

Temporal evolution of surface and sub-surface geochemistry and microbial communities of Pb-rich mine tailings during phytostabilization: A one-year pilot-scale study

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 geochemistry and microbial communities of Pb rich mine tailings during phytostabilization: a one year pilot-scale study

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14 Graphical abstract



17 Abstract

18 Old mine waste repositories can present health and/or environmental issues linked to their 19 erosion, inducing dissemination of metals and metalloids in air and water that can be attenuated 20 through phytostabilization. Here, the effect of this widespread phytomanagement option on the 21 biogeochemistry of a Pb-rich mine waste was evaluated with a laboratory pilot-scale experiment 22 giving access to the non-saturated and saturated zones below the rhizosphere compartment. 23 Amendment of the tailings surface with biochar, manure and iron-oxide-rich ochre promoted 24 growth of the seeded Agrostis capillaris plants. These events were accompanied by an increase 25 of pH and a decrease of Pb concentration in pore water of the surface layer, and by a transient increase of Pb, Zn, and Ba concentrations in the deeper saturated levels. Macroscopic and 26 27 microscopic observations (SEM) suggest that Pb was immobilized in A. capillaris rhizosphere 28 through mechanical entrapment of tailing particles. Microbial taxonomic and metabolic 29 diversities increased in the amended phytostabilized surface levels, with an increase of the 30 proportion of heterotrophic micro-organisms. Below the surface, a transient modification of 31 microbial communities was observed in the non-saturated and saturated levels, however 11 32 months after seeding, the prokaryotic community of the deepest saturated zone was close to that 33 of the initial tailings. pH and water saturation seemed to be the main parameters driving 34 prokaryotic communities' structures. Results obtained at pilot-scale will help to precisely 35 evaluate the impacts of phytostabilization on the temporal evolution of reactions driving the 36 fate of pollutants inside the tailings dumps.

³⁸ Key words: mine tailings, metals, assisted phytostabilization, laboratory pilot, microbial
39 diversity.

40 **1** Introduction

41 Unrestored mine tailing sites are a worldwide problem since they generally remain bare for decades or even centuries. For abandoned mine tailing heaps, the establishment of a 42 43 spontaneous vegetation cover is usually considered beneficial as it represents a sort of 44 phytostabilization (Houben et al., 2013). Indeed, revegetation of these degraded sites appears 45 as a suitable long-term solution: the plant cover limits eolian dispersion, while the roots prevent 46 water erosion and leaching, and provide a rhizosphere wherein metals can precipitate and 47 stabilize (Mendez and Maier, 2008). However, for lead (Pb)/zinc (Zn) mine sites, metal toxicity, especially available Pb, nutrient deficiency, and poor physical structure of the substrate are 48 49 major constraints for plant establishment and colonization (Ye et al., 2002). Therefore, a 50 suitable strategy consists of aided phytostabilization by using pioneer plants (Lago-Vila et al., 51 2019) coupled with efficient organic and inorganic amendments (Sikdar et al., 2020).

In this context, numerous laboratory studies with pot experiments have recently been devoted to test the efficiency of plants and several amendments, alone or in combination, on Pb and Zn retention and plant growth in mine tailings (Ciarkowska et al., 2017; Al-Lami et al., 2019; Norini et al., 2019; Gao et al., 2020). Although different combinations may rapidly be tested, these pot experiments only focused on plant biomass and metal availability in the rhizosphere area, and are far from the field scale.

Other small-scale experiments include laboratory percolation tests enabling to measure/assess the transfer of contaminants through mine residues and to evaluate the effect of amendments (Rodriguez et al., 2018; Thouin et al., 2019), whereas influence of water saturation can be studied in columns (Ouangrawa et al., 2010). These systems are well constrained and they give insight on small-scale processes but it is necessary to increase the scale in order to understand interactions between different compartments and reflect field conditions. In this regard, field experiments have been conducted to optimize the combination of plants and amendments in phytostabilization of metal(loid)s pollutants in mine sites (Yang et al., 2016; Acosta et al., 2018; Li et al., 2019). However, some key parameters could not be precisely controlled and accounted for, such as soil water saturation and incoming water flow. Moreover, some types of samples, such as pore water at different depths are difficult to collect during onsite studies and spatial heterogeneities of mine tailings often represent an obstacle for the interpretation of results obtained on-site (Constantinescu et al., 2019).

71 To overcome these limitations, lysimeters directly installed in the field give access to real 72 conditions of watering, temperature and light, and allow the possibility to sample percolated 73 water (Nicoara et al., 2014; Bilibio et al., 2017; Constantinescu et al., 2019). In such systems, 74 some environmental factors cannot be controlled. Mesocosm scale laboratory pilot 75 experiments, as an intermediary step between pot and field experiments, combine the advantage 76 of controlled conditions of temperature, watering and water saturation with the possibility of 77 simultaneously observing the amended and planted surface levels (Valentin-Vargas et al., 2014) 78 and the underlying non saturated and saturated zones (Thouin et al., 2017; 2018).

79 Among the multiple parameters evaluated during these multi-scale experiments, microbial 80 communities can give indications on the biogeochemical processes. Phytostabilization always 81 induces modifications of the microbial diversity and metabolic profiles in the mining wastes 82 colonized by plants, amended or not (Asemaninejad et al., 2020). The ability of diverse bacterial 83 and fungal species to thrive in mining residues improves soil functions and encourages 84 sustainable plant growth (Harris et al., 2009). Common effects of phytostabilization on 85 microbial diversity were observed in diverse conditions of amendments and plants species, both 86 in greenhouse experiments (Mendez et al., 2007, You et al., 2018) or field conditions (Li et al., 87 2015; Asemaninejad et al., 2020). They include increases in microbial diversity, biomass and 88 relative increase of heterotrophic/organotrophic genera versus litho-autotrophic organisms.

These last organisms, mainly sulfur and iron oxidizers, are common hosts of mining wastes and contribute to environmental issues linked to production of sulfuric acid and mobilization of metals, thus a decrease of their abundance and activity in mine waste dumps, during phytostabilization, should be beneficial (Valentín-Vargas et al., 2018).

However, most of the previous studies provided data on the microbial communities of the surface environment, in the rhizosphere, and in the close non saturated zones. Thus, the influence of re-vegetation on the underlying microbial communities and on the main biogeochemical cycles driving the fate of pollutants in the depth of the heaps is not well documented.

A methodology to promote stabilization of metals and plant growth with amendments, in the surface of aged tailings from a former lead (Pb)-silver (Ag) mine tailing, was previously developed through microcosm scale experiments (Thouin et al., 2019). The objectives of the present laboratory pilot experiment were to evaluate in environmental compartments rarely studied, from the surface to the water saturated level, the influence of amendments and plants growth on temporal evolution of (1) pore water physico-chemistry, and (2) microbial diversity along the vertical profile of this tailing.

105

106 2 Material and methods

107 **2.1** Characteristics of the mine tailings, the amendments and the plants

Tailings from the site of the former Ag and Pb mine at Pontgibaud (Puy de Dôme, France) were used for the pilot-scale experiment. Mine tailings were sampled to a depth of 0–60 cm from a mine waste heap, using a power shovel (SM1a). Residues were taken in 7 discrete zones within a 25 m² area. The material was homogenized and sieved at 2 cm; it presented the following particle size distribution: 7% (by weight) < 60 μ m, 15% in the 60–315 μ m range, and 78% >315 113 μ m. The detailed characteristics of this moderately acidic sandy material are given in Thouin 114 et al. (2019) and Table 1. The pH of the tailings – consisting mainly of quartz, orthoclase and 115 phyllosilicate – was 4.9 (measured in water according to ISO 10390). Tailings contain about 116 2.6% Pb and Pb bearing phases were detected by X-ray diffraction (XRD): anglesite (PbSO₄) 117 and beudantite ((PbFe₃(AsO₄)(SO₄)(OH)₆).

118 Table 1. Main chemical characteristic of the tailings, ochre, manure and biochar. (a) pH was measured in water according to

119 ISO 10390. (b) Element concentrations in solids were analyzed by high resolution inductively coupled plasma mass

120 spectrometry and elemental flash pyrolyser. (c) Standard leaching test was performed with demineralized water according to

121 ISO/TS 21268–2. Pb, Ba and As were analyzed by oven atomic absorption spectroscopy, and Fe and Zn by flame atomic

¹²² absorption spectroscopy. DOC and DON were determined using a TOC elemental analyzer.

	Tailings	Ochre	Manure	Biochar
pH ^a	4.9	8.1	9.9	9.5
Solid chemical analyses ^b				
Pb (mg.kg ⁻¹)	26432	10.1	2.82	n.d.
Fe (%)	1.5	26.1	n.d.	n.d.
Zn (mg.kg ⁻¹)	265	14717	53.8	n.d.
Ba (mg.kg ⁻¹)	1063	69.8	78.9	n.d.
As (mg.kg ⁻¹)	1134	505.7	0.75	n.d.
C (%)	0.13	1.99	35.39	81.2
N (%)	<lq< td=""><td><lq< td=""><td>2.47</td><td>0.6</td></lq<></td></lq<>	<lq< td=""><td>2.47</td><td>0.6</td></lq<>	2.47	0.6
S (%)	1.58	0.69	0.18	<lq< td=""></lq<>
Standard leaching testing ^c				
Pb (mg.L ⁻¹)	19.6	0.23	0.33	n.d.
Fe (mg. L^{-1})	<lq< td=""><td>0.4</td><td><lq< td=""><td>n.d.</td></lq<></td></lq<>	0.4	<lq< td=""><td>n.d.</td></lq<>	n.d.
Zn (mg.L ⁻¹)	0.17	<lq< td=""><td>0.33</td><td>n.d.</td></lq<>	0.33	n.d.
Ba (mg.L ⁻¹)	<lq< td=""><td><lq< td=""><td>0.27</td><td>n.d.</td></lq<></td></lq<>	<lq< td=""><td>0.27</td><td>n.d.</td></lq<>	0.27	n.d.
As (µg.L ⁻¹)	2.3	2.5	7.0	n.d.
DOC (mg. L^{-1})	63.5	44.1	1134.1	n.d.
DON (mg.L ⁻¹)	8.1	4.4	131.1	n.d.

123 DOC: dissolved organic carbon, DON: dissolved organic nitrogen, LQ: limit of quantification (SM2) and n.d.: not determined.

124 The first amendment used was an oxy-hydroxide sludge, called ochre, collected in a coal mine 125 water treatment in the south of France, mainly composed of 2-6 µm particles. It contained 26.1 126 % Fe, and had a pH value in water of 8.1 (measured according to ISO 10390; Thouin et al., 127 2019). A poorly crystalline goethite (FeO(OH)) and aragonite (CaCO₃) were identified, and the 128 presence of zincite (ZnO) was suspected (Mertz et al., 2021). The second amendment was cow 129 manure, collected from a farm near Orléans (France). Its pH in water was 9.9 (according to ISO 10390). The manure was dried at 30°C, ground in a mortar and sieved at 2 mm before using. 130 The third amendment was biochar, produced from the slow pyrolysis at 500 °C of hardwood 131 132 biomass (mixture of oak, beech, and hornbeam), followed by a sieving to obtain a particle size between 0.5 and 1 mm (Norini et al., 2019). The pH in water was 9.5 measured (according to
ISO 10390) and the CEC of biochar (using the 0.05 N cobaltihexamine method) was 0.65
cmol.kg⁻¹. Chemical composition of amendments are detailed in Table 1.

Agrostis capillaris naturally present on the site was selected for this study. Nandillon et al.,
2019 showed that this indigenous *A. capillaris* is a metallicolous ecotype, more tolerant to the
stress condition of these mine tailings than a commercial non metallicolous ecotype. The seeds
of *A. capillaris* were directly sampled on the Pontgibaud mine site close to the tailings sampling
site.

141 2.2 Pilot experiment



Figure 1. Design of the laboratory pilot experiment. (a); Analyses performed during the experiment (b) and experimental
program presenting the chronology of the main events (c).

The instrumented laboratory pilot device consisted of a closed stainless-steel column (100 cm in diameter and 145 cm high), placed in a temperature-regulated room at $23 \pm 1^{\circ}$ C. The pilot design was previously described in Thouin et al. (2017). A layer of inert gravel (centimetric quartz) and a geotextile membrane were placed at the bottom of the column to facilitate outflow drainage without loss of tailing particles. This single metric pilot experiment (Fig. 1), represents the up-scaling of the assisted phytostabilization method previously developed in multiplemicrocosms (Thouin et al., 2019).

152 The column was filled with 814 kg (equivalent to 670 kg of dry weight (dw.)) of the < 2 cm 153 fraction of homogenized mine tailings (SM1b), and was equipped during the process at three 154 levels (Lv2: 26.2 cm, Lv3: 43.7 cm, and Lv4: 61.2 cm measured from the surface and after 155 solids settlement) with time domain reflectrometry probes (TDR) (TRIME-PICO32, IMKO) to 156 measure moisture and temperature in tailings, and with inert Polytetrafluoroethylene/Quartz 157 porous probes (pore size 2 µm, three probes for each depth, regularly spaced) to sample pore 158 water. These probes were inserted into mine tailings at 30 cm from the column wall (Fig. 1a). 159 Five horticultural light-emitting diodes (LED) at the top of the column simulated 12-h day/night 160 cycles. Rainfall was simulated by a sprinkler system connected to a water reservoir and fed by pumps, and consisted in the watering of 5.5 L of Mont Roucous[®] water each time and three 161 162 times a week. This mineral water was used because of its chemical composition close to that of rainwater (pH = 6.38, electrical conductivity = 160 μ S.cm⁻¹, Cl⁻ = 56 μ M, NO₃⁻ = 24 μ M, SO₄²⁻ 163 = 16 μ M, Na⁺ = 109 μ M, K⁺ = 7.7 μ M, Ca²⁺ = 25 μ M, Mg²⁺ = 21 μ M). 164

After three months of stabilization, the tailings settled by 12.5 % in height, and the thickness of 165 166 the tailings in the column reached a constant value of 68 cm. The first experimental step 167 corresponded to the control non-phytostabilized condition, which lasted 6 months (t_{-6m} to t_{0m}). 168 Both levels Lv4 and Lv3 were always in the saturated part of the column all along the 169 experiment, whereas the Lv2 level was located in the unsaturated zone. The leachate was 170 collected in a steel tank at the bottom, noted Lch, and quantified using scales. This outflow 171 weight as well as moisture and temperature at the three levels (Lv2, Lv3 and Lv4) were 172 continuously monitored.

After 6 months (t_{0m} , corresponding to 167 days after starting the monitoring of the experiment, Fig. 1c), 19 cm of tailings surface were stripped (195 kg, equivalent to 173 kg of dw.), and amended with 1 wt% ochre, 1 wt% cow manure and 1 wt% biochar (i.e. 1.73 kg of each amendment), homogenized, then replaced at the top of the column (SM1c). This tailing amended level (Lv1) was instrumented with one TDR and three porous probes at 9.5 cm from the surface, regularly spaced. The amendment was followed by the seeding of 4.1 g of *A*. *capillaris* on all the tailings surface.

Sampling of pore water from levels Lv1, Lv2, Lv3, Lv4 and Lch was performed (before water sprinkling) via fluorinated ethylene propylene (FEP) tubing connected to a peristaltic pump. The 3 water samples from the 3 independent regularly spaced porous probes of each level (Lv1, Lv2, Lv3 and Lv4) were pooled before determination of physicochemical and geochemical parameters. In Lv1, water sampling was limited due to the low water contents and to the competition with *A. capillaris* roots, this is why there were no water analyses after 231 days.

186 Solid sampling was performed in the column by coring at t_{-6m}, t_{0m}, t_{3m} and t_{11m} (at t_{0m} sampling 187 was performed before amendment). Coring was performed with 5 cm diameter and 1m length 188 stainless steel pipes. Only one coring was carried out at each experimental step, in order to 189 minimize disruptive effects. After sampling, the pipes were clogged and put back in place in 190 the tailings to fill the empty space. Each core was separated into four samples, Lv1 at a depth 191 of 0-19 cm, Lv2 at 19-34 cm, Lv3 at 34-51 cm, and Lv4 at 51-68 cm, corresponding to the pore 192 water sampling levels (Fig. 1c). These samples were homogenized, sub-sampled in sterile tubes 193 stored at -20°C for DNA extractions then stored at 5°C in tightly closed sterile jars for the 194 determination of functional diversity of microbial communities.

The sampling of *A. capillaris* biomass and rhizospheric and non-rhizospheric materials were
performed at t_{3m}, t_{6m} and t_{11m}, (respectively 92, 193 and 338 days after seeding). Five plastic
(polystyrene) tubes with an area of 19.6 cm² and a height of 7 cm were used to make small

198 cores of level Lv1. For each sampled A. capillaris, non-rhizospheric (Lv1 NR) tailings fraction 199 was separated from rhizospheric (Lv1 R) materials by shaking, and rhizospheric-associated 200 tailings particles were manually isolated from A. capillaris roots. The plants of A. capillaris 201 were washed with 0.5 mM CaCl₂ and deionized water and plant biomass was measured after 202 plant drying at 60°C during 48h (roots and leaves separately). The total plant cover of the 203 tailings surface was estimated by eye in percentage and was used to extrapolate the total 204 biomass of A. capillaris defined in gram of dry leaves and roots by m^2 . At t_{3m} and t_{6m} , the A. 205 capillaris plants were cut at about 5 cm above the tailings' surface to simulate mowing and 206 leaves were recovered.

207 2.3 Water analyses

208 Electrical conductivity (EC), redox potential (Eh), pH, dissolved oxygen (DO), and temperature 209 of interstitial water were measured with a benchtop meter (multiparameter analyser C3040, 210 Consort) connected to an ORP sensor (platinum electrode, with an Ag/AgCl reference 211 electrode, 3M KCl, Eh value refers to the normal hydrogen electrode (NHE)), oxygen probes 212 (Fisherbrand), pH electrode (Fisherbrand) and conductivity probe (Sentek) (Fig. 1b, SM2). As, 213 Pb and Ba were analyzed by oven atomic absorption spectroscopy (AAS) 214 (Varian AA220Z), and Zn and Fe by flame AAS (Varian AA22FS) (SM2).

215 2.4 Analyses of solids

216 **2.4.1 Mineralogy and chemical composition**

Solid samples were dried and ground to 70 µm in an agate mortar for X-ray diffraction (XRD)
and chemical analyses (SM2). XRD was performed using an INEL CPS120 diffractometer
equipped with a Co anode. Measurements of total carbon (C), hydrogen (H), nitrogen (N), and
sulfur (S) were obtained using an elemental flash pyrolyser analyzer (Flash 2000, Thermo
Fisher Scientific) (SM2). Total organic carbon (TOC) contents were determined by Rock-Eval
pyrolysis (Rock-Eval 6 Turbo, Vinci Technologies) (SM2). Elemental concentration

223 measurements of all solid samples were performed on high resolution inductively coupled 224 plasma mass spectrometry (HR-ICP-MS ; Thermo Element-XR, details in SM2) after complete 225 dissolution by hydrofluoric acid attack (HNO₃ 65%, HCl 37% and HF 40%) using a pressurized 226 closed-vessel microwave system (MARS 6, CEM).

227 2

2.4.2 Optical and SEM-EDS observations

Fresh tailings material sampled in Lv1 at the end of the experiment was observed with a binocular magnifier Leica 2616 APO equipped with a photography and video device and connected to a computer equipped with Leica Mycrosystem software. Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) observations were performed on a TM 3000 (Hitachi) at 15 kV accelerating voltage. SEM was coupled to a SwiftED3000 X-Stream module (Hitachi). The acquisition time of EDS point analyses was 300 seconds and 15 minutes for maps with a resolution of 512 x 384 pixels.

235 **2.4.3 DNA extraction and next generation sequencing**

Total nucleic acids were extracted in duplicate from 0.5 g (wet weight) of each solid sample with a FastDNA® SPIN Kit for soil (MP Biomedicals, Illkirch, France). DNA was quantified with the Promega Quantifluor®, using the QuantusTM fluorometer (Promega, France).

For next generation sequencing, a fragment of the gene coding 16S rRNA (bacteria and archaea) was amplified using the barcoded, universal primer set (515WF/918WR) (Wang et al., 2009). The primers used to amplify the specific fungal barcode ITS2 were w404 and w40 (Degois et al., 2019). PCR reactions were performed using AccuStart II PCR ToughMix kit and cleaned (HighPrep PCR beads, Mokascience). Pools were submitted for sequencing on Illumina MiSeq instrument at GeT-PlaGe (Auzeville, France). The raw datasets are available on the European Nucleotide Archive system under project accession number PRJEB52405 and PRJEB52412. 246 Sequences were processed using the FROGS (v.3.0) bioinformatics pipeline (Escudié et al., 247 2017), implemented into the Genotoul plateform of the Galaxy server of Toulouse. Fastq paired 248 reads were merged with FLASH software, and clustering into OTU with Swarm and an 249 aggregation distance clustering of 3. Chimera and operational taxonomic unit (OTU) with a 250 proportion less than 0.005 % of all sequences were removed. Taxonomic affiliation was 251 performed using BLASTn and the Silva 132 database for 16S rRNA sequences and the Unite 252 Fungi 8.0 database for ITS2 sequences. Filtration on taxonomic affiliation was done at 253 minimum identity of 100% and minimum coverage of 95%. The FROGS pipeline implemented 254 Phyloseq R package was used for OTU structure visualization, rarefaction curves, and alpha 255 diversity index calculations. Chao 1 index (non-parametric measurements) estimates the 256 number of unobserved species from those observed 1 or 2 times while the Shannon and 257 invSimpson diversity indexes give respectively a measure of heterogeneity of microbial 258 community and the probability that two randomly picked individuals do not belong to the same 259 species.

260 **2.4.4** Functional diversity of heterotroph microorganisms

BiologTM Ecoplates community-level substrate utilization assays were used to study the functional diversity of microbial communities(Garland, 1999). The BiologTM Ecoplates were incubated at 25 °C and the absorbance values (optical density (OD) at 590 nm) were monitored using an Omega SPECTROstar (BMG Labtech) microplate spectrophotometer at t=0 and 168 h (7 days). The well absorbance values were adjusted by subtracting the average absorbance of the control well (water only) at t=0. After subtracting the control well, an OD590nm > 0.25 was considered as positive. Negative readings (OD < 0) were set to zero for all subsequent analyses.

268 2.5 Statistical analysis

269 Statistical analysis were performed using R (<u>http://www.r-project.org/</u>; version 4.3.0) with the 270 vegan package (version 2.5-7). To visualize differences in the OTUs-based community

271 composition, we performed an agglomerative hierarchical clustering (AHC) on the 100 most 272 abundant OTUs of 16S rRNA and ITS 2 sequencing data, using Bray-Curtis dissimilarity 273 metric, and Ward's minimum variance. Redundance analysis (RDA) was performed to identify 274 the relationships among samples, environmental variables, and relative abundance of phyla. 275 This analysis was calculated based on Hellinger - transformed phylum abundance basis of 16S 276 rRNA and ITS 2 data (Borcard et al., 2011). Environmental explanatory tested variables were 277 as follows: humidity, pH, TOC, [S], [N], [Fe], [Pb], [Zn], [As], [Cu], [Ba] (solid concentrations 278 at t_{0m} , t_{3m} , t_{11m}) and were standardized. The function ordistep in vegan package was used to 279 identify the significant explanatory forward environmental variable. The significance of the 280 RDA model was tested by ANOVA based on Monte Carlo test with 999 permutations. This analysis helps to determine the most influential factors and the extent that various 281 282 environmental parameters affected microbial composition.

283 **3 Results**

284 **3.1** Percolated water composition

285 **3.1.1 Physico-chemical parameters**

Pore-waters in the subsurface levels of the column (Lv2, Lv3, and Lv4) were characterized by constant pH values between 4 and 3, whether before or after the amendment (Fig. 2). In Lch, the pH was higher but decreased from 6 to 4 after amendment of the tailings. In Lv1, the pore water pH was higher than in Lv2, Lv3, and Lv4. Values of redox potential was positive (>300 mV NHE) before amendment, even in the saturated levels (Fig. 2). After amendment, Eh NHE increased in all sampling level and remained positive during the experiment.





297 **3.1.2** Metal(loid)s concentrations

Pb was the metal with the highest concentration in tailings' water and leachate. Before t_{0m}, Pb 298 concentrations were similar and constant in Lv2, Lv3, and Lv4 with values of 14.4 mg.L⁻¹ (Fig. 299 2). In Lch, Pb concentrations were lower and increased to reach 2.3 mg.L⁻¹ just before the 300 amendment. In the amended level Lv1, mean Pb concentration was $532 \pm 77 \,\mu g.L^{-1}$. In Lv2 and 301 Lv3. Pb rapidly decreased after the amendment to values close to 3 300 and 4 000 μ g.L⁻¹ 302 (respectively), then between t_{3m} and t_{11m} , Pb slightly increased to reach 8 160 and 7 150 μ g.L⁻¹ 303 304 at the end of the experiment. At the bottom of the column, in Lv4, Pb concentration rapidly increased after the amendment to reach a maximum of 88 900 µg.L⁻¹ on day (d) 207 then 305 decreased down to 4 100 µg.L⁻¹ on d 319. As for Lv4, Pb concentration in Lch increased after 306 the amendment to reach a maximum of 14 775 µg.L⁻¹ at d 214 and then decreased down to 3 307 700 μ g.L⁻¹ at t_{11m}. 308

309 During the period before the amendment, Ba concentrations were between 8.2 and $18 \ \mu g.L^{-1}$ in 310 Lv2, Lv3 and Lv4, and between 101 and 65 $\mu g.L^{-1}$ in Lch (Fig. 2). After t_{0m}, Ba concentration 311 rapidly decreased below 2 $\mu g.L^{-1}$ in Lv2 and Lv3, while in Lv4 it increased to a maximum of 312 77.6 $\mu g.L^{-1}$ on d 207 and decreased under 2 $\mu g.L^{-1}$ on d 300. Between d 300 and the end of the 313 experiment, Ba concentration in Lv2, Lv3 and Lv4 slowly increased to about 10 $\mu g.L^{-1}$. In Lch, 314 two peaks were observed after the amendment, at 207 days (145.6 $\mu g.L^{-1}$) and at 252 (51.4 315 $\mu g.L^{-1}$).

As concentrations in pore water in Lv2, Lv3 and Lv4 were less than 15 μ g.L⁻¹ throughout the experiment (SM3). In Lch, As concentration decreased from 233 to 13 μ g.L⁻¹ between t_{-6m} and t_{0m}. After the amendment As decreased below 5 μ g.L⁻¹ and then increased at the end of experiment to reach 26.7 μ g.L⁻¹.

321 **3.2** Evolution of the solids

322 The evolution of parameters related to organic matter and the main contaminants in solids is 323 presented in Table 2. Amendment slightly increased the concentration in carbon (total C, TOC) 324 in Lv1 and in Lv2, but not at all in the deeper levels Lv3 and Lv4. The other analysed elements 325 did not vary according to experimental time and depth of layer in the column. In particular, Pb, 326 the main contaminant, did not exhibit significant trends as a function of depth and time. One 327 can only note slightly lower Pb contents in the rhizospheric area compared to the non-328 rhizospheric one. Zn contents slightly increased in the upper levels (Lv1 and Lv2) just after the 329 addition of amendments (t_{3m}), due to the presence of Zn in ochre, then returned to values close 330 to that of the tailings.

X-ray diffractograms of the tailings sampled at different depths (Lv1, Lv2, Lv3, and Lv4) at
the end of the pilot experiment revealed no change of the mineralogy (SM4): quartz, orthoclase,
anglesite, beudantite and a phyllosilicate (presumably muscovite) were the main crystalline
phases, as previously identified in the initial tailings (Mertz et al., 2021).

Table 2 Concentrations of the main interesting constituents in the solids sampled in four depths and at different periods of the
pilot experiment, and analyzed by *a Flash pyrolyzer analyzer*, *b Rock-Eval 6 and c HR-ICP-MS*.

	Depth	C ^a (%)	N ^a (%)	S ^a (%)	TOC ^b (%)	Fe ^c (mg.kg ⁻¹)	Pb ^c (mg.kg ⁻¹)	Zn ^c (mg.kg ⁻¹)	As ^c (mg.kg ⁻¹)	Cu ^c (mg.kg ⁻¹)	Ba ^c (mg.kg ⁻¹)
	Lv1	0.10	0.06	0.68	0.09	15 085	23 817	321	1 384	124	3 631
t0m	Lv2	0.09	0.06	n.d.	0.08	19 450	24 166	327	1 398	74	3 4 4 4
tom	Lv3	0.23	0.04	0.71	0.08	15 493	26 234	347	1 381	131	4 635
	Lv4	0.04	0.02	0.72	n.d.	15 246	22 712	381	1 319	128	4 133
4	Lv1 R	1.00	0.05	1.12	0.96	14 166	19722	521	1 127	119	3 452
t _{3m}	Lv1 NR	1.04	0.04	1.05	1.02	12 793	27 594	419	969	107	7 706

	Lv2	0.99	0.06	0.65	0.97	19 000	28 479	484	1 404	69	3 974	
	Lv3	0.18	0.06	n.d.	0.11	17 304	27 857	371	1 598	71	4 535	
	Lv4	0.09	0.05	0.77	n.d.	14 336	20 848	345	1 235	126	4955	
	Lv1 R	1.28	0.07	1.04	0.98	13 280	21 644	360	988	101	2 503	
	Lv1 NR	1.00	0.07	0.68	0.85	16912	29 835	308	1 083	52	3 340	
t _{11m}	Lv2	0.61	0.05	0.72	0.56	18 386	27 786	434	1 515	73	4 321	
	Lv3	0.15	0.03	0.70	0.11	13 935	30 940	285	1 059	52	3 605	
	Lv4	0.07	0.02	0.74	n.d.	12766	27 696	368	1 054	91	3 051	

337 Initial tailing composition is available in table 1. TOC: total organic carbon, Lv1 to Lv4 represent the sampling depths in the 338 tailings. Lv1 R: solid sampled in the rhizospheric zone, Lv1 NR: solid sampled in the non rhizospheric zone, tom: 6 months after 339 the beginning of the monitoring without amendments and plants, t_{3m}: 3 months after adding amendments and seeding Agrostis 340 capillaris, t_{11m}: end of the experiment, 11 months after adding amendments and seeding A. capillaris, and n.d.: not determined.

341

342

Agrostis capillaris development 3.3

343 Three months after seeding (t_{3m}) , thin leaves of Agrostis capillaris have grown and a dense 344 implantation with about 80% of surface cover was observed (Table 3). The biomass was about 190 g.m² for leaves and stems and 57 g.m² for roots. At t_{3m} , A. capillaris was cut, and 3 months 345 346 later, a reduced plant cover (50%) was observed but with larger and better developed leaves. 347 Root biomass has also increased between t_{3m} and t_{6m}. A second cut was made at t_{6m}. At the end 348 of the pilot experiment, 11 months after seeding, the biomass of aerial parts was multiplied by 349 four (SM1d). A. capillaris with stolon was observed and plant cover increased between t_{6m} and 350 t_{11m} . Measured root biomass seemed to drastically decrease, but only roots in the first 7 351 centimeters were weighted whereas roots had colonized the entire thickness of the amended 352 level and were observed up to 40 cm deep.

353 Table 3 Agrostis capillaris development. Plant cover was estimated visually and the biomass was measured for a determined

354 surface where the A. capillaris plants were well developed. The extrapolated biomass was determined with biomass and plant

355 *cover.* * *Root biomass was determined only in the first 7 cm.*

		Biomass sa	mpling	Extrapolated biomass		
	Plant cover (%)	Aerial Parts (g)	Roots* (g)	Aerial Parts (g.m ²)	Roots* (g.m ²)	
t _{3m}	80	2.4	0.7	191.5	57.0	
t _{6m}	50	2.4	1.2	119.7	73.3	
t_{11m}	70	9.8	0.2	700.9	11.4	
		C 1 1.1.1	1 C 11	1 1 1 1		

356

 t_{3m} , t_{6m} , t_{11m} : respectively 3 months, 6 months and 11 months after adding amendments and seeding A. capillaris.

357 **3.4** Tailings stabilization by Agrostis capillaris root system

The surface of the tailings at the end of the pilot experiment was visually invaded by the 358 359 Agrostis capillaris root system. The binocular magnifier enabled to observe solid particles 360 forming immobilized tailing aggregates associated with the roots (Fig. 3a). Some fragments of roots observed with SEM-EDS presented fibrous coverage (Fig. 3b) enriched in P and Pb (Fig. 361 3c point 1) compared with the simple solid particles present at the root surface (Fig. 3c point 362 363 2). Fine filaments of 3-4 µm diameter were observed in the rhizosphere (Fig. 3d, e and f). They 364 might be fungal hyphae. EDS mapping revealed the presence of Fe and Pb on their surface and 365 inside their membrane (Fig. 3e point 1, and f).



366

Figure 3. Observations of Agrostis capillaris root samples at the end of the pilot experiment (t_{11m}): (a) aggregates of tailings
associated with roots (binocular magnifier); (b) and (c) backscattered electron images of a root (SEM); (d) and (e)
backscattered electron images of a presumed fungal hyphae, and (f) backscattered electron images and elemental mapping of
Fe and Pb of presumed fungal hyphae; Table present the results of SEM-EDS semi-quantification of elements at the surface of
the (c) observed root and (e)inside hyphae membrane.

372

373 **3.5 Microbial diversity and structure**

374 **3.5.1** Community species richness and diversity

After treatment steps, a total of 790 820 16S and 920 302 ITS 2 sequences were obtained from the 19 samples. The rarefaction curves reach a plateau for all samples showing that the diversity of communities was well sampled (SM5). The richness of 16S OTUs was homogeneous before amendment at t_{-6m} and t_{0m} , with values varying between 431 in Lv3 t_{-6m} and 752 in Lv4 t_{0m} . After amendment, an increase of richness was observed except in Lv4 t_{11m} . Chao1 index shows 380 that the majority of OTUs was sequenced (Table 4). The richness of ITS2 sequences was lower 381 than 16S sequences. As with bacteria, the number of OTUs increased after amendment in all 382 levels except in the deepest level at the end of the experiment. The bacterial species diversity, 383 based on the Shannon and InvSimpson indexes (Table 4) increased after amendment in the 384 surface levels Lv1. In Lv2 and Lv3 an increase of diversity indexes was also observed at t_{3m} 385 and t_{11m}. The lowest Shannon and InvSimpson indexes were observed in Lv4 at t_{11m}. For the 386 fungi, the evolution of Shannon and InvSimpson indexes were less obviously related to the 387 experimental conditions.

Table 4: 16S and ITS 2 sequences diversities estimation of the solids sampled in four depths and at different periods of the pilot
 experiment.

16S		Richness	Chao1	Shannon	InvSimpson
	Lv1	576	612.7	4.2	16.4
	Lv2	621	673.9	4.4	20.6
L -6m	Lv3	598	635.8	4.3	18.8
	Lv4	752	810.1	4.4	18.6
	Am	448	561.8	4.7	45.4
	Lv1	613	652.1	4.6	28.1
t _{0m}	Lv2	549	572.8	4.1	14.8
	Lv3	431	444.0	4.3	19.0
	Lv4	550	595.2	4.1	15.4
	Lv1 R	918	1022.2	5.4	86.6
	Lv1 NR	902	997.5	5.4	85.7
t _{3m}	Lv2	953	1046.9	5.4	81.8
	Lv3	880	986.0	4.7	28.5
	Lv4	938	1022.5	4.1	14.3
	Lv1 R	747	825.5	5.2	58.4
ι_{11m}	Lv1 NR	829	927.3	4.9	42.1

Lv	2 91	0 1	1031.9	5.4	76.2
Lv	3 87	75 9	971.2	4.3	18.6
Lv	4 31	.8 3	366.4	3.4	14.3

ITS 2		Richness	Chao1	Shannon	InvSimpson
	Lv1	56	61.0	2.3	4.8
	Lv2	51	58.5	1.9	4.2
L -6m	Lv3	61	61.5	1.8	2.9
	Lv4	56	63.0	2.1	4.2
	Am	91	91.1	2.3	6.4
	Lv1	42	48.0	1.7	3.0
t _{0m}	Lv2	35	45.0	2.2	5.4
	Lv3	26	35.3	1.8	3.4
	Lv4	65	70.3	1.8	2.7
	Lv1 R	107	116.2	2.2	5.2
	Lv1 NR	105	106.9	2.6	8.4
t _{3m}	Lv2	115	118.5	2.7	8.7
	Lv3	93	93.0	2.7	7.5
	Lv4	82	92.0	2.8	9.7
	Lv1 R	105	115.5	2.1	3.6
t _{11m}	Lv1 NR	113	117.2	3.0	13.0
	Lv2	117	119.5	2.5	6.2
	Lv3	78	81.0	2.9	8.6
	Lv4	16	22.0	2.3	8.3

Lv1 to Lv4 represent the sampling depths in the tailings. Am: tailings amended with 1 wt% ochre, 1 wt% cow manure and 1
wt% biochar, Lv1 R: solid sampled in the rhizospheric zone, Lv1 NR: solid sampled in the non rhizospheric zone, t-6m: start of
the experiment, tom: 6 months after the beginning of the monitoring without amendments and plants, t3m: 3 months after adding
amendments and seeding Agrostis capillaris, t11m: end of the experiment, 11 months after adding amendments and seeding A.

capillaris.

395 **3.5.2** Microbial community structure

396 Samples of solids from the pilot experiment hosted bacterial and fungal communities presenting 397 different spatial and temporal distributions (Fig. 4). The 100 most represented phyla of 398 procaryotes (Fig. 4A) included Proteobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, 399 Gemmatimonadetes, Actinobacteriaceae, Planctomycetes, Cyanobacteria, Firmicutes, 400 members of the Deinococcus-Thermus group for the bacteria, then Thaumarchaeota and 401 Euryarchaeota for the archaea. The AHC analysis, that groups the OTUs according to 402 similarities of occurrence in samples, shows a cluster of OTUs, gathered in the upper part of 403 the Fig. 4A, that were mainly present in the tailings before amendment (t_{-6m} and t₀) and in the 404 deepest levels of the column (Lv3 and Lv4) all along the experiment (OTUs n°9 to n°96). The 405 ten most abundant procaryote OTUs (in bold) were present in this group that included 406 Acidobacteria, Chloroflexi, Proteobacteria and Thaumarchaeota, however none of them was 407 identified at the genus level (SM6). Procaryote OTUs more represented in surface levels from 408 t_{3m} to t_{11m} and influenced by the amendment and plant growth, are grouped in three clusters in 409 the bottom part of Fig. 4A. The cumulative list of the ten most represented known bacterial 410 genera in each sample (SM7), included three sulfur-oxidizing bacteria, namely Sulfuriferula, 411 Sulfurifustis and Thiomonas, and two autotrophic Fe-oxidizing bacteria, i.e. Gallionella (only 412 in Lv4 at t_{3m}) and Sideroxydans (only in Lv4 at t_{11m}). All the 44 other major genera are 413 heterotrophic organisms. A known nitrogen fixing genus, Mesorhizobium, was present in the 414 list of the 10 most represented procaryote OTUs in Lv1, both in the rhizospheric and non 415 rhizospheric samples. The contribution of the 3 sulfur-oxidizing bacteria, expressed as the sum 416 of their relative abundance in the 10 most abundant identified genera (SM8 and SM9), shows 417 their preponderance in the initial tailings. Their relative abundance strongly decreased in Lv1, 418 Lv2 and Lv3 at t_{3m} . At the end of experiment their contribution rose in Lv3 and Lv4.





Figure 4. AHC analysis of the 100 most abundant OTUs (10 most abundant in bold) in solid samples from the pilot experiment.
A: bacteria and archaea (based on 16S sequences), B: fungi (based on ITS sequences) (Lv1to Lv4 represent the sampling
depths in the tailings, Am: tailings amended with 1 wt% ochre, 1 wt% cow manure and 1 wt% biochar, Lv1 R: solid sampled
in the rhizospheric zone, Lv1 NR: solid sampled in the non rhizospheric zone, t-6m: start of the experiment, tom: 6 months after
the beginning of the monitoring without amendments and plants, t_{3m}: 3 months after adding amendments and seeding Agrostis

426 *capillaris, t*_{11m}: *end of the experiment,* 11 *months after adding amendments and seeding A. capillaris).*

427 The distribution of the 100 most abundant fungal OTUs (Fig. 4B) shows some tendencies 428 already observed with bacterial OTUs, such as a group of fungal OTUs mainly found in the 429 initial tailings, at t_{-6m} in all levels, and t_{0m} from Lv2 to Lv4 gathered at the top of Fig. 4B. 430 Another group of OTUs was mainly found in the surface levels after amendment (bottom of 431 Fig. 4B). The 10 most abundant fungal OTUs were distributed between these two groups (Fig. 432 4B, SM10). A group of fungal OTUs (OTU 14 to OTU 93), probably resulting from the 433 amendment, was found at t_{3m} and t_{11m} in deeper levels (up to Lv3 at t_{11m}). The fungi present in 434 the rhizospheric materials at t_{3m} and t_{11m} are different from those found in non rhizospheric Lv1 435 samples. The classification of the ten most represented known fungal genera (SM11) shows 436 that the genera *Mortierella* and *Talaromyces* were strongly represented in the original tailings 437 (t-6m) and detected in all samples. The genera Clonostachys, Coniochaeta and Pisolithus were 438 found among the 12 dominant fungal genera at t_{-6m}. The most represented genus in the freshly 439 amended Am Lv1 was Remersonia. The genera Zopfiella and Mortierella were also found in 440 this sample, in small proportions. At t_{3m}, the genera Mortierella, Clonostachys and Zopfiella 441 were still present. The genus *Podospora* was present in all levels at this sampling time, , together 442 with Giberella, emerging t in Lv1 R and Lv4. The fungal diversity of the rhizospheric Lv1 at 443 t_{3m} differed from that of the other levels, in particular because it was the only level containing 444 Saccharomyces as one of the 10 dominant fungal genera. At t_{11m}, Podospora represented nearly 445 50% of the fungal genera present in Lv1 R andLv4 exclusively included Malassezia as a 446 dominant fungal genera.

447 A RDA was performed to identify the influence on microbial community structure of 12 448 physico-chemical characteristics of solid phases (Fig. 5). For Bacteria and Archaea (Fig. 5A) 449 the results indicated that pH, humidity, Zn, and As were explanatory variables that significantly 450 influenced the community structure, which explained 88.6 % (p = 0.001) of the total variance. 451 In particular, the highest pH and lowest water content seemed to favour the presence of 452 Bacteroidetes and Proteobacteria, whereas low pH and high water saturation seemed to induce 453 higher proportions of Acidobacteria and Thaumarchaeota. Zn and As concentrations in the 454 solids exerted antagonist influences. For Fungi (Fig. 5B), As and humidity were the only

455 significant explanatory variables that influenced the community structure. These parameters 456 significantly explain (p = 0.011) 37.7 % of the total variance of fungal community structure. 457 High As concentrations seemed to favorise the development of Mortierellomycota while low 458 water content favorised the presence of Ascomycota.



Figure 5. RDA of sequence abundance assigned at phylum level of microbial communities in the solids samples in relation to
environmental variables. A: Bacteria and Archaea (based on 16S sequences), B: Fungi (based on ITS sequences). Red lines
represent the significant environmental variable, blue lines represent the Phylum and circles represent the samples. (Lv1 to
Lv4 represent the sampling depths in the tailings, Lv1 R: solid sampled in the rhizospheric zone, Lv1 NR: solid sampled in the
non rhizospheric zone, t_{0m}: 6 months after the beginning of the monitoring without amendments and plants, t_{3m}: 3 months after
adding amendments and seeding Agrostis capillaris, t_{11m}: end of the experiment, 11 months after adding amendments and
seeding A. capillaris).

467 **3.5.3 Functional diversity of heterotrophic microorganisms**

The substrate spectrum of active cultivable heterotrophs was mainly driven by polymers and carbohydrate degradation in the initial tailings (Fig. 6). Amended surface level clearly displayed higher heterotrophic activities and a deployment of polymer and carboxylic acids degradation was observed at t_{0m} . Increase of carboxylic acids biodegradation activities was shared by all levels at t_{3m} . A large spectrum of heterotrophic activities was detectable in non-rhizospheric compartments Lv1, Lv2 and Lv3 at t_{11m} , when heterotrophic activity was lower in the rhizospheric samples, and nearly inexistent in the deepest level Lv4.



477 Figure 6. Functional diversity of cultivable heterotroph microorganisms in solid samples from the pilot experiment (Lv1to Lv4
478 represent the sampling depths in the tailings, Am: tailings amended with 1 wt% ochre, 1 wt% cow manure and 1 wt% biochar,
479 Lv1 R: solid sampled in the rhizospheric zone, Lv1 NR: solid sampled in the non rhizospheric zone, tom: 6 months after the
480 beginning of the monitoring without amendments and plants, t_{3m}: 3 months after adding amendments and seeding Agrostis
481 capillaris, t_{11m}: end of the experiment, 11 months after adding amendments and seeding A. capillaris).

482 **4 Discussion**

483 **4.1** Stabilization of Pb linked to the development of Agrostis capillaris

484 The Agrostis capillaris plants grown for 11 months in the column developed an abundant root 485 system that macroscopically retained tailing particles. A. capillaris was already reported as a 486 good candidate for phytostabilization, often present as a pioneer plant of polluted sites, resistant 487 to metals and metalloids including Pb and As (Bradshaw, 1952; Meharg and Macnair, 1991; 488 Ernst, 2006; Nandillon et al., 2019). A. capillaris has been found to grow spontaneously on 489 contaminated soils, and was recognized as an interesting candidate for the phytostabilization of 490 As and Pb polluted sites (Austry et al., 2013; Rodriguez-Seijo et al., 2016). When using pioneer 491 plants for phytostabilization purposes, preference should be given to pseudo-metallophyte over 492 hyperaccumulator species to minimize the mobilization of metals (Houben et al., 2013). Lead-493 rich minerals associated with roots of our A. capillaris plants were observed by SEM-EDS, co-494 occurring with high either iron or phosphate concentrations. Pyromorphite (Pb₅(PO₄)₃(C1) or 495 (OH)⁻ grains have been observed in the outer roots' cell-walls of A. capillaris grown in a mine 496 waste contaminated soil (Cotter-Howells et al., 1999). The authors suggested that the 497 precipitation of Pb phosphate might be related to a mechanism of resistance. As a fact, 498 Nandillon et al. (2019) found higher Pb concentrations in roots of this metallicolous ecotype of 499 *A. capillaris* than in a non-metallicolous one, grown on Pontgibaud tailings, suggesting that Pb 500 immobilization in roots might increase the resistance of plants to this toxic element. Apoplastic 501 sequestration of chloropyromorphite was observed by transmission electron microscopy (TEM) 502 in roots of Pb-resistant *Brachiaria decumbens* (Kopittke et al., 2008).

503 **4.2** Impact of phytostabilization on water and solids composition

504 Amendment of the tailings surface layer was performed to facilitate phytostabilization through 505 the development of A. capillaris. This event induced a local increase of solids and pore water 506 pH, accompanied with a decrease of Pb concentration in pore water. This phenomenon, in 507 agreement with results of microcosm experiments (Thouin et al., 2019), was more pronounced 508 in the planted layer, and its amplitude decreased with depth. In the water saturated deeper level 509 Lv4, conversely, a sharp peak of Pb concentration in pore water was observed at the beginning 510 of the phytostabilization process. This phenomenon might be linked to different processes. First, amendment brought several soluble salts releasing cations $(Ca^{2+}, Na^+, Mg^{2+}...)$ that could 511 compete with Pb²⁺ for adsorption on mineral surfaces (Li et al., 2012). This competition might 512 513 have been compensated by the pH increase in the surface layers, but not in the deeper layer 514 whose pH was not influenced. Another phenomenon that could explain Pb release in Lv4 might 515 be a slight pH decrease linked with the production of CO_2 by the organic matter (manure) 516 degradation. This phenomenon was observed at microcosm scale (Thouin et al., 2019) and the 517 associated Pb mobilization was numerically simulated (Mertz et al., 2021). Again, this 518 acidification might have been negligible in the upper levels because of the buffering effect of 519 the amendment, that were not efficient in Lv4.

520 The redox potential in sampled water never decreased significantly, suggesting that reducing 521 processes, such as ferric iron or sulfate bio-reduction, did not establish at a macroscopic scale, 522 whereas they might have occurred locally in micro-environments.

523 Interestingly, Ba behaved in the same way as Pb with a peak of concentration in Lv4 observed 524 following the amendment and seeding. Yet, both Ba and Pb form low-solubility solid phases 525 with sulfate (PbSO₄ and BaSO₄). In a previous pot experiment (Norini et al., 2019) correlations 526 between Ba and major ions concentrations were highlighted. This could suggest that soluble 527 ions derived from amendment (and more precisely from biochar) induced an increase of Ba 528 concentration in pore water. Zinc release was also observed in all levels except the surface. This 529 might be related both with the transport of Zn-containing ochre in the underlying acidic layers, 530 inducing release of Zn from this amendment, and/or with the release of Zn from the tailings due 531 to competition for adsorption sites with the salts released by amendment (Voegelin et al., 2001).

532 **4.3** Impact of phytostabilization on microbial communities

The development of a plant cover during phytostabilization of mine tailings is generally 533 534 associated with a diversification of microbial communities in the colonized surface material. 535 This shift can be associated with the amendments provided to help plant growth (Valentin-536 Vargas et al., 2014), however survey of long-term phytostabilized mine tailings shows that 537 plants alone can induce a complexification of the microbial communities' structure 538 (Asemaninejad et al., 2020). The observed evolutions linked with phytostabilization of mine 539 tailings include the increase of the proportion of heterotrophic bacteria linked to a decrease of 540 the abundance of chemo-autotrophic or mixotrophic bacteria involved in iron and sulfur cycles 541 (Mendez et al., 2007; Valentin-Vargas et al., 2014; Gil-Loaiza et al., 2016; Li et al., 2015), and 542 the development of nitrogen-fixing and plant growth promoting bacteria (Honeker et al., 2019; 543 Asemaninejad et al., 2020). Our moderately acid tailings hosted three main sulfur oxidizers, i.e. 544 Sulfuriferula, Sulfurifustis and Thiomonas, and no FeII-oxidizing acidophiles, whereas many

545 previous studies reported the occurrence of the genera Leptospirillum, Acidithiobacillus, 546 Alicyclobacillus, Sulfobacillus, or Ferrithrix (Li et al., 2016; Li et al., 2017; Honeker et al., 547 2019; Asemaninejad et al., 2020). Sufuriferula genus includes the species Sulfuriferula 548 plumbophilus, formely Thiobacillus plumbophilus, a chemolithotrophic bacterium growing 549 between pH 4.0 and pH 6.5 by oxidation of galena, producing anglesite (Drobner et al., 1992). 550 Sulfurifustis genus (Kojima et al., 2015) gathers autotrophic species oxidizing reduced sulfur 551 compounds (thiosulfate, tetrathionate, elemental sulfur) at near neutral pH conditions. 552 Thiomonas species are acid-tolerant mixotrophic organisms presenting optimal growth in 553 presence of both organic substrates and sulfur compounds or arsenite (Battaglia-Brunet et al., 554 2011). The main Pb minerals identified by XRD in Pontgibaud tailings were the oxidized forms 555 anglesite and beudantite. However, a minor fraction of Pb probably remained in the form of the 556 sulfide galena. As a fact, a darker zone that was sampled from the tailing was enriched in Pb, 557 suggesting the presence of less oxidized materials. The absence of acidophilic iron-oxidizing 558 bacteria, that distinguishes our system from many other described autotrophic microbial 559 communities of tailings, may be explained by the low proportion of Fe-bearing sulfides (pyrite 560 and arsenopyrite) compared with galena. Amendment with material providing organic 561 substrates and neutralizing acidity had an immediate effect on microbial communities' 562 composition in the surface level, as previously observed (Valentin-Vargas et al., 2014), with an 563 increase of the proportion of heterotrophic phyla and an activation of the metabolism of organic 564 substrates, in terms of activities and diversity. Moreover, our pilot experiment highlighted the 565 effect of amendments in the underlying zones, with clear changes of global community 566 composition in the non-saturated zone below roots. The two deeper saturated levels were less 567 influenced in terms of global bacterial diversity, however the proportion of sulfur-oxidizers in 568 the 10 most abundant OTUs declined both in Lv2 (non-saturated) and Lv3 (saturated just below) 569 3 months after the amendment and seeding event. Only the deepest level Lv4 always kept a 570 significant proportion of sulfur-oxidizers. However, at t_{11m} , the proportion of sulfur-oxidizers 571 was restored in Lv3, suggesting an attenuation of the organic substrates supply from the surface. 572 Honeker et al. (2019) already observed re-acidification of phytostabilized mine tailings during 573 phytostabilization, in the initially amended surface levels. Here, we showed a transient 574 influence of surface amendments and plant growth on the underlying levels, however limited 575 spatially as the deeper saturated zone retained the characteristics of initial tailings. From the 576 perspective of Fe cycling, the typical acidophile FeII-oxidizers Leptospirillum were not 577 represented in the 100 most abundant OTUs, neither in the 10 most abundant identified genera, 578 however they were detected by the sequencing as minor OTUs typically present in the tailings 579 signature (t_{-6m} and Lv3-Lv4). Two other less acidophile chemolithotrophic FeII-oxidizers, 580 Gallionella and Sideroxydans, emerged (list of 10 most abundant identified genera) in the 581 deepest level Lv4 at t_{3m} and t_{11m} . In parallel, the presence of an acidophilic FeIII-reducer, 582 Acidibacter, was noticed (list of 10 most abundant identified genera) in Lv3 at t_{3m} and Lv1R at 583 t_{11m}. Acidibacter was previously mentioned as part of the acid-generating group in 584 phytostabilized pyritic mine tailings (Honeker et al., 2019). Its presence in our column suggests 585 that amendments and plant growth could induce activation of Fe cycling, including FeIII-586 reduction in the surface levels fueled by new organic electron donors, providing energy (FeII) 587 for moderately acidophilic FeII-oxidizers in the deepest zones.

In the surface level colonized by plants, plant growth promoted bacteria of the genus *Mesorhizobium* which emerged in the 10 most abundant OTUs at t_{11m} , suggesting that conditions favorable to long-term plant maintenance were established, and that N₂-fixing bacteria could take over from the manure as a source of nitrogen necessary for plant development. Asemaninejad et al. (2020) already observed groups of N₂-fixing bacteria including *Mesorhizobium* in long-term vegetated tailings. Bacterial diversity of rhizospheric and non rhizospheric materials did not differ significantly, and evolved similarly from t_{3m} to

595 t_{11m} , both in terms of global diversity and representation of the major known bacteria. According 596 to Honeker et al. (2019), the microbial diversity in a phytostabilized soil can be similar in the 597 rhizospheric and bulk soils when pH is near neutral; differences appear when the pH is acidic 598 (<4). In our case, amendment enabled to maintain pH > 4 in Lv1 along the experiment. However, 599 differences in the metabolism of organic compounds were observed at t_{11m} , the metabolic 600 activity being higher in the bulk than in the rhizospheric material. Whereas root exudates should 601 feed the heterotrophic microbes (Navarro-Noya et al., 2010), here, not the organic substrate 602 availability but other type of limitation of microbial activity might be linked to rhizospheric 603 processes. In particular, phosphate might be depleted in the root vicinity, because of uptake by 604 plants. In a context of low phosphate bioavailability linked to low solubility of Pb phosphate, 605 competition between plants and microbes for this essential nutrient might occur.

606 Concerning fungi, about 90 species of *Mortierella* are known mainly from soil, the rhizosphere, 607 and plant or animal remains in contact with soil (Domsch et al., 1980). Members of Mortierella 608 (phylum Zygomycota) are rapidly growing organisms which have been suggested to be 609 saprobes in forest ecosystems (Heitefuss, 2011) and that are important decomposers in soil. The 610 genus Mortierella also comprises highly chitinolytic fungi. Due to their potentially endophytic 611 trophic lifestyle, Mortierella may be tracking changes in nutrients driven by vegetation turnover 612 (Veach et al., 2017). It is therefore not surprising that this fungal genus was found in the initial 613 tailings that were surrounded by a forest. The adaptive capacities and oligotrophic lifestyle 614 allowed these fungi to be present throughout the experiment. Talaromyces are saprotrophic 615 Ascomyceta which contribute to the spoilage of dead or decaying organic material. They are 616 notoriously tolerant to extreme conditions. Talaromyces species are commonly distributed in a 617 wide range of substrates, mostly in soil. In an As-contaminated mine soil, Nam et al. (2019) 618 showed that a fungal strain hyper-tolerant to arsenic of *Talaromyces* sp. efficiently removed As 619 from aqueous medium (>70%) with significant improvement by the surface modification of the

fungi with Fe(III) (hydr)oxides. Thus, the hypothesis of a contribution of fungal biomass to the 620 621 removal of As and/or metals from the pore water of our system cannot be excluded, as our 622 SEM-EDS observations suggested the presence of Fe and Pb at the surface of fungal hyphae. 623 *Pisolithus* is a genus of fungi within the family *Sclerodermataceae*. It is an ectomycorrhizal 624 (ECM) Gasteromycetes able to realize symbiosis with various trees (Cairney et al., 2013). We 625 found this genus in Lv1 tom, in the amended level, but also in Lv4 at tom, thus it was not 626 exclusively present in the amendments. This genus was detected in different levels and at 627 different times thereafter. The spores of this ectomycorrhizal fungus were probably initially 628 present in the tailings and were maintained throughout the experiment thanks to their great 629 resistance.

630 **5** Conclusion

631 The first objective of phytostabilization is the decrease of pollutants spreading through surface 632 water runoff and wind erosion. However, the modification of the transfer of metals and 633 metalloids linked with infiltration processes may have important implications in terms of impact 634 on downstream groundwater quality or mine drainage. Here, the phytostabilization technology 635 was examined not only in the surface zones but also from the perspective of the evolution of 636 underlying compartments. The metallicolous A. capillaris grew and efficiently trapped tailing 637 particles in its root system. The most mobile pollutant, i.e. lead, was actually stabilized in the 638 surface tailings layer. Beyond these phenomena previously reported for planted tailing systems, 639 our pilot experiment revealed some phenomena occurring in the underlying zones, following 640 the amendment/seeding event, such as a transient increase of the pore water concentrations in 641 Pb, Zn, and Ba. Geochemical data acquired at this laboratory pilot scale will find application in 642 the development of numerical model, helpful to foresee the future evolution of pollutant fluxes 643 in the waste heap. Meanwhile, the phytomanagement process strongly influenced the microbial diversity and metabolic activity in the surface layer but less intensely in the deeper ones. The 644

645 main parameters influencing bacterial communities' structure, namely pH and water saturation 646 could be easily related to amendment in the surface compartment, and to the experimental 647 simulation of a groundwater zone. The evolution of fungal community and their potential 648 influence on metalloids and metals dynamics would deserve deeper investigation. Thus, the 649 overall influence of a phytomanagement strategy should be considered and evaluated not only 650 in the surface compartments, but also considering the potential diffusion of phytostabilization-651 related substances that could influence both abiotic phenomena and microbial processes, toward 652 underlying non-saturated and saturated zones of the mining site.

653

CRediT author statement

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