO), the few sludge iron-rich particles larger than  
 372  $10\ \mu\text{m}$  (less than 15 % of the mud according to the laser granulometer) did not fix Pb  
 373 efficiently (SM.4), unlike the vast majority of the sludge particles (SM.4), smaller than

374 10  $\mu\text{m}$ , which contained on average 9 % Pb (Standard Deviation (SD) = 2.96; n = 15) and also  
375 1.5 % of As (SD = 0.75; n = 15).

376 Locally, concentrations reached 12.6 % for Pb and 2.3 % for As (Fig. 3c and e, spectrum 1).  
377 This sludge, rich in Pb and As, also contained S: 4.9 % and 2.6 % in the analyses presented in  
378 Fig. 3e, and 3.5 % as an average of 15 point analyses (SD = 1.5; n = 15).

379 Carbon fragments identifiable with certainty as manure particles were not found in the  
380 microcosm amended with manure, even in the microcosm incubated for 7 days only. Particles  
381 evoked possible morphologies or textures of manure fragments (arrangements reminiscent of  
382 alignments of cells in plant tissues) but contained very low amounts of carbon not allowing  
383 direct comparison with intact manure. Nevertheless, these pieces, enriched in phosphorus  
384 compared to the other components (soil, ochre), were rich in varied Pb-rich minerals also  
385 containing S, P and As. The loosely packed agglutinate shown in Fig. 3 was a commonly  
386 found situation, with carbonaceous matter mixed intimately with iron-rich ochre and fine soil  
387 particles. Carbon in such agglutinates reached 40 % locally (Fig. 3d, red arrow), but the  
388 distribution of Pb clearly matched with Fe.

389 Scarce Pb-Ca rich particles were present in microcosms amended with manure, even after 7  
390 days of incubation. Ochre contained  $\text{CaCO}_3$  rod-like particles that remained easily  
391 recognizable in the TO and TOM samples but, unlike the Ca-rich particles, they did not  
392 contain any trace of Pb, as shown in Fig. 3d (C and Ca maps, white arrows).

### 393 **Biological characterization**

394 Total microbial biomass, measured by the total double-strand DNA (Table 2), showed that the  
395 addition of 2% of manure significantly increased the microbial biomass present in the  
396 microcosms after 7 and 28 days of incubation ( $p < 0.05$ ). Even if, overall, the data are not  
397 significant, the addition of amendment induced an increase in the amount of dsDNA,  
398 regardless of incubation time. However, after 84 days of incubation, the TOM 1% and TOM  
399 2% conditions showed a significantly higher microbial biomass ( $p < 0.05$ ).

400 The proportion of bacteria in the microbial community is expressed as 16S rDNA gene copies  
401 (Table 2). Bacteria were significantly influenced by addition of 1% of cow manure in samples  
402 after 7 days of incubation ( $p < 0.05$ ). The variations were not significant in the other  
403 conditions, but amendments did tend to increase the amount of bacteria. After 28 days, the  
404 amount of bacteria quantified increased with the amount of amendment. After 84 days, all

405 samples contained an equivalent amount of 16S rDNA gene copies, with the T sample having  
406 more bacteria than at the beginning of the experiment.

407 The abundance of As(III)-oxidizing microbes in the solid phases after 84 days (Table 2),  
408 tended to decrease with amendment, the decrease being significant for the highest manure  
409 concentration (2%). Conversely, the abundance of As(V)-reducing microorganisms tended to  
410 increase with the addition of amendments. This increase was not significant for the conditions  
411 with manure because of large differences between the samples. However, a significant  
412 increase was observed between the T and TO amendment conditions.

413 **Table 2** : Total soil DNA, bacterial communities quantification, and As(III)-oxidizing and  
414 As(V)-reducing bacteria enumerated by the Most Probable Number (MPN) method in the five  
415 test-condition microcosms after 7, 28 and 84 days of experiment (d). Analysis of variance was  
416 by post-hoc Tukey-HSD test at different levels  $P < 0.05$  ( $n=3$ ). Different letters indicate the  
417 differences between samples for a given time.

418 T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure.  
419 TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

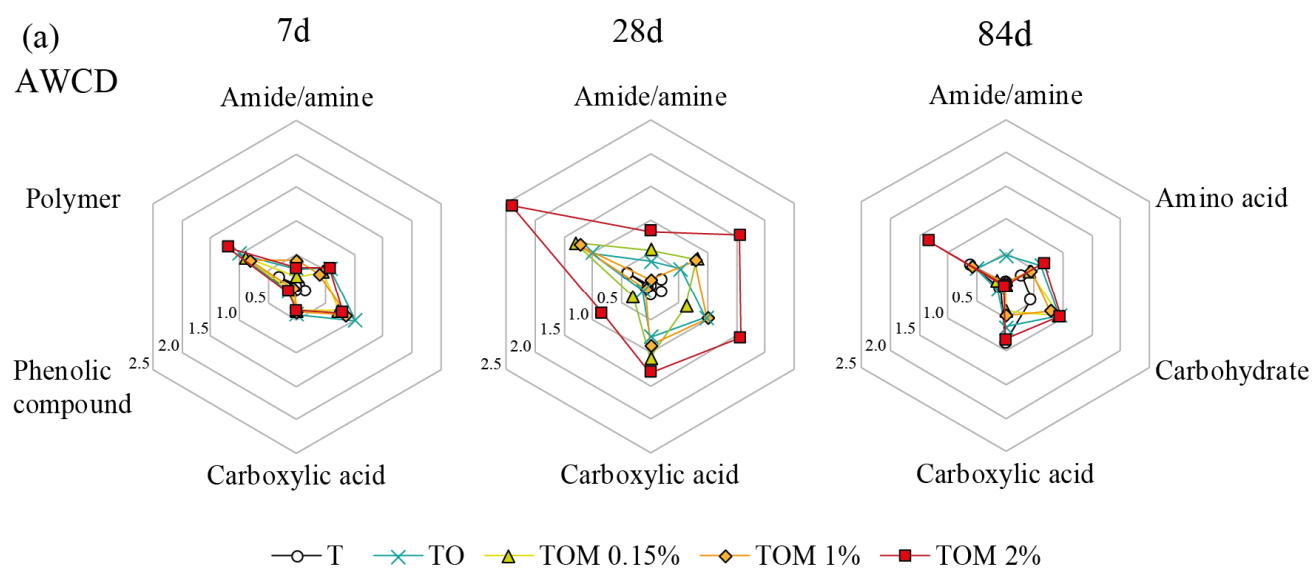
	T	TO	TOM 0.15%	TOM 1%	TOM 2%
<b>7 d</b> Total dsDNA ng.dw soil <sup>-1</sup>	298 b	497 b	1282 b	3687 ab	9273 a
16S rDNA gene copies.g dw soil <sup>-1</sup>	8.9E+6 b	1.5E+7 ab	1.6E+7 ab	2.3E+7 a	1.0E+7 ab
<b>28 d</b> Total dsDNA ng.dw soil <sup>-1</sup>	123 b	558 b	1746 b	4722 b	13,485 a
16S rDNA gene copies.g dw soil <sup>-1</sup>	4.2E+6 c	1.0E+7 bc	1.6E+7 b	2.5E+7 a	2.4E+7 a
<b>84 d</b> Total dsDNA ng.dw soil <sup>-1</sup>	90 b	304 b	1100 b	5907 a	7396 a
16S rDNA gene copies.g dw soil <sup>-1</sup>	1.1E+7 a	2.5E+7 a	7.5E+7 a	1.9E+7 a	2.3E+7 a
MPN of As(III)-oxidizing microorganisms microorganisms.g <sup>-1</sup> dw soil	1.7E+5 a	1.3E+5 ab	1.4E+4 ab	1.9E+4 ab	1.5E+4 b
MPN of As(V)-reducing microorganisms microorganisms.g <sup>-1</sup> dw soil	1.2E+1 b	5.9E+4 a	1.4E+4 ab	1.1E+4 b	1.1E+4 b

420

#### 421 **Average well color development (AWCD) and diversity of the microbial communities in** 422 **the solid phases**

423 The ability of fast growing heterotrophs present in the samples (mainly composed of rapid  
424 growing gram-negative bacteria) to utilize different C substrates are presented with the  
425 functional richness ( $S'$ ), the AWCD calculated per substrate and for the whole Biolog™  
426 Ecoplates, and with the Shannon-Weaver diversity index ( $H'$ ) (Fig. 4, SM.5). The AWCD is  
427 an expression of the microbial activity in the sample and integrates cell density and diversity

428 in substrate utilization, while  $H'$  expresses the diversity of the microbial communities and  $S'$   
 429 the number of degraded compounds. The measurements of  $S'$  provide information about  
 430 microbial activities without providing any information on the type of substrate used. The  
 431 community-level physiological profile (CLPP) demonstrates changes in the structure and  
 432 function of microbial communities with the addition of amendments, and with time.



433

434 **Figure 4:** (a) Radar diagrams of the bacterial average well color development (AWCD) of all  
 435 classes of carbon substrates, identified by Biolog™ EcoPlates for the five test-condition  
 436 microcosms after 7, 28 and 84 days of experiment ( $n=3$ ). (b) Metabolic profiling of samples  
 437 using Biolog™ EcoPlates based on pattern of utilization based on average functional richness  
 438 ( $S'$ ), mean of AWCD, and Shannon-Weaver diversity index ( $H'$ ) of substrates and on  
 439 functional diversity.

440 T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure.  
 441 TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

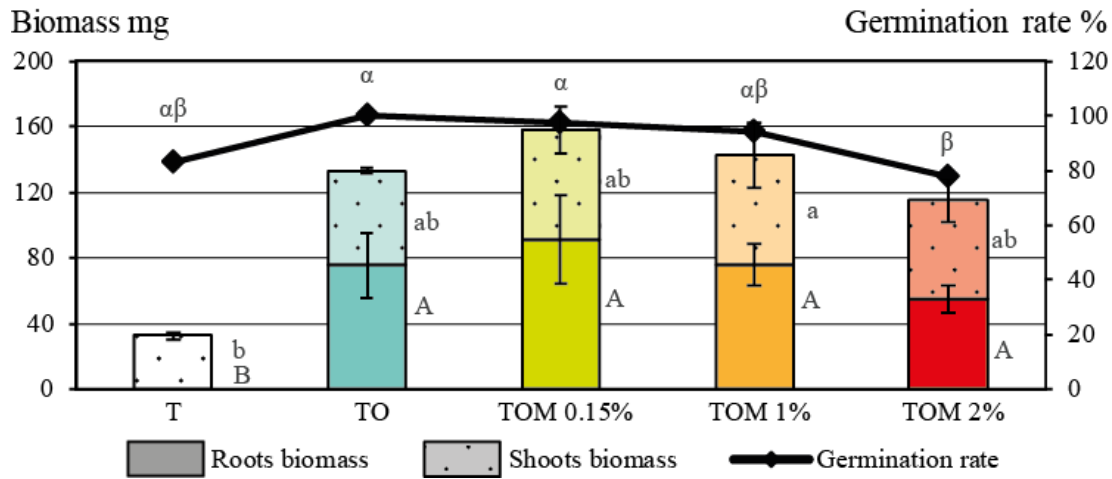
442 Over time, only a few substrates supported the growth of microbial communities, especially  
 443 those belonging to the carbohydrates and polymers chemical groups, while amides/amines  
 444 and phenolic compounds were not (or were only slightly) degraded by the bacteria that  
 445 catabolize the C-sources of the Biolog™ EcoPlates (Fig. 4, SM.5).

446 After 7 days of incubation, the relative abundance based on AWCD for each substrate (Fig. 4,  
447 SM.5) indicated quantitative differences in the use of 31 carbon sources between the samples  
448 without amendment and those amended with iron hydroxide sludge and cow manure. The  
449 AWCDs of the tailings amended with iron hydroxide and iron hydroxide-cow manure were  
450 greater than those of the tailings without amendments. These samples also showed the highest  
451 values for functional richness  $S'$  and Shannon diversity index  $H'$ . This indicates a greater rate  
452 of substrate utilization (catabolic potential) by the microbial community and a greater  
453 functional diversity in the amended tailings than in the non-amended tailings. Even though the  
454 functional richness value for the TOM 0.15% sample was not the highest, its Shannon-Weiner  
455 diversity index and its AWCD value were the highest from amongst the amended samples.  
456 After 28 days of incubation, TOM 2% differed from the other samples with greater values for  
457  $S'$ , AWCD (for each substrate and for the whole Biolog<sup>TM</sup> Ecoplates) and  $H'$  (Fig. 4, SM.5).  
458 The  $S'$ , AWCD, and  $H'$  values were greater for the T sample at day 28 than at day 7. After 84  
459 days of incubation, the AWCD (for each substrate and for the whole Biolog<sup>TM</sup> Ecoplates) had  
460 decreased for all amended samples.

461 The CLPPs indicated by Biolog<sup>TM</sup> Ecoplates data were affected by amendment and by  
462 incubation time. Again, the diversity parameters of T increased (Fig. 4, SM.5). The TO  
463 sample appeared to have the greatest microbial community-level substrate utilization diversity  
464 with the highest  $S'$  and  $H'$  values. The TO samples stood out at this time step with  
465 communities able to catabolize amides/amines. The AWCD remained highest for the TOM  
466 2% sample.

#### 467 **Phytotoxicity test**

468 Ryegrass survival was improved by amendment but a concentration of 1% and 2% manure  
469 tended to reduce plant germination (Fig. 5). The highest survival rate for ryegrass was for the  
470 TO and TOM 0.15% amended samples. The addition of ochre and cow manure contributed to  
471 the development of roots, since the plants did not develop roots (or did so poorly) when they  
472 grew on tailings without amendment. There were significant differences in total plant biomass  
473 between plants growing on tailings without amendment and the other samples (statistical data  
474 not shown). TO and TOM 0.15% amendments favored higher plant biomasses, while  
475 TOM 1% and TOM 2% were least favorable for the development of ryegrass shoots (Fig. 5).



476

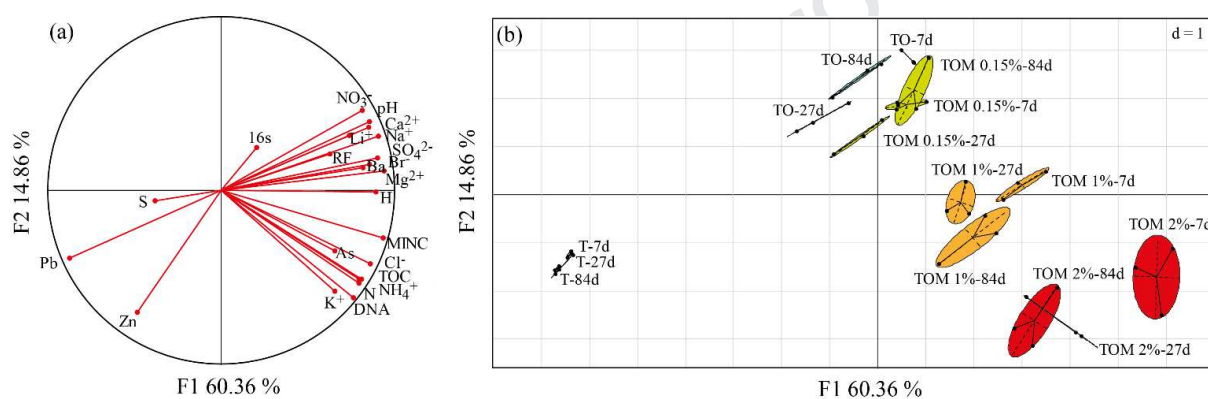
477 **Figure 5:** Germination rate (n=12) and average biomass dry weight (DW) of ryegrass (mg)  
 478 (n=3). Multiple comparisons were performed by a post-hoc Tukey-HSD test at different levels  
 479  $P < 0.05$  (n=3), to compare the five microcosm conditions with a 95% confidence level.  
 480 Different letters indicate significant differences between samples for each parameter  
 481 according to the test (capital letters for roots biomass, minuscule letters for shoots biomass  
 482 and Greek letters for germination rate).

483 T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure.

484 TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure

## 485 Discussion

486 The tailings sample from the Pontgibaud mine used for the experiment was characterized by  
 487 relatively high content of metals and metalloids with Pb, As and Ba at respectively  
 488 26,432 mg.kg<sup>-1</sup>; 1,134 mg.kg<sup>-1</sup>; and 1,063 mg.kg<sup>-1</sup>. These were higher contents than those  
 489 found in other tailings from the Pontgibaud mine (Pascaud et al., 2014) but were of the same  
 490 order of magnitude as those from other former mines in the Massif Central (Bodéan et al.,  
 491 2004; Courtin-Nomade et al., 2016). Tailings from the Pontgibaud mine, like other mining  
 492 wastes, were acidic (pH=4) (SM.2) (Pascaud et al., 2014), a condition that contributed to the  
 493 high mobility of Pb (Fig.6), with concentrations reaching 14,000 µg.L<sup>-1</sup> in percolation water  
 494 in the microcosms (Fig. 2). Sulfates and Pb were the major chemical species in solution. It  
 495 seems that these species solubility were controlled by the stability of anglesite and beudantite,  
 496 the Pb sulfates detected in the initial material.



497  
 498 **Figure 6:** Correlation circle (a) and principal component analysis (PCA) (b) applied to the data  
 499 matrix formed by 23 variables, namely: total metals and metalloid (Pb, Zn, Ba, and As) concentrations  
 500 and major ions (Cl<sup>-</sup>, Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Li<sup>+</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) in the percolation water,  
 501 constituents of organic matter (TOC, MinC), pH, DNA quantification, bacterial abundance (16S), and  
 502 parameters from Biolog<sup>TM</sup> EcoPlates (RF,H<sup>+</sup>) of the five test-condition microcosms after 7, 28 and 84  
 503 days of experiment.  
 504 T: tailings without amendment. TO: tailings + 5% ochre. TOM 0.15%: tailings + 5% ochre + 0.15% manure.  
 505 TOM 1%: tailings + 5% ochre + 1% manure. TOM 2%: tailings + 5% ochre + 2% manure.

506 The tailings had low microbial biomass and both microbial functional richness and diversity  
 507 were poor. Moreover, the germination of *Lolium perenne L.* was greatly inhibited by this  
 508 material. The characteristics of the tailings – very low pH, low content of nutrients (organic  
 509 carbon content between 0.05 and 0.09 %) and severe inorganic contamination – seemed to  
 510 contribute to their specific biological properties. Londry and Sherriff (2005) have shown that  
 511 microbial activities are limited by carbon availability in this type of material, and that  
 512 revegetated areas can provide a source of organic matter. The current geochemical state of the  
 513 tailings limits the development of both microorganism activity and vegetation cover.

Pb was the most concentrated pollutant in the tailings and in the pore water of this mine site. Immobilization of Pb was the first aim of the amendments. Phosphate amendments are often used to reduce the mobility of Pb by ionic exchange and precipitation of pyromorphite (Brown et al., 2005; Kumpiene et al., 2008). Such amendments were not tested because of the high As content of the tailings, phosphate being able to compete with arsenate for sorption sites (Manning and Goldberg, 1996; Darland and Inskeep, 1997). However, the manure may have introduced some phosphate that contributed to the precipitation of a minor fraction of the mobile As whereas, in our microcosms, Pb immobilization seemed to be mainly linked to the ochre amendment (Fig.6). The addition to soils of iron oxide-hydroxide wastes (ochres) from water treatment facilities is known to induce immobilization of Pb as a result of pH increase (Doi et al., 2005; Nielsen et al., 2011; Olimah et al. 2015). In the experiment, the ochre induced a rise in pH to neutral values in the amended microcosms. This phenomenon, together with the efficient sorption capacity of goethite (Wu et al., 2003, Rahimi et al., 2015), was beneficial for the stabilization of Pb. SEM observations of Pb associated with iron-rich particles of ochre supported this hypothesis. In order to evaluate the proportion of Pb precipitation in the amended tailings, the geochemical PHREEQC 3.0.6.7757 code was used with the minteq.v4 thermodynamic database, by integrating the complete chemistry of the percolation water (SM.6). Simulation performed with the data from the amended conditions indicated no precipitation of Pb minerals. However, when Pb concentration was adjusted to that of the non-amended tailings, the simulation predicted the Pb hydroxide and Pb sulfate precipitation. This phenomenon, made possible by the high quantity of sulfate brought with ochre, could also contribute to the immobilization of Pb, this hypothesis being in line with the observation by SEM-EDS of relatively high S concentrations associated with Pb.

One process that may control soluble Ba is the precipitation of barite in the case of high sulfate content (Ippolito and Barbarick, 2006). The PHREEQC simulation sustained the occurrence of this phenomenon with the addition of ochre (SM.6). Also, barite had already been detected in the Pontgibaud mine tailings (Pascaud et al., 2014). A second process was linked to the presence of As, which favored the formation of  $BaHAsO_4^-$  and  $Ba_3(AsO_4)_2$  (Lu and Zhu, 2011).

Although all amendments induced a strong decrease in Pb concentration in the water phase, the evolution of Pb concentration differed with the composition of amendments. In the presence of 1% and 2% manure, Pb concentration increased during the first 28 days of the experiment. It then decreased and even dropped below the detection limit with 2% manure



547 (Fig. 2). In parallel, the maximum of metabolic potential for degradation of organic molecules  
548 was observed after 28 days of incubation (Fig. 4, SM.5). These results suggest a relationship  
549 between the dynamics of mobile Pb and the evolution of the organic amendment. Previous  
550 studies have shown that the solubility of Pb is at a minimum at pH 6, and that at higher pH  
551 values soil organic matter is more soluble, inducing the release of organo-Pb complexes  
552 (Wang and Benoit, 1996; Sauvé et al., 1998). This type of soluble complex may have been  
553 produced in our manure-amended microcosms, as they both had pH higher than 6 (between 7  
554 and 8) and had the highest organic matter concentration for this experiment. In addition to  
555 these dissolved species, the formation of Pb-bearing colloids is expected in this pH range:  
556 Klitzke et al. (2008) observed an increase of both colloidal and dissolved Pb when they raised  
557 the pH of a highly polluted forest soil that was rich in organic matter. The effect of pH on the  
558 mobility of colloids is linked to the change of soil surface charge, decreasing inter-particle  
559 attraction. Colloidal soil minerals are mobilized by changes in charges and stabilized by  
560 organic coating. During the microcosm experiment, the more mobile fraction of organic  
561 matter from manure may have been released during the first 28 days of experiment, inducing  
562 the mobilization of both Pb-organic molecule complexes and organic-coated Pb colloids.  
563 Bacterial activity may have contributed to these processes through the biodegradation of  
564 insoluble molecules with high molecular weights into more soluble small metabolites. The  
565 assumed kinetics of manure biodegradation in our experiment fitted well with those observed  
566 in previous laboratory studies: Bernal and Kirchmann (1992) observed a maximum rate of  
567 mineralization of pig manure in soil during the first month of incubation, and Calderon et al.  
568 (2005) showed that the maximum degradation rate of cow manures occurred during the first 8  
569 weeks of contact with soil.

570 Immobilization of As by ochre in soils polluted with As has already been observed (Doi et al.,  
571 2005; Nielsen et al., 2011; Olimah et al. 2015). However, the experiment described here was  
572 able to show that ochre can simultaneously decrease Pb and As leachability. As was not  
573 greatly mobilized during the experiment, its concentration in the percolated water always  
574 remaining lower than the standard for drinking water ( $10 \mu\text{g.L}^{-1}$ ). However, in every case, As  
575 concentration was higher in the manure-amended conditions than in T and TO conditions  
576 (Fig. 1). In parallel, the range of concentration of As(III)-oxidizing microorganisms tended to  
577 be lower in the manure-amended microcosms. Previous studies have shown that organic  
578 matter can negatively influence microbial As(III)-oxidizing activities (Challan-Belval et al.,  
579 2009; Bachate et al., 2012; Lescure et al. 2016). The abundance of As(V)-reducing organisms

580 was highest in TO and TOM 0.15% manure, and of the same (lower) range in the other  
581 conditions. The increase in pH appeared to stimulate growth of microorganisms able to reduce  
582 As(V). Taking into account that As(V) adsorption on iron oxides is optimum at pHs lower  
583 than 6, whereas As(III) adsorption is favored at pHs above 7, the evolution of As-  
584 transforming communities with amendments was quite positive in terms of As adsorption on  
585 solid phases. Globally, the total number of As-transforming microbes was higher in the  
586 conditions without manure, i.e. when As was found in lower concentration in the percolating  
587 water, suggesting that they were involved in biogeochemical processes favoring As stability.

588 Quantification of microorganisms by measuring the total DNA and 16S rDNA gene copies  
589 number showed that tailings without cow manure and iron hydroxide sludge had lower  
590 microbial biomass than tailings with amendment whatever the time step. Similarly, microbial  
591 diversity, evaluated by average functional richness (S'), AWCD and Shannon-Weaver  
592 diversity index (H'), was lower for tailings without amendment. These results are not  
593 surprising since, typically, tailings are known to have low diversity of microbial species and  
594 functions and low microbial biomass and activity. Thus, Chen et al. (2005) considered that  
595 microbial metabolic activity in tailings is lower than in soils. Tailings can therefore be  
596 considered as an extreme and geochemically dynamic environment exerting a strong selection  
597 pressure on microbial communities that are suited to stressful conditions and considered as  
598 extremophiles, since the microorganisms rely on chemical energy from minerals in tailings  
599 due to the lack of organic matter (Schimel et al., 2007). Therefore, it is not surprising that the  
600 microbial communities in this experiment strongly metabolized complex and more  
601 recalcitrant C sources such as Tween 80, Tween 40,  $\alpha$ -cyclodextrin, and glycogen.

602 Organic matter is a crucial component of the soil sorption complex that immobilizes toxic  
603 metals and metalloids and increases the soil's capacity to store and supply nutrients.  
604 Increasing organic matter content of tailings by supplying cow manure may contribute to  
605 increasing biomass and microbial activity. In this experiment, after 7 days of incubation, the  
606 cow manure and the ochre input provided the tailings with more biomass and diverse  
607 microbial communities. Microorganisms were thus shown to respond rapidly to  
608 environmental changes. The ability of the microbial communities to use substrates seems to  
609 have been increased by the ochre supply alone, since there was little or no difference between  
610 the TO and TOM samples. Amendments may induce shifts in the soil microbial communities  
611 (Pérez de Mora et al., 2006). In a long-term fertilization study of a soil completely eroded and  
612 with a very low organic matter content, Zhong et al. (2010) showed that amendment with

613 manure led to shifts in C utilization patterns and increased soil microbial functional diversity.  
614 The differences in AWCD for each substrate observed on day 7 between microcosms with or  
615 without amendment may be partially due to nutrients (i.e. major ions) brought by the  
616 amendments and consumed by microorganisms. However, according to Bolan et al. (2017),  
617 microbial diversity increases with increase in pH in the tailings, as neutrophilic  
618 microorganisms are more diverse than acidophilic ones. The application of cow manure and  
619 ochre increased pH and major ion content while improving the carbon and hydrogen status of  
620 the mine tailings, thus increasing the biomass of microorganisms and their activities. It has  
621 already been shown that the C/N ratio of soil also has an impact on microbial community  
622 dynamics (Wang et al., 2015).

623 The greater richness and diversity of microbial communities on day 28 for the TOM 2%  
624 sample may be due to the higher percentage of amendment of this sample. Thus, 2%  
625 amendment led to a greater supply of major ions and possibly of viable microorganisms. The  
626 reduction in the activity of microorganisms over time in the other amended microcosms may  
627 be due to the decrease of nutrients available for the microflora. This is in agreement with a  
628 study on the composting of cow manure, the organic matter contained in the manure being  
629 gradually decomposed by microorganisms (Wang et al., 2018). The tailings without  
630 amendments remained at the lowest community-level substrate utilization level.

631 Amendments should contribute to decreasing the availability of metals and metalloids and  
632 should enhance microbial biomass and diversity, but they must also allow revegetation of the  
633 tailings. This is why a test of *Lolium perenne* L. (perennial ryegrass) growth was conducted to  
634 evaluate the effect of cow manure and ochre sludge on the phytotoxicity of the tailings.  
635 Ryegrass is one of the highest quality forage grasses and its primary use is for pasturing cattle  
636 and sheep. This herbaceous plant is also routinely used to investigate plant growth responses  
637 (germination, root and shoot growth) to changing environmental conditions (Gregory et al.,  
638 2014). While ryegrass roots had serious difficulties in growing in the tailings without  
639 amendments – probably because of acidity, toxicity of metals and metalloids and/or nutrient  
640 deficiency – amendments appeared to facilitate plant growth. However, ryegrass seemed to  
641 prefer the test condition with 0.15% of cow manure. This result highlights that an excess of  
642 nutrients might not be beneficial, and that organic amendment dose should be optimized for  
643 the specific plant species selected for the remediation strategy.

## 644 **Conclusion**

645 The results of the microcosm experiments indicate the efficiency of combining ochre and cow  
646 manure amendments to greatly decrease Pb solubility in acidic mine tailings while avoiding  
647 any dramatic mobilization of other elements, namely As, Zn and Ba. Stabilization of As-rich  
648 mine tailings may be an interesting way to make use of the sludge produced during the  
649 treatment of the iron-containing coal mine waters. Short-term monitoring of the amended  
650 tailings revealed a transient increase in both Pb leaching and functional microbial diversity,  
651 both phenomena linked to the maturation of manure. However, 84 days after the amendment  
652 event, Pb immobilization was strengthened by 2% manure, whereas the benefits of this  
653 amendment on functional microbial biodiversity were greatly attenuated. This latter result  
654 shows that amendments alone are not sufficient to induce an enduring improvement in the soil  
655 microbial biodiversity necessary for the development of its functions. However, the  
656 amendments were shown to stimulate the growth of ryegrass, and the colonization of tailings  
657 by plants should contribute to improving their structure and functions. Further experiments  
658 could throw light on the long-term biogeochemical evolution of amendments in the context of  
659 plant cover development. In addition, it will be necessary to take account of on-site  
660 management practices since, as the amendments would be applied only at the surface of the  
661 tailings dumps, there is a strong need to investigate the evolution of the underlying material.  
662 With future upscaling of the process in mind, this study provides key information about the  
663 biogeochemical processes that drive the dynamics of inorganic pollutants in acidic mine  
664 tailings amended with iron-rich ochre and organic matter.

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- Ochre from coal mine water treatment increases pH of acid tailings
- Pb is stabilized and As is not mobilized in tailings by ochre and manure amendment
- Manure induces mobilization of Pb for 35 days then its stabilization
- Microbial functional biodiversity is transiently increased by amendment
- Ochre favors short-term growth of ray-grass on tailings

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