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1 **Rehabilitation of mine soils by phytostabilization: does soil inoculation with microbial**  
2 **consortia stimulate *Agrostis* growth and metal(loid) immobilization?**

3

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12

13 Running title: microbial inoculation improves mine soil remediation

14

15 **Abstract**

16 Metal(loid) soil pollution resulting from mining activities is an important issue that has negative effects  
17 on the environment (soil acidification, lack of vegetation, groundwater pollution) and human health  
18 (cancer, chronic diseases). In the context of a phytostabilization process for the bioremediation of a mine  
19 soil highly contaminated by arsenic (As) and lead (Pb), a pot experiment was set up to study the effect of  
20 plant sowing and microbial inoculation on soil properties, metal(loid) (im)mobilization in soil and  
21 accumulation in plant, and plant growth. For this, mine soil was sown with endemic metalicolous  
22 *Agrostis* seeds and/or inoculated with endogenous microbial consortia previously selected for their As  
23 and Pb tolerance. *Agrostis* was able to develop on the contaminated mine soil and immobilized  
24 metal(loid)s through metal(loid) accumulation in the roots. Its growth was improved by microbial

25 consortium inoculation. Moreover, microbial consortium inoculation increased soil organic content and  
26 electrical conductivity, and led to an increase in soil microbial activities (linked to C and P cycles);  
27 however, it also induced a metal(loid) mobilization. In conclusion, microbial consortium inoculation  
28 stimulated the growth of endemic *Agrostis* plants and thus ameliorated the phytostabilization of a  
29 former mine soil highly polluted by As and Pb. This study is thus a good example of the benefits of  
30 coupling several approaches such as phytostabilization and bioaugmentation for the bioremediation of  
31 former mine contaminated sites.

32

### 33 **Keywords**

34 Arsenic; Lead; phytoremediation; bioaugmentation; microbial consortium inoculation; metalicolous  
35 *Agrostis*

36

### 37 **Highlights**

- 38 • A new bioremediation approach was carried out, coupling phytostabilization and  
39 bioaugmentation
- 40 • Metal(loid) tolerant microbial consortia and endemic metalicolous *Agrostis* were used
- 41 • Consortium inoculation increased soil DOC and microbial activities (NCP cycles)
- 42 • Microbial inoculation stimulated *Agrostis* growth and phytostabilization
- 43 • Microbial inoculation mobilized As and Pb

44

### 45 **1. Introduction**

46 Past mining activities have induced a significant contamination of soils by metals and metalloids, at both  
47 the extraction sites and the surrounding areas (Vareda et al. 2019). This is mainly due to the fact that  
48 mining activities generated large amounts of wastes that were deposited on soils around the mines,

49 leading to the formation of technosols (Sheoran et al. 2010, Wong 2003). Such areas are generally  
50 characterized by low levels of macronutrients such as NPK (Sheoran et al. 2010), a lack of organic matter  
51 (Wong 2003), and a sandy texture, leading to a low ability to retain water (Sheoran et al. 2010).  
52 Therefore, the biological functionality of the soil is disturbed, and the growth of microorganisms and  
53 plants is impaired (Sheoran et al. 2010, Wong 2003). Due to the lack of vegetation, erosion on these  
54 areas is elevated, leading to a contamination of the surrounding areas due, for instance, to the transport  
55 of contaminated dusts by the wind. In addition, the underground water can be contaminated through  
56 leaching. Such spreading of the contamination can potentially be a threat to human health if the  
57 pollution enters the food chain through drinking water and crops. All these consequences were observed  
58 on mining sites such as the Pontgibaud site, a former French extraction mine site, located in the Massif-  
59 Central (France). This region is characterized by soils made of galena, arsenopyrite, and gray coppers,  
60 and a geochemical background showing the presence of Pb and arsenic (As). The mine district extracted  
61 argentiferous Galena until the end of the nineteenth century. It was the main mine producing silver (Ag)  
62 and Pb during the nineteenth century, and the largest metallic mine in France, extracting 50 000 tons of  
63 Pb and 100 tons of Ag. Previous studies on this site highlighted an acidic pH (pH 3-4), elevated  
64 concentrations in Pb and As and low organic matter and nutrient contents (Cottard 2010, Lebrun et al.  
65 2017, 2018, Nandillon et al. 2019a, b, Thouin et al. 2019). This contaminated site is in need of  
66 remediation, to ameliorate its physical, chemical and biological parameters and regain functionality.  
67 Compared to the conventional physical and chemical techniques that have been used over the last  
68 decades to treat metal(loid) polluted sites, bioremediation techniques using plants and/or  
69 microorganisms are cheaper and more environmentally friendly (Gong et al. 2018). Cristaldi et al. (2017)  
70 defined bioremediation as “the use of living organisms to clean up oil spills or remove other pollutants  
71 from soil, water or wastewater”. In the case of bioremediation of mine sites, coupling microbial  
72 bioremediation with phytoremediation is potentially a good option. Indeed, metal(loid)s cannot be

73 degraded but plants can immobilize them in the soil and microorganisms can convert metal(loid)s into a  
74 form which is potentially less toxic, less bioavailable and/or less mobile than the initial form (Ashraf et al.  
75 2019, Gong et al. 2018). Moreover, microorganisms can interact with plants, ameliorating their growth  
76 and thus making the phytoremediation process more efficient (Cristaldi et al. 2017). When plants are  
77 grown on a contaminated soil, they can uptake metal(loid)s and store them in their different tissues  
78 (Cristaldi et al. 2017). In addition, the vegetation cover will stabilize the soil and thus reduce erosion and  
79 leaching. Many plant species were shown to be efficient for the remediation of former mine  
80 contaminated soils, such as willows and poplars (Lebrun et al. 2017, 2018, 2019, Nandillon et al. 2019a),  
81 white mustard (Foucault et al. 2013) and birch (Alagić et al. 2013). However, these studies used species  
82 which were non-native to the polluted site. Interestingly, some other studies showed it is more effective  
83 to use species which are endemic to the site. For instance, Fahr et al. (2015) observed that the  
84 metallicolous populations of *Hirschfeldia incana* were adapted to tolerate and accumulate Pb and thus  
85 had good potential for the phytoremediation of Pb contaminated soils. Similarly, the metallicolous  
86 population of *Arabidopsis halleri* had a higher zinc (Zn) tolerance than the non-metallicolous population  
87 of the same species (Meyer et al. 2010). *Agrostis* plants, which are also found scarcely on the studied site  
88 of Pontgibaud, showed an adaptive tolerance, which allows a local adaptation to the soil contamination  
89 as demonstrated in the study of Austruy et al. (2013). At last, Nandillon et al. (2019b) found that *Agrostis*  
90 seeds collected from the contaminated soil of Pontgibaud showed a better growth on the amended mine  
91 soil compared to commercial seeds. Therefore, the use of indigenous plant species is strongly  
92 encouraged because they have a tolerance to the stress conditions of the soil, are adapted to the  
93 climatic conditions encountered, and thus require less maintenance and have a lower environment risk  
94 (introduction of non-native species) than the non-native species (Gerhardt et al. 2017, Laghlimi et al.  
95 2015).

96 Microbial remediation is defined as the use of microorganisms to adsorb, precipitate, oxidize or reduce  
97 metal(loid)s, thus lowering their (bio)availability and toxicity (Gong et al. 2018). Although they cannot  
98 degrade metal(loid)s, microorganisms can affect their behavior (Ashraf et al. 2019). In particular, in  
99 metal(loid) contaminated mining sites, microorganisms able to oxidize or reduce metal(loid)s are well  
100 represented (Battaglia et al. 2002, Lescure 2016). Such an ability can reduce their mobility and toxicity,  
101 which is particularly the case for As, whose toxicity depends on its speciation. For instance, Thouin et al.  
102 (2019) identified As(III)-oxidizing and As(V)-reducing bacteria in the Pontgibaud technosol. Resistance  
103 mechanisms used by metal(loid) resistant bacteria include: metal(loid) extracellular complexation,  
104 intracellular accumulation, oxido-reduction reactions and precipitations (Ashraf et al. 2019). In addition,  
105 microorganisms can benefit the plants, increasing their growth (Akhtar et al. 2018, Li et al. 2019).  
106 Therefore, microorganisms can be inoculated to the soil in order to improve plant growth and  
107 detoxifying activities and reduce the toxicity of metal(loid)s. The process involving the inoculation of  
108 microorganisms which are selected and acclimated to the soil conditions is called bioaugmentation  
109 (Emenike et al. 2018, Mishra et al. 2020). To increase the survival of the inoculated microorganisms and  
110 thus improve the remediation, endemic bacteria already adapted to the physical and chemical conditions  
111 of the studied site are preferred over non-native ones. For instance, Abdelkrim et al. (2019) isolated  
112 several strains from polluted soils, which showed an adaptive response against metal(loid)s. They  
113 showed that contaminated soils were a source for naturally resistant bacteria (metallotolerance). They  
114 also concluded that the adaptation of bacteria exposed to high levels of toxic pollutants was caused by  
115 the development of resistance mechanisms, therefore native bacteria could help in the bioremediation  
116 of metal(loid) contaminated sites. Finally, some studies showed that using several strains which formed a  
117 consortium gave better results than the inoculation of a single strain. For instance, Abdelkrim et al.  
118 (2018) isolated 12 bacterial strains from a contaminated soil based on their resistance to Pb and  
119 evaluated their ability to produce IAA (indole acetic acid), siderophores, solubilize P and accumulate Pb.

120 They found that when *Lathyrus stiivus* plants were inoculated with different combinations of these  
121 strains inoculated plant growth increased and there was a great potential for Pb bioaccumulation.  
122 Similarly, Dary et al. (2010) measured a 29 % increase in yellow lupine growth following soil inoculation  
123 with *Bradyrhizobium* strains, whereas the inoculation of a microbial consortium containing several plant  
124 growth promoting rhizobacteria capable of nodulation and N fixation increased plant growth by 109 %.  
125 Finally, the association of microorganisms and plants bring mutual benefits. Microorganisms give an  
126 external protection to the plants and bring nutrients, which stimulates plant growth, whereas  
127 microorganisms benefit from the root exudates of the plants for their development (Escalante-Espinosa  
128 et al. 2005).

129 In the context of rehabilitation of polluted mining sites, the objective of this study was to evaluate the  
130 impact of microbial consortia inoculation on a phytostabilization process. For this, two endemic  
131 microbial consortia were selected (based on their tolerance to As and Pb) from soils (both with and  
132 without presence of *Agrostis* plants) collected at the Pontgibaud metal(loid) contaminated former  
133 mining site. The effects of inoculating these soils with their respective microbial consortium was then  
134 evaluated through (i) soil physico-chemical properties, (ii) metal(loid) immobilization in the soil, (iii)  
135 growth of endemic *Agrostis* plants and their ability for metal(loid) accumulation, and (iv) soil bacterial  
136 community activity and structure, with the aim to verify whether microbial inoculation brought about  
137 the re-functionalization of the soil.

138

## 139 **2. Materials and methods**

### 140 **2.1. Soil sampling and characterization**

141 This study focused on the former Ag-Pb extraction mining district of Pontgibaud (Puy-de-Dome,  
142 Auvergne-Rhône-Alpes, France). This mining district was very active until the nineteenth century and

143 lead to 87000 m<sup>3</sup> of waste tailings highly contaminated with As (539 mg.kg<sup>-1</sup>) and Pb (11453 mg.kg<sup>-1</sup>)  
144 (Lebrun et al. 2019, Nandillon et al. 2019b).

145 Two types of soils were collected from the second settling pond of this mine district, near the village of  
146 Roure-les-Rosiers: soil C sampled in a zone that had no history of vegetation; soil A which corresponded  
147 to the rhizosphere soil of *Agrostis* plants found on a vegetated zone next to the zone used to collect soil  
148 C. Soil A was collected by shaking the roots of *Agrostis* plants in a container in order to recover soil  
149 adhering to the roots.

150 The two soils had similar water holding capacities (WHC) and pseudo-total As and Pb concentrations  
151 (determined as described in Lebrun et al. 2018, 2019) (Table 1). Soil As and Pb concentrations were 31-  
152 fold and 36-fold higher, respectively, than the maximum permissible concentrations in soil, given by the  
153 European Union and World Health Organization (Ashraf et al. 2019), thus highlighting the high pollution  
154 level of Pontgibaud technosol.

## 155 2.2. Microbial consortium selection

156 A previous study in our laboratory has demonstrated the presence of metal(loid) tolerant  
157 microorganisms as well as As(III)-oxidizing bacteria in the soils of the Pontgibaud site (Thouin et al. 2019).  
158 The first step here was to recover such microorganisms from soils C and A for microcosm experiments.  
159 For this, seeds of *Agrostis* collected on the Pontgibaud site were sown on soils A and C, in 500 g pots. The  
160 sowing density was 220 seeds per m<sup>2</sup>. Plants were grown for five weeks under greenhouse conditions: 20  
161 ± 5 °C, 16 h photoperiod and 800 µmol.m<sup>-1</sup>.s<sup>-1</sup> light intensity. After this growing period, the soil attached  
162 to the roots of *Agrostis* plants, *i.e.* rhizosphere soil, was collected by shaking the roots inside a sterile bag  
163 for a few minutes. Five grams of rhizosphere soil were mixed with 50 mL sterile NaCl (0.9 %), shaken for  
164 two hours (28 °C at 150 rpm), then gently centrifuged (2500xg during 5 min). The supernatant was  
165 recovered and constituted the soil microbial extracts. Next, microbial extracts were inoculated (at 10 %  
166 v/v) in liquid Luria-Bertani (LB) medium (composition for 1 L: 5 g yeast extract, 10 g NaCl, 10 g peptone)



167 supplemented with 0.10 mg.L<sup>-1</sup> sodium arsenate (Na<sub>2</sub>HAsO<sub>4</sub>) (As(III)) and 40 mg.L<sup>-1</sup> nitrate lead (Pb(NO<sub>3</sub>)<sub>2</sub>)  
168 (Pb(II)). Both As(III) and Pb(II) concentrations were chosen based on the concentrations usually found in  
169 the soil pore waters of the Pontgibaud technosol (Lebrun et al. 2019, Nandillon et al. 2019). Such  
170 cultures were done in order to maintain a selective pressure and allow the selection of a microbial  
171 community adapted to the presence of As and Pb. Cultures were incubated under aerobic conditions at  
172 28 °C and subjected to shaking (150 rpm). Five sub-cultures were carried out successively (every 2 days  
173 after checking the microbial growth using the absorbance value at 600 nm). Microorganisms that grew in  
174 the final sub-culture constituted the two consortia named Cons A (coming from soil A) and Cons C  
175 (coming from soil C).

## 176 2.3. Mesocosm pot experiment

### 177 2.3.1. Experimental design

178 Four treatments per soil were applied (Table S1): soil alone (named A or C), soil inoculated with its  
179 corresponding microbial consortium (*i.e.* Cons C or Cons A) (named AM or CM (M for Microorganisms)),  
180 soil sown with *Agrostis* (named AP or CP (P for Plant)), soil inoculated with its corresponding microbial  
181 consortium and sown with *Agrostis* seeds (named AMP or CMP). Pots were filled with 500 g of soil.  
182 Inoculation of microbial consortium, when needed, was performed by adding 30 mL of inoculum per pot;  
183 the inoculum contained 10<sup>9</sup> CFU (colony forming units) per mL. The number of CFU was determined by  
184 serial dilution plating onto LB medium after colonies numeration. Seeding with *Agrostis capillaris*, when  
185 needed, was done at a density of 220 seeds per m<sup>2</sup>. Each treatment was tested in four replicates (n=4) so  
186 that a total of 16 pots were prepared for each soil. Pots were then incubated for 28 days under  
187 greenhouse conditions: temperature of 20 ± 5 °C, photoperiod of 16 h and light intensity of 800 μmol.m<sup>-2</sup>.  
188 s<sup>-1</sup>.

189 At the end of the experiment (T28), all of the soil of the pot from each pot was collected, and an equal  
190 amount of soil of the four replicates (n = 4) of a given treatment were mixed together to obtain a  
191 composite sample for each treatment for soil microbial analysis.

#### 192 2.3.2. Plant analysis

193 In pots sown with *Agrostis* seeds, plants were harvested at the end of the incubation (T28). The aerial  
194 and root parts were separated and rinsed several times with tap water and distilled water to remove soil  
195 particles. Organs were dried for three days at 60 °C to measure dry weight. Finally, plant samples were  
196 subjected to acid digestion in microwave: 6 mL of 65 % HNO<sub>3</sub> and 3 mL of 35 % HCl were mixed with 0.2 g  
197 of plant sample and the mixtures were heated into a microwave, with a 15 min heating rate up to 180 °C,  
198 15 min resting at 180 °C and a 15 min cool down. The digested samples were recovered and diluted to 50  
199 mL with distilled water. As and Pb concentrations were measured using ICP-AES (Inductively Coupled  
200 Plasma Atomic Emission Spectroscopy) (ULTIMA 2, HORIBA, San Francisco, USA).

201

#### 202 2.3.3. Soil pore water sampling and analysis

203 Soil pore waters (SPWs) were sampled in all the pots twice during the pot experiment: before  
204 consortium inoculation and seed sowing (T0) and at the end of the experiment (T28). Sampling was done  
205 using soil moisture samplers (Rhizon®, model MOM, Rhizosphere Research Products, Wageningen, The  
206 Netherlands). pH, electrical conductivity (EC) and redox potential (Eh) were measured using a multimeter  
207 (Serveur Excellence). SPWs were then acidified (83 µL of 65 % HNO<sub>3</sub> in a 5 mL sample) to determine As  
208 and Pb concentrations by ICP-AES.

#### 209 2.3.4. As and Pb content in soils

210 Concentrations of CaCl<sub>2</sub> extractable As and Pb, corresponding to the plant's available amounts of  
211 metal(loid)s, were measured by mixing a 0.01 M CaCl<sub>2</sub> solution with soil (1:10 solid:liquid ratio) and

212 shaking at 150 rpm for 2 h at room temperature, as described in Lebrun et al. (2019). Solutions were  
213 then filtrated and As and Pb concentrations were measured by ICP-AES after acidification of the samples.

#### 214 2.3.5. Soil microbial activity

##### 215 *2.3.5.1 Soil enzyme activities linked to the C and P cycles*

216 The activity of four soil enzymes (alkaline phosphatase, acid phosphatase,  $\beta$ -glucosidase and hydrolytic  
217 activity towards fluorescein diacetate) were measured at the end of the pot experiment (T28), as  
218 described in Lebrun et al. (2021). For acid and alkaline phosphatase activities (related to the P cycle in  
219 soil), two grams of soil were mixed with two mL of buffer. The buffer was sodium acetate (0.1 M, pH 5)  
220 for acid phosphatase, and Tris-HCl (0.1 M, pH 8) for alkaline phosphatase. Extracellular extracts  
221 containing the enzymes of interest were then recovered by centrifugation (10 000 rpm, 10 min) and 100  
222  $\mu$ L were mixed with 100  $\mu$ L of 5 mM PNPP (4-nitrophenyl phosphate disodium salt hexahydrate) as  
223 substrate and incubated at 25 °C for three hours. The enzymatic reaction was stopped by adding 0.1 M  
224 NaOH (100  $\mu$ L) and absorbance was read at 410 nm, using a spectrometer  $\mu$ Quant (Bio-Tek Instruments,  
225 Inc., Winooski, Vermont, USA). The activity was calculated using the extinction coefficient of PNPP at 410  
226 nm,  $\epsilon = 19500 \text{ L.mol}^{-1}.\text{cm}^{-1}$  and expressed as  $\text{mU.g}^{-1} \text{ soil}$  (1 mU = 1  $\mu\text{g}.\text{min}^{-1}$ ).

227 The  $\beta$ -glucosidase activity, related to the carbon cycle, was assessed by mixing 0.1 g soil with citrate  
228 phosphate buffer (0.15 M, pH 4-5) and 10 mM PNPG (4-nitrophenyl  $\beta$ -D glucopyranoside) as substrate.  
229 After two hours of incubation at 37 °C, the supernatant was recovered by centrifugation (14000 x g for 3  
230 min), and 2 %  $\text{Na}_2\text{CO}_3$  was added before an absorbance measurement at 410 nm. The activity was  
231 calculated using  $\epsilon = 18400 \text{ L.mol}^{-1}.\text{cm}^{-1}$ .

232 The hydrolytic activity, which represents the overall microbial activity, was assessed by the FDA  
233 (fluorescein diacetate) test. For this, 0.1 g of soil were mixed with potassium phosphate buffer (60 mM,  
234 pH 7.6) and FDA solution (50 mM prepared in acetone) as substrate. The mixtures were incubated for 3 h

235 at 37 °C and 105 rpm on a stirring platform. The absorbance was read at 490 nm after a centrifugation  
236 step (10000rpm, 10 min) and the activity was calculated using  $\epsilon = 8000 \text{ L}\cdot\text{mol}^{-1}\cdot\text{min}^{-1}$ .

#### 237 *2.3.5.2 Biolog Ecoplates*

238 The community level physiological profile of the soil microbial community was determined using Biolog  
239 Ecoplates™ tests in microplates containing 31 carbon substrates present in triplicates.

240 Two grams of soil were vortexed with 10 mL sterile NaCl (0.9 %) for three minutes. The microbial extracts  
241 obtained after centrifugation at 3000 rpm were used to inoculate the microplates, 150  $\mu\text{L}$  per well.

242 Plates were incubated at 25 °C for one week and the absorbance values were measured at 590 nm after  
243 96 h were used to calculate the following parameters:

- 244 - Average well color development, AWCD = mean of absorbance
- 245 - Shannon-Weaver index,  $H' = - \sum p_i * \ln p_i$ , with  $p_i = \text{Abs}_i / \sum \text{Abs}$  and  $i$  representing the substrate
- 246 - Evenness,  $E = H' / \ln 31$
- 247 - Richness = number of wells with  $\text{Abs}_{590} > 0.25$

248

#### 249 *2.3.5.3 Microbial As(III)-oxidase activity*

250 The As(III) oxidation (into As(V)) activity of the soils microbial communities was evaluated using the  
251 As(III)-chelation properties of pyrrolidine dithiocarbamate (PDTC), according to Michel et al. (2020). Soil  
252 was vortexed briefly in NaCl (0.9 %) (1:10 solid:liquid ratio) for three minutes followed by a filtration  
253 (0.22  $\mu\text{m}$ ) at 3000 rpm. The microbial extract (supernatant) was inoculated into LB medium containing  
254 100  $\text{mg}\cdot\text{L}^{-1}$  As(III) and the culture was incubated at 25 °C until the end of the exponential phase of  
255 growth. Then, 0.5 mL of filtered growth medium were mixed with 0.5 mL of acetate buffer (0.1 M Na-  
256 acetate, pH 5) and 0.1 mL of a PDTC stock solution (5 g of PDTC (Sigma) dissolved in 1 L of demineralized  
257 water). In the presence of As(III), white precipitates appear immediately (whereas there are no  
258 precipitates with As(V)). The higher the concentration of As(III), the higher the amount and size of the

259 white precipitates. This test therefore allows a semi-quantitative detection (by visual appreciation) of  
260 As(III) concentration and thus As(III)-oxidizing activity. The presence of a large amount of precipitates  
261 means that no As(III) oxidation took place (designed as “ – “ in Table 3), whereas the absence of  
262 precipitates, or a small or medium amount of them indicate a high (“ +++ “), medium (“ ++ “) or weak  
263 (“ + “ in Table 3) As(III)-oxidising activity, respectively.

264

#### 265 2.3.6. Soil microbial community analyses.

266 Soil DNA was extracted from (i) initial rhizosphere soils used for microbial consortium selection, (ii) Cons  
267 A and Cons C consortia, and (iii) soils sampled at the end of the pot experiment (T28). For soil samples,  
268 0.5 g of soil were used, and for consortia, 1.5 mL of culture were centrifuged to obtain the pellet. DNA  
269 was extracted using the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) according to the  
270 manufacturer’s recommendations. Microbial DNA concentrations were measured using a NanoDrop  
271 (NanoDrop 1000 spectrophotometer, ThermoFisher Scientific, Watham, USA).

272 For soil DNAs analysis through quantitative PCR (qPCR) at the end of the pot experiment, soil microbial  
273 DNA concentrations were adjusted to  $1 \text{ ng} \cdot \mu\text{L}^{-1}$  and qPCR targeting 16S rRNA genes for bacterial biomass  
274 quantification was performed using a CFX Connect™ Real-Time PCR Detection System (Bio-Rad, France)  
275 and the following thermocycling conditions: 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 60 °C for 30 s, 72  
276 °C for 30 s and a data acquisition step at 80 °C for 30 s at each cycle. The reaction volume was 20  $\mu\text{L}$  and  
277 contained 7.6  $\mu\text{L}$  RNase and DNase free-water, 10  $\mu\text{L}$  SYBR Green IQ Supermix (Bio-Rad, France), 500 nM  
278 primers (341F and 515R) and 2 ng of DNA extracts. A calibration curve was constructed from 10-fold  
279 serial dilutions of a linear plasmid, containing  $2.2 \cdot 10^3$  to  $2.2 \cdot 10^7$  16S rRNA gene copies. All samples were  
280 analyzed in duplicates. Results were expressed as gene copies per g of soil.

281 For NGS (new generation sequencing), DNA samples were sent to INRAE Transfert (Narbonne, France) in  
282 order to generate amplicon libraries and MiSeq Illumina sequences of the V4-V5 variable region of the  
283 16S rRNA gene.

284 Fastq sequences were analyzed using the FROGS pipeline (Escudié et al. 2018). First, the pre-process tool  
285 was applied to delete primer sequences, sequences that were not of the expected length and those with  
286 ambiguous bases, and to merge the paired reads using VSEARCH. Sequences were clustered into OTUs  
287 (Operational Taxonomic Units) using SWARM and an aggregation distance of 3. After chimera removal  
288 and filtering for OTU abundance (threshold of 0.00005%), the remaining OTUs were affiliated using  
289 BLASTn and the Silva 132 16S database. A total of 1325398 sequences were retrieved, made up of 897  
290 OTUs. Samples contained between 52658 and 106321 sequences, and between 21 and 764 OTUs. Next,  
291 sequence abundance was normalized through random resampling in order to obtain an equal number of  
292 52 658 sequences per sample. FROGSSTAT was used to calculate the alpha and beta diversities, after  
293 normalization of the data. Finally, the affiliation of the most abundant OTUs (relative abundance > 5%)  
294 was verified using BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

#### 295 2.4. Statistical analysis

296 Data were analyzed using R software version 3.5.1 (R Core Team, 2017). The normality and  
297 homoscedasticity of the data were evaluated using Shapiro and Bartlett or Fligner tests, respectively.  
298 Means were then compared using Anova tests for parametric data and Kruskal tests for non-parametric  
299 data, followed by a post-hoc test, TukeyHSD test or Pairwise Wilcox test, respectively. Difference was  
300 considered significant at  $p < 0.05$ .

301 Finally, a Principal Component Analysis was performed on the soil and soil pore water parameters using  
302 the software PAST.

303

### 304 **3. Results**

305 3.1. Impact of microbial inoculation and *Agrostis* growth on the soil pore water chemical properties.

306 At the beginning of the experiment (T0), soil A pore water had an acidic pH, a low EC and an Eh of 366  
307 mV. These parameters were stable after 28 days as no evolution was observed in the absence of  
308 microbial inoculation and *Agrostis* seeding (treatment A) (Table 2). Microbial inoculation did not affect  
309 pH, EC and Eh, whereas it increased DOC level, both in presence and absence of *Agrostis*. The growth of  
310 *Agrostis* had no effect on pH, EC and Eh while it decreased DOC content in the condition without  
311 microbial inoculation (AP compared to A) (Table 2).

312 At T0, soil C pore water also had an acidic pH and a low EC, and its Eh was 393 mV. After 28 days, in the  
313 non-inoculated and non-seeded condition (C), no change in pH and EC was measured whereas Eh  
314 decreased compared to T0. Microbial inoculation had no influence on pH and EC, but gave a decreased  
315 Eh value in the presence of *Agrostis* (CMP compared to CP) and increased DOC content in both the  
316 presence and absence of *Agrostis* (CMP and CM). *Agrostis* growth had no effect on pH, EC and Eh but  
317 decreased DOC content in both inoculated and non-inoculated pots (CP and CMP) (Table 2).

318 These results thus showed that both soils reacted to microbial inoculation and *Agrostis* growth in the  
319 same way: microbial inoculation had no impact on soil pore water parameters (pH, EC and Eh), except  
320 for the DOC content which increased, and similarly *Agrostis* growth had no impact on pH and EC, but  
321 decreased DOC content.

322 3.2. Impact of microbial inoculation and *Agrostis* growth on metal(loid)s.

323 Arsenic and Pb concentrations were measured in SPW and CaCl<sub>2</sub> extractions, which give information on  
324 their mobility in soil, and availability to the plant, respectively.

325 In soil A, no difference was observed in As and Pb SPW concentrations between T0 and T28 for the  
326 condition A (no inoculation and no plant) suggesting that neither metal(loid)s was leached from soil with  
327 time (Table 2). Microbial inoculation had no effect on SPW and CaCl<sub>2</sub> As concentrations, but its presence  
328 led to a decrease in As availability in the presence of *Agrostis* (Table S2) and to an increase in SPW and

329 CaCl<sub>2</sub> Pb concentration in the presence or absence of *Agrostis* plants. When alone (in the absence of  
330 microbial inoculation) *Agrostis* growth had no effect on SPW As and Pb concentration but increased  
331 CaCl<sub>2</sub> As and Pb concentrations, compared to treatment A. When coupled to microbial inoculation,  
332 *Agrostis* growth led to an increase in SPW Pb (but not As) concentration, and CaCl<sub>2</sub> Pb concentration  
333 (Tables 2 and S2).

334 For soil C, after 28 days, the condition without consortium or plant had a lower As SPW concentration  
335 compared to T0 and the same Pb concentration, suggesting that As (but not Pb) was leached from the  
336 soil. In the presence of microbial inoculation, As leaching was not observed anymore as As in the SPW  
337 was the same in CM, CP and CMP conditions compared to the condition C at T0. Microbial inoculation  
338 increased the SPW Pb concentrations in the absence of *Agrostis*, while it had no effect on CaCl<sub>2</sub> As and  
339 Pb concentrations. *Agrostis* growth only affected the CaCl<sub>2</sub> Pb concentrations, leading to a rise in  
340 available Pb (Tables 2 and S2).

341 These results showed that As was leached from soil C with time, and this leaching was reduced with  
342 microbial inoculation and *Agrostis* growth. For both soils, microbial inoculation tended to mobilize and  
343 render the Pb more available (SPW and CaCl<sub>2</sub>) while *Agrostis* growth tended to mainly increase the  
344 availability of As and Pb (CaCl<sub>2</sub>).

345 Soil and soil pore water chemical data obtained at T0 and T28 during batch experiments were submitted  
346 to Principal Component Analysis (Figure S1). On this biplot, treatments clustered into three groups  
347 separated according to the first axe (PC1), explaining 99.67% of the variability, which was constrained by  
348 SPW EC, Eh, and Pb concentrations (Figure S2). Group 1 was composed of the initial and final soils (A and  
349 C) and the final vegetated soils (CP and AP), suggesting that *Agrostis* growth alone did not modify soil  
350 and SPW chemical properties. Group 2 was composed of the vegetated and inoculated final soils (AMP  
351 and CMP). Finally, soils inoculated with their respective inoculum (AM and CM) formed the 3<sup>rd</sup> group.  
352 PCA thus shows that *Agrostis* seeding and microbial inoculation had a similar effect on both soils, and



353 that *Agrostis* growth did not affect soil or SPW properties (soils with and without plants clustering  
354 together), while consortium inoculation affected soil and SPW.

### 355 3.3. Impact of microbial inoculation on plant parameters.

356 Dry weight (DW) production was measured after 28 days of growth. *Agrostis* aerial DW was higher in  
357 both inoculated soils (AMP and CMP) compared to the non-inoculated ones (AP and CP) (Figure  
358 1). However, no impact on *Agrostis* root DW was observed after microbial inoculation. Microbial  
359 inoculation also had no effect on As accumulation in *Agrostis* plants, whatever the soil (Figure 1).  
360 Concerning Pb accumulation in *Agrostis* plants, microbial inoculation decreased Pb aerial concentrations  
361 and increased root Pb concentrations of soil A (Figure 1), but had no effect on metal(loid) accumulation  
362 in *Agrostis* plants grown on soil C.

363

### 364 3.4. Impact of microbial inoculation and *Agrostis* growth on soil microbial activity.

365 In soil A, results showed a decrease in FDA activity and alkaline phosphatase activity when *Agrostis* was  
366 seeded alone or with microbial inoculation (AP and AMP) (Figure S3). *Agrostis* seeding also had an  
367 impact on some enzyme activities in soil C, with or without microbial inoculation, but no clear trends  
368 could be established.

369 Concerning microbial inoculation, it had no impact on the four tested enzyme activities in soil A, whereas  
370 in soil C, it impacted (decrease or increase) all the tested enzymes when in the absence of *Agrostis*  
371 (Figure S3).

372 These results thus suggest that the impact of bioaugmentation and plant seeding on soil enzyme  
373 activities varies according to the soil properties. It is therefore not possible at this point for us to  
374 determine which treatment is the best for the restoration of soil enzyme activities and the re-  
375 functionalization of soil.

376 Biolog Ecoplates™ were used to assess the community level physiological profiles. On soil A, microbial  
377 inoculation increased AWCD value, in both the presence and absence of plant, whereas Shannon Weaver  
378 H, Evenness E and richness increased only in the non-seeded condition. *Agrostis* growth did not affect the  
379 measured parameters (Table 3). On soil C, all diversity indices increased after microbial inoculation,  
380 whereas *Agrostis* growth had no effect (Table 3).

381 Both soils reacted the same to microbial inoculation, with an increase in diversity indices (AWCD, H, E  
382 and richness) whereas *Agrostis* growth had no significant effect.

383 Finally, the capacity of the soil microbial community to oxidize As(III) into As(V) was evaluated (Table 3).  
384 Soil A had a weak As(III) oxidation capacity in the absence of inoculation and plants (condition A); both  
385 microbial inoculation and *Agrostis* growth increased As oxidation capacity in this soil; the highest  
386 oxidation capacity was measured in the AMP condition.

387 Soil C had no As(III) oxidation capacity on its own. Microbial inoculation led to the detection of an As(III)  
388 oxidation activity. *Agrostis* growth only increased this activity in the inoculated condition (CMP  
389 compared to CM).

390 Overall, both the microbial inoculation and *Agrostis* growth were beneficial for the As(III)-oxidizing  
391 capacity of the microbial community of soils A and C.

392

393 3.5. Impact of microbial inoculation and *Agrostis* growth on soil bacterial community.

394 3.5.1 *Bacterial biomass*.

395 Microbial inoculation and *Agrostis* growth had no effect on bacterial biomass in either of the soils (Table  
396 4).

397 3.5.2 *Main OTUs*. Twelve OTUs had a relative abundance over 5 % in at least one sample (Figure 2).

398 In consortium C, three major OTUs were found: two OTUs affiliated to the *Lysinibacillus* genus and  
399 representing more than 85 % of the sequences, and one OTU affiliated to the *Bacillus* genus (8%

400 abundance). The two *Lysinibacillus* affiliated OTUs were also found at a high relative abundance (around  
401 29 % and 18 %) in soil C when inoculated with this consortium and seeded (CMP) or not (CM) with  
402 *Agrostis*, which suggested that both OTUs were able to grow once inoculated, and that bioaugmentation  
403 was successful. At the end of mesocosm experiments, the diversity profile of Soil C which hadn't been  
404 inoculated with its microbial consortium (C and CP) was closer to the initial profile of Soil C and the  
405 dominant *Lysinibacillus* OTU found in Cons C was in low abundance (< 5 %). Results also underlined the  
406 presence of one OTU, which was also also found in final soil C, and related to the *Chitinophagaceae*  
407 family, but its relative abundance was reduced with *Agrostis* growth (CP and CMP).

408 Consortium A contained one major OTU (96 %), affiliated to the *Bacillus* genus. This genus was found in  
409 higher abundance in soil A after consortium inoculation (AM and AMP compared to A and AP) suggesting  
410 that this OTU was able to develop once inoculated into soil A. Again, similarities were found between the  
411 vegetated and non-vegetated conditions of the soil inoculated with Cons A: *Bacillus*, *Rhodanobacter*,  
412 *Dyella*, and *Lysinobacillus* were found with (AMP) and without (AM) *Agrostis*. Divergent profiles were  
413 obtained in both conditions without the microbial consortium (A and AP): absence of *Bacillus*, a larger  
414 relative abundance of *Lysinobacillus*, and the presence of a *Mucolaginibacter* OTU.

415 **3.5.3 Alpha diversity.** The two initial soils used for pot experiments and consortium selection showed  
416 small variations in their alpha diversity: all four indices (Observed OTUs, Chao1, Shannon and  
417 InvSimpson) were higher for soil A than soil C; whereas on the other hand Cons C had higher alpha  
418 diversity indexes than Cons A (Table 4). Moreover, as expected and due to the selection pressure applied  
419 to obtain the consortia, the diversity of the consortia was much lower than that of their respective soils.  
420 At the end of the experiment, alpha diversity indexes were lowered following the inoculation of the  
421 consortium C compared to the non-inoculated soil C. For the InvSimpson index, a decrease was also  
422 observed after plant growth. Similarly, for soil A, the inoculation of consortium A decreased alpha

423 diversity indexes compared to the non-inoculated soil A. Moreover, the growth of *Agrostis* plant  
424 increased Chao1 and InvSimpson indexes compared the non-vegetated soil A.

425 In summary, in both soils, initial soils had higher diversity than consortia and microbial inoculation  
426 decreased the alpha diversity.

427 *3.5.4 Beta diversity.* The  $\beta$ -diversity of soils and consortia was analyzed by clustering (Figure S4). This  
428 clustering dendrogram showed two groups: one composed of both consortia, and the other one including  
429 all the soil samples. The second group can be further divided into two subgroups: one composed of soil A  
430 samples and the other of soil C samples. This underlines the impact of soil properties on bacterial  
431 diversity. Finally, it can be seen that plant growth had a greater effect on the beta diversity of soil C than  
432 microbial inoculation.

433

#### 434 **4. Discussion**

435 Waste amounts from extractive industries represent about 29 % of total waste generated in the  
436 European Union (EU) each year, with an annual volume in excess of 400 million tons (COM 2003, 319).

437 These mining wastes generate many environmental problems (Joran and Abdaal 2013). Some studies  
438 have even estimated that up to 1500 km of watercourses are polluted by metal mine discharges in the  
439 EU (Younger et al. 2002). For the remediation of such sites, both plants and microorganisms can be used,  
440 alone or combined, in order to reduce the risk induced by metal(loid)s and thus allow the rehabilitation  
441 of such sites. The efficiency of the bioremediation process will notably depend on the ability of the plants  
442 and microorganisms to tolerate the metal(loid) stress and to develop on the site. That is why species  
443 (plants as well as microorganisms) that are found on polluted mining sites, and already tolerant to the  
444 soil conditions and pollution, are a better option than foreign species selected at the laboratory scale.

445 When taken all together, the results of the present study globally led to the conclusion that the two  
446 tested soils exhibited the same response to inoculation (Table S2) which could suggest that the present

447 conclusions could be applied to other mine soils. Indeed, consortium inoculation was beneficial for soil  
448 by increasing DOC content, As(III) oxidation, microbial activity, and plant root DW, but it was negative for  
449 As and Pb mobility as well as microbial diversity. *Agrostis* growth increased the As(III) oxidation potential  
450 and the soil microbial diversity of soil A but it had negative effects on the microbial diversity of soil C as  
451 well as on soil As and Pb availability (Table S1). Such results demonstrated the positive effects of the  
452 inoculation of microbial biomass for the phytoremediation of a former mine technosol using endemic  
453 *Agrostis* plants.

454

455 4.1. Bioaugmentation (microbial inoculation) for improving soil properties and plants growth.

456 4.1.1. Impacts of consortium inoculation on soil properties.

457 Our results demonstrated an impact of the microbial inoculation on several soil parameters, such as DOC  
458 content and soil enzyme activities, which both increased. In our case, these impacts are positive as they  
459 improve soil quality for (micro)biological development. They can be considered as microbial bio-  
460 indicators of an effective soil rehabilitation (Baldrian 2009). The impact of bioaugmentation on soil  
461 properties has already been underlined in other studies. As an example, the increase in DOC content  
462 following microbial biomass addition has also been observed on Pb-Zn mine tailings by Wu et al. (2006a).  
463 Such result can be related to a better plant root development thanks to the microbial inoculation, which  
464 leads to more root exudates. Moreover, microorganisms can decompose organic matter and release  
465 soluble low molecular weight organic compounds (Wu et al. 2006b).

466 In our work, no impact on pH was observed for microbial inoculation when applied alone. Schoebitz and  
467 Vidal (2016) also observed that microbial consortium inoculation did not affect soil pH for inoculations  
468 performed on a slightly acidic sandy loam soil and a slightly acidic clay soil. However, other studies  
469 demonstrated that the inoculation of other types of soils with microorganisms can affect soil pH. For  
470 instance, the inoculation of a neutral phosphate mine soil with *Pseudomonas chlororaphis* and *Bacillus*

471 *megaterium* decreased soil pH whereas the strain *Arthrobacter pascens* had no effect (Yu et al. 2012). On  
472 the contrary, soil pH increased when the strains *Serratia liquefaciens* CL-1 and *Bacillus thuringiensis* X30  
473 were inoculated to a metal(loid) contaminated agricultural soil with a slightly acidic pH (Han et al. 2018).  
474 The observed pH increase following microbial inoculation of soil, in combination with *Agrostis* growth,  
475 could potentially be explained by the production of polyamines by the bacteria, such as putrescine,  
476 spermine and spermidine, as demonstrated by Han et al. (2018). When all information from these  
477 studies is combined, the results suggest that pH variation following bioaugmentation can mainly be  
478 expected in soils with an initial pH close to neutrality, but not in mine soils characterized by acidic  
479 conditions.

480 The third soil parameters known to be potentially impacted by soil bacterial inoculation is soil EC, which  
481 was shown to increase following several bioaugmentation approaches (Rojas-Tapias et al. 2014, Wu et al.  
482 2006b). Such an effect can be attributed to the synthesis of organic acids, the exclusion of protons, the  
483 production of chelating agents, as well as the production of various metabolites by the bacteria added to  
484 the soil (Wu et al. 2006b, Munir and Faisal 2016). However, in the present work, EC was shown to not be  
485 affected by microbial inoculation, demonstrating that contaminated mining technosols can have  
486 different responses to plant and microbial inoculation compared to other types of metal(loid) polluted  
487 soils.

488

#### 489 4.1.2. Impact on metal(loid) mobility.

490 In addition to its impact on soil properties, this study showed that bioaugmentation can also impact soil  
491 metal(loid) behavior by increasing As and Pb mobility. This is in agreement with previous studies,  
492 demonstrating that the addition of microorganisms to polluted soil can either mobilize, immobilize or  
493 have no effect on metal(loid) behaviors (Han et al. 2018, Li et al. 2017, Touceda- González et al. 2015).  
494 Microorganisms can affect metal(loid) behavior by secreting organic acids, which mobilize metal(loid)s

495 (Rojas-Tapias et al. 2014, He et al. 2009, Munir and Faisal 2016) or induce the production of siderophores  
496 that can chelate metal(loid)s, immobilizing them (Nicoară et al. 2014).

497 In addition to its impact on metal(loid) mobility, our results demonstrated that microbial inoculation also  
498 increased the soil's potential to oxidize As(III) into As(V). This can be related to the fact that consortia  
499 were selected based on their tolerance to As(III) and thus their potential oxidizing capacity. This is a  
500 beneficial reaction (Tirry et al. 2018), since As(V) is less toxic than As(III). The higher As(III) oxidation  
501 capacity following a consortium inoculation indicates that the inoculated soils had more microorganisms  
502 capable of As(III) oxidation (Ghosh et al. 2011), coming from the inoculum. Therefore, even if As was  
503 mobilized by the microbial inoculation, the higher As(III) oxidation activity of inoculated soil probably  
504 explains the improved plant growth in the presence of microbial inoculation.

505

#### 506 4.1.3. Impact of microbial inoculation on plants

507 In addition to improving soil properties, microbial inoculation ameliorated *Agrostis* growth on the  
508 polluted soil and increased *Agrostis*' capacity to accumulate As and Pb. Many studies also showed that  
509 inoculating a polluted soil with microorganisms was beneficial to plant growth. For instance, Chen et al.  
510 (2014) observed, in a hydroponic experiment, that the plants of *Sedum alfredii* collected on an old Pb/Zn  
511 mine, produced a higher aerial biomass when the endophytic bacteria *Sphingomonas* SaMR12 was  
512 inoculated. Similarly, in another hydroponic experiment and in a pot experiment using sand,  
513 supplemented with Pb, as a growing substrate, Abdelkrim et al. (2018) inoculated different consortia and  
514 found that *Lathyrus sativus* shoot biomass increased by 7 to 172 % and root biomass increased between  
515 10 and 85 % compared to non-inoculated conditions. Finally, on a Zn and Cd contaminated soil, De Maria  
516 et al. (2011) did not measure any effect of a microbial treatment (*Streptomyces* AR17 and *Agromyces*  
517 AR33) on the shoot biomass of *Salix caprea* whereas the inoculation with *Agromyces* AR33 increased  
518 root biomass. Such amelioration of plant growth after inoculation can be attributed to a better water

519 and nutrient availability following soil inoculation (Akhtar et al. 2018, Coccozza et al. 2015), which are low  
520 in the PG mine technosol used in this study (Lebrun et al. 2019, 2020, Nandillon et al. 2019b).  
521 Microorganisms produce organic acids that can ameliorate the availability of essential ions and nutrients  
522 for the plants (Munir and Faisal 2016). In particular, some microorganisms had the ability to solubilize  
523 phosphate and secrete hormone-like substances such as IAA and thus improve plant growth (Nicoară et  
524 al. 2014).

525 Lastly, the increase in metal(loid) accumulation was also demonstrated in previous studies such as that  
526 of Abdelkrim et al. (2018) in which microbial inoculation of a sandy soil increased *Lathyrus sativus* Pb  
527 uptake by 39 % in the shoots and 47 % in the roots. The shoots of *Averrhoa carambola* plants  
528 accumulated more Cd with the inoculation of a bacterial consortium to a paddy field contaminated with  
529 Cd (Li et al. 2017). Finally, soil inoculation increased Mn, Pb and Zn concentrations in the roots of  
530 *Agrostis capillaris* grown on mine tailings (Nicoară et al. 2014). Such an increase in metal(loid) plant root  
531 accumulation can be explained by the mobilization of metal(loid)s following inoculation (Wu et al.  
532 2006a). However, translocation towards the aerial parts was reduced in the presence of the inoculated  
533 microorganisms, which reduces the risk of contamination entry into the food chain.

534

#### 535 4.1.4. Impact of microbial inoculation on soil microbial community: activity and biodiversity

536 Regarding the bacterial community of the soil, its activity (linked to C and P cycles) generally increased  
537 with consortium inoculation, which was consistent with the studies of Teng et al. (2010) and Nicoară et  
538 al. (2014), who worked on an agricultural land contaminated with hydrocarbons and mine tailings,  
539 respectively. These observations can be attributed to the introduced microorganisms that have acquired  
540 a resistance to metal(loid) stress as an evolution trait, due to the presence of high metal(loid)  
541 concentrations in their growing environment over a long time (Nicoară et al. 2014). Consortium  
542 inoculation also affected the structure of the soil bacterial community. This can be due to the added



543 microorganisms (He et al. 2018 and Escalante-Espinosa et al. 2005), and also to the effects that these  
544 microorganisms have on the soil properties and metal(loid) mobility. Indeed, soil properties are  
545 important drivers of the bacterial community structure (Guo et al. 2017, Touceda- González et al. 2015).  
546 Another important microbiological parameter is the composition of the microbial community. When  
547 looking at the most present OTUs found in our samples, consortia and soils, many were affiliated to taxa  
548 already known to be metal(loid) tolerant, and/or found on mining sites. For instance, although absent  
549 from consortia, OTUs affiliated to the *Rhodanobacter* genus were found in the soil samples. This genus  
550 includes some species known to be acidotolerant and/or to be tolerant to several metal(loid)s, including  
551 As(III) (Dahal and Kim 2017). Other OTUs belonging to genera previously found on metal(loid)-  
552 contaminated soils were also recovered in the soil samples but not in the consortia. This was the case for  
553 the OTUs linked to the *Mucilaginibacter* genus, which includes some species already detected on mining  
554 sites and known to carry arsenic and metal resistant genes (Fan et al. 2018, Li et al. 2018).  
555 The two main OTUs of consortium C belonged to the *Lysinibacillus* genus. Some members of this genus  
556 are known to carry arsenic and other metals (Cd, Zn...) resistance genes (Peña-Montenegro and Dussàn  
557 2013, Rahman et al. 2014). Their high abundance in the inoculated C soils at the end of the experiment  
558 suggested that they were able to persist and grow once inoculated into the soil. The main OTU of  
559 consortium A was affiliated to the *Bacillus* genus. This genus is widely represented in many environments  
560 including As(III) contaminated ones, and several *Bacillus* species have been shown to be As resistant  
561 (Anderson and Cook 2004, Poudel et al. 2019). Microbial inoculation using consortia previously enriched  
562 from the site probably explains the good persistence of the inoculated OTUs. It thus positively impacts  
563 soil biodiversity as it leads to soil enrichment in tolerant strains.

564

565 4.2. Impact of plant seeding on the soil physicochemical and biological properties

566 Compared to consortium inoculation, the growth of *Agrostis* plants on both soils, inoculated or not with  
567 microbial biomass, had less effect on soil parameters, such as pH, EC and metal(loid) mobility. The main  
568 impacts were observed on soil As(III) oxidizing activity and bacterial community.

569 Soil As(III) oxidizing capacity increased with plant growth. This indicated that the presence of plants, and  
570 more probably their root exudates, could support microorganisms capable of oxidizing As(III), that were  
571 not present or only poorly present in the non-vegetated soil. *Agrostis* plant growth also affected soil  
572 bacterial community structure. This can be also attributed to the plant root exudates, which can strongly  
573 impact the composition of the bacterial soil community (Lucisine et al. 2014). The quantity and quality of  
574 root exudates are known to affect the bacterial community directly and also indirectly, by altering the soil  
575 properties, such as pH (Touceda- González et al. 2015). However here, as soil pH was not affected by  
576 plant presence, the compounds released by the roots may have directly affected the bacterial activity.

577 Regarding the potential impact of *Agrostis* growth on microbial biomass, our results suggested that  
578 bacterial biomass did not increase following the addition of microorganisms to the soil and plant  
579 development. Previous studies showed that microbial biomass was higher in inoculated and vegetated  
580 pots, and was noticed, according to the study, after 30 days (Cocozza et al. 2015) or more (180 days in  
581 the case of Escalante-Espinosa et al. 2005). Authors attributed this to a larger crop biomass and thus the  
582 return of more organic residues and exudates to the soil, which accelerates microbial biomass  
583 accumulation and activity (Ju et al. 2019). However, these studies were conducted on non-contaminated  
584 or artificially contaminated soils, with better growing conditions than those available on mine soils.  
585 Indeed, the soil used in the present study exhibited extreme conditions of pH (very acidic), organic  
586 matter content (low), nutrient content and availability (low) and metal(loid) contaminations levels (high),  
587 which are not in favor of high and rapid microorganism and plant growths. It can be hypothesized that  
588 on mining sites, a long period is needed after microorganism inoculation and plant seeding to allow

589 significant microbial and plant growth, due to the extreme physical and chemical conditions  
590 characterizing these kind of soils.

591

## 592 **5. Conclusion**

593 This study is the first which gives a complete picture of the association of microbial inoculation and  
594 endemic plant species, in a remediation process, and its effects on the soil properties, the metal(loid)  
595 mobility and availability, the plant growth as well as the microbial community structure and activity.  
596 Moreover, the study focused on a mining site, which is little studied compared to other soil types, and  
597 has particular characteristics, with elevated metal(loid) concentrations and acidic pH, making it very  
598 difficult to remediate. It underlines the interest of coupling phytoremediation with bioaugmentation to  
599 improve the phytoremediation process for the stabilization of polluted sites. Indeed, increasing soil  
600 microbial biomass via the addition of microorganisms significantly enhances soil revegetalization and  
601 increases metal(loid) accumulation in plant roots without increasing metal(loid) translocation and thus  
602 the risk of metal(loid) entry into the food chain. Soil microbial inoculation helps soil re-functionalization  
603 through the acquisition and/or increase in microbial activities such as those linked to the C and P cycles  
604 as well as the metal(loid) cycles (here As(III) oxidation). Thus, microbial bioaugmentation favors its  
605 rehabilitation by increasing soil organic matter content. In addition, endemic *Agrostis* seeds are good  
606 candidates for growing on such sites, and accumulated elevated concentrations of As and Pb in their  
607 roots, demonstrating the potential of this *Agrostis* ecotype for the phytoremediation of the Pontgibaud  
608 technosol, especially when associated with microbial inoculation.

609 In conclusion, this work suggests that a rapid and efficient phytostabilization-bioaugmentation approach  
610 has to be based on the use of endemic plant species and microorganisms adapted to the physical and  
611 chemical conditions of the polluted site to be treated, and selected for their tolerance and even redox  
612 activity towards metal(loid)s (in the case of microorganisms).

613

614 **Conflict of interest:** The author declares no conflict of interest.

615

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619

620 **References**

621 Abdelkrim, S., Jebara, S.H., Saadani, O., Chiboub, M., Abid, G., Jebara, M., 2018. Effect of Pb-resistant  
622 plant growth-promoting rhizobacteria inoculation on growth and lead uptake by *Lathyrus sativus*. *Journal*  
623 *of basic microbiology*, 58(7), 579-589.

624 Abdelkrim, S., Jebara, S.H., Saadani, O., Chiboub, M., Abid, G., Mannai, K. Jebara, M., 2019. Heavy metal  
625 accumulation in *Lathyrus sativus* growing in contaminated soils and identification of symbiotic resistant  
626 bacteria. *Archives of microbiology*, 201(1), 107-121.

627 Abujabhah, I.S., Doyle, R.B., Bound, S.A. Bowman, J.P., 2018. Assessment of bacterial community  
628 composition, methanotrophic and nitrogen-cycling bacteria in three soils with different biochar  
629 application rates. *Journal of soils and sediments*, 18(1), 148-158.

630 Akhtar, M.J., Ullah, S., Ahmad, I., Rauf, A., Nadeem, S.M., Khan, M.Y., Hussain, S. Bulgariu, L., 2018. Nickel  
631 phytoextraction through bacterial inoculation in *Raphanus sativus*. *Chemosphere*, 190, 234-242.

632 Alagić, S.Č., Šerbula, S.S., Tošić, S.B., Pavlović, A.N. Petrović, J.V., 2013. Bioaccumulation of arsenic and  
633 cadmium in birch and lime from the Bor region. *Archives of environmental contamination and toxicology*,  
634 65(4), 671-682.

635 Anderson C.R., Cook G.M. 2004. Isolation and characterization of arsenate-reducing bacteria from  
636 arsenic-contaminated sites in New Zealand. *Curr Microbiol*, 48(5), 341-7.

637 Ashraf, S., Ali, Q., Zahir, Z.A., Ashraf, S. Asghar, H.N., 2019. Phytoremediation: Environmentally  
638 sustainable way for reclamation of heavy metal polluted soils. *Ecotoxicology and Environmental Safety*,  
639 174, 714-727.

640 Austruy, A., Wanat, N., Moussard, C., Vernay, P., Joussein, E., Ledoigt, G. Hitmi, A., 2013. Physiological  
641 impacts of soil pollution and arsenic uptake in three plant species: *Agrostis capillaris*, *Solanum nigrum*  
642 and *Vicia faba*. *Ecotoxicology and environmental safety*, 90, 28-34.

643 Baldrian, P., 2009. Microbial enzyme-catalyzed processes in soils and their analysis. *Plant Soil Environ*,  
644 55(9), 370-378.

645 Battaglia-Brunet F., Dictor M.C., Garrido F., Crouzet C., Morin D., Dekeyser K., Clarens M., Baranger P.,  
646 2002. An arsenic(III)-oxidizing bacterial population: selection, characterisation, and performance in  
647 reactors. *J. Appl. Microbiol.*, 93, 656-667.

648 Chen, B., Zhang, Y., Rafiq, M.T., Khan, K. Y., Pan, F., Yang, X. and Feng, Y., 2014. Improvement of  
649 cadmium uptake and accumulation in *Sedum alfredii* by endophytic bacteria *Sphingomonas* SaMR12:  
650 effects on plant growth and root exudates. *Chemosphere*, 117, 367-373.

651 Coccozza, C., Trupiano, D., Lustrato, G., Alfano, G., Vitullo, D., Falasca, A., Lomaglio, T., De Felice, V., Lima,  
652 G., Ranalli, G.S, Scippa, Tognetti, R., 2015. Challenging synergistic activity of poplar–bacteria association  
653 for the Cd phytostabilization. *Environmental Science and Pollution Research*, 22(24), 19546-19561.

654 COM(2003) 319 final. Proposal for a directive of the European parliament and of the council on the  
655 management of waste from the extractive industries. COM (2003) 319 final, 2003/0107 (COD),  
656 Commission of the European Communities, Brussels. [https://urldefense.com/v3/\\_\\_http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2003:0319:FIN:EN:PDF\\_\\_;!!KbSiYrE!yYZRr0VWLgTmyPXc0cUlmIM3XuZlQIoPSRYyFvt8QbaUUJ-Gg24UzhOdR3rKu94\\$](https://urldefense.com/v3/__http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2003:0319:FIN:EN:PDF__;!!KbSiYrE!yYZRr0VWLgTmyPXc0cUlmIM3XuZlQIoPSRYyFvt8QbaUUJ-Gg24UzhOdR3rKu94$)

659 Cottard, F., 2010. Résultats des caractérisations complémentaires effectuées sur différents milieux dans  
660 le district minier de Pontgibaud, (63). BRGM/RP-58571-FR.

661 Cristaldi, A., Conti, G.O., Jho, E.H., Zuccarello, P., Grasso, A., Copat, C., Ferrante, M., 2017.  
662 Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. *Environmental*  
663 *Technology & Innovation*, 8, 309-326.

664 Dahal, R.H., Chaudhary, D.K., Kim, J., 2018. *Rhodanobacter hydrolyticus* sp. nov., a novel DNA-and  
665 tyrosine-hydrolysing gamma proteobacterium isolated from forest soil. *International journal of*  
666 *systematic and evolutionary microbiology*, 68(8), 2580-2586.

667 Dary, M., Chamber-Pérez, M.A., Palomares, A. J., Pajuelo, E., 2010. "In situ" phytostabilisation of heavy  
668 metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting  
669 rhizobacteria. *Journal of Hazardous Materials*, 177(1-3), 323-330.

670 De Maria, S., Rivelli, A. R., Kuffner, M., Sessitsch, A., Wenzel, W. ., Gorfer, M., Straus, J., Puschenreiter,  
671 M., 2011. Interactions between accumulation of trace elements and macronutrients in *Salix caprea* after  
672 inoculation with rhizosphere microorganisms. *Chemosphere*, 84(9), 1256-1261.

673 Emenike, C.U., Jayanthi, B., Agamuthu, P. and Fauziah, S.H., 2018. Biotransformation and removal of  
674 heavy metals: a review of phytoremediation and microbial remediation assessment on contaminated  
675 soil. *Environmental Reviews*, 26(2), 156-168.

676 Escalante-Espinosa, E., Gallegos-Martínez, M.E., Favela-Torres, E., Gutiérrez-Rojas, M., 2005.  
677 Improvement of the hydrocarbon phytoremediation rate by *Cyperus laxus* Lam. inoculated with a  
678 microbial consortium in a model system. *Chemosphere*, 59(3), 405-413.

679 Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., Maman, S., Hernandez-Raquet,  
680 G., Combes, S., Pascal, G., 2018. FROGS: find, rapidly, OTUs with galaxy solution. *Bioinformatics*, 34(8),  
681 1287-1294.

682 Fahr, M., Laplaze, L., El Mzibri, M., Doumas, P., Bendaou, N., Hocher, V., Bogusz, D., Smouni, A., 2015.  
683 Assessment of lead tolerance and accumulation in metallicolous and non-metallicolous populations of  
684 *Hirschfeldia incana*. *Environmental and experimental botany*, 109, 186-192.

685 Fan, X., Tang, J., Nie, L., Huang, J., Wang, G., 2018. High-quality-draft genome sequence of the heavy  
686 metal resistant and exopolysaccharides producing bacterium *Mucilaginibacter pedocola* TBZ30 T.  
687 *Standards in genomic sciences*, 13(1), 1-8.

688 Festa, S., Coppotelli, B.M., Morelli, I.S., 2016. Comparative bioaugmentation with a consortium and a  
689 single strain in a phenanthrene-contaminated soil: Impact on the bacterial community and  
690 biodegradation. *Applied Soil Ecology*, 98, 8-19.

691 Foucault, Y., Lévêque, T., Xiong, T., Schreck, E., Austruy, A., Shahid, M., Dumat, C., 2013. Green manure  
692 plants for remediation of soils polluted by metals and metalloids: Ecotoxicity and human bioavailability  
693 assessment. *Chemosphere*, 93(7), 1430-1435.

694 Gerhardt, K., Gerwing, P., Greenberg, B., 2017. Opinion: Taking phytoremediation from proven  
695 technology to accepted practice. *Plant Science*, 256, 170-185.

696 Ghosh, P., Rathinasabapathi, B., Ma, L. Q., 2011. Arsenic-resistant bacteria solubilized arsenic in the  
697 growth media and increased growth of arsenic hyperaccumulator *Pteris vittata* L. *Bioresource*  
698 *technology*, 102(19), 8756-8761.

699 Gong, Y., Zhao, D., Wang, Q., 2018. An overview of field-scale studies on remediation of soil  
700 contaminated with heavy metals and metalloids: Technical progress over the last decade. *Water*  
701 *research*.

702 Guo, H., Nasir, M., Lv, J., Dai, Y., Gao, J., 2017. Understanding the variation of microbial community in  
703 heavy metals contaminated soil using high throughput sequencing. *Ecotoxicology and environmental*  
704 *safety*, 144, 300-306.

705 Han, H., Sheng, X., Hu, J., He, L., Wang, Q., 2018. Metal-immobilizing *Serratia liquefaciens* CL-1 and  
706 *Bacillus thuringiensis* X30 increase biomass and reduce heavy metal accumulation of radish under field  
707 conditions. *Ecotoxicology and environmental safety*, 161, 526-533.

708 He, L.Y., Chen, Z.J., Ren, G.D., Zhang, Y.F., Qian, M., Sheng, X.F., 2009. Increased cadmium and lead  
709 uptake of a cadmium hyperaccumulator tomato by cadmium-resistant bacteria. *Ecotoxicology and*  
710 *Environmental Safety*, 72(5), 1343-1348.

711 He, R., Peng, Z., Lyu, H., Huang, H., Nan, Q., Tang, J., 2018. Synthesis and characterization of an iron-  
712 impregnated biochar for aqueous arsenic removal. *Science of The Total Environment*, 612, 1177-1186.

713 Ji, H., Zhang, Y., Bararunyeretse, P., Li, H., 2018. Characterization of microbial communities of soils from  
714 gold mine tailings and identification of mercury-resistant strain. *Ecotoxicology and environmental safety*,  
715 165, 182-193.

716 Jordan, G., Abdaal, A., 2013. Decision support methods for the environmental assessment of  
717 contamination at mining sites. *Environmental Monitoring and Assessment*, 185, 7809-7832

718 Laghlimi, M., Baghdad, B., Hadi, H., Bouabdli, A., 2015. Phytoremediation mechanisms of heavy metal  
719 contaminated soils: a review. *Open Journal of Ecology*, 05(08), 375-388.

720 Langella, F., Grawunder, A., Stark, R., Weist, A., Merten, D., Haferburg, G., Büchel, G., Kothe, E., 2014.  
721 Microbially assisted phytoremediation approaches for two multi-element contaminated sites.  
722 *Environmental Science and Pollution Research*, 21(11), 6845-6858.

723 Lebrun, M., Macri, C., Miard, F., Hattab-Hambli, N., Motelica-Heino, M., Morabito, D., Bourgerie, S.,  
724 2017. Effect of biochar amendments on As and Pb mobility and phytoavailability in contaminated mine  
725 technosols phytoremediated by *Salix*. *Journal of Geochemical Exploration*, 182, 149-156.

726 Lebrun, M., Miard, F., Nandillon, R., Scippa, G.S., Bourgerie, S., Morabito, D., 2019. Biochar effect  
727 associated with compost and iron to promote Pb and As soil stabilization and *Salix viminalis* L. growth.  
728 *Chemosphere*, 222, 810-822.

729 Lebrun, M., Miard, F., Nandillon, R., Hattab-Hambli, N., Scippa, G.S., Bourgerie, S., Morabito, D., 2018.  
730 Eco-restoration of a mine technosol according to biochar particle size and dose application: study of soil



731 physico-chemical properties and phytostabilization capacities of *Salix viminalis*. *Journal of Soils and*  
732 *Sediments*, 18(6), 2188-2202.

733 Lebrun, M., Nandillon, R., Miard, F., Le Forestier, L., Morabito, D., Bourgerie, S., 2020. Effects of biochar,  
734 ochre and manure amendments associated with a metallicolous ecotype of *Agrostis capillaris* on As and  
735 Pb stabilization of a former mine technosol. *Environmental Geochemistry and Health*.

736 Lebrun, M., Miard, F., Van Poucke, R., Tack, F. M., Scippa, G.S., Bourgerie, S., Morabito, D., 2021. Effect of  
737 fertilization, carbon-based material and redmud amendments on the bacterial activity and diversity of a  
738 metal (loid) contaminated mining soil. *Land Degradation & Development* (in press)

739 Lescure, T., Moreau J., Charles, C., Ben Ali Saanda, T., Thouin, H., Pillas, N., Bauda, P., Lamy, I., Battaglia-  
740 Brunet, F., 2016. Influence of organic matters on AsIII oxidation by the microflora of polluted soils.  
741 *Environ Geochem Health*, 38(3), 911–925.

742 Li, J.T., Liang, Z.W., Jia, P., Liu, J., Xu, Y.J., Chen, Y.J., Chu, H.Y., Kuang, J.L., Liao, B., Shu, W.S., 2017.  
743 Effects of a bacterial consortium from acid mine drainage on cadmium phytoextraction and indigenous  
744 soil microbial community. *Plant and Soil*, 415(1-2), 347-358.

745 Li, X., Yan, Z., Gu, D., Li, D., Tao, Y., Zhang, D., Su, L., Ao, Y., 2019. Characterization of cadmium-resistant  
746 rhizobacteria and their promotion effects on *Brassica napus* growth and cadmium uptake. *Journal of*  
747 *basic microbiology*, 59(6), 579-590.

748 Li, Y.P., Carraro, N., Yang, N., Liu, B., Xia, X., Feng, R., Saquib, Q., AL-Wathnani, H.A., van der Meer, J.R.,  
749 Rensing, C., 2018. Genomic islands confer heavy metal resistance in *Mucilaginibacter kameinonensis* and  
750 *Mucilaginibacter rubeus* isolated from a gold/copper mine. *Genes*, 9(12), 573.

751 Lucisine, P., Echevarria, G., Sterckeman, T., Vallance, J., Rey, P., Benizri, E., 2014. Effect of  
752 hyperaccumulating plant cover composition and rhizosphere-associated bacteria on the efficiency of  
753 nickel extraction from soil. *Applied Soil Ecology*, 81, 30-36.

754 Meyer, C.L., KostECKa, A. A., Saumitou-Laprade, P., Créach, A., Castric, V., Pauwels, M., Frérot, H., 2010.  
755 Variability of zinc tolerance among and within populations of the pseudometallophyte species  
756 *Arabidopsis halleri* and possible role of directional selection. *New Phytologist*, 185(1), 130-142.

757 Michel, C., Jouliau, C, Hafida, T., Charnois, F., Battaglia-Brunet, F., 2020. Rapid and simple As(III)  
758 quantification using a turbidimetric test for the monitoring of microbial arsenic bio-transformation. *J*  
759 *Microbiol Meth*, 177, 106026.

760 Mishra, J., Singh, R., Arora, N.K., 2017. Alleviation of heavy metal stress in plants and remediation of soil  
761 by rhizosphere microorganisms. *Frontiers in Microbiology*, 8, 1706.

762 Munir, I., Faisal, M., 2016. Plant growth-promoting bacteria: a good source for phytoremediation of  
763 metal-contaminated soil. In *Phytoremediation* (pp. 119-129). Springer International Publishing.

764 Nandillon, R., Lebrun, M., Miard, F., Gaillard, M., Sabatier, S., Villar, M., Bourgerie, S. Morabito, D., 2019.  
765 Capability of amendments (biochar, compost and garden soil) added to a mining technosol contaminated  
766 by Pb and As to allow poplar seed (*Populus nigra* L.) germination. *Environmental monitoring and*  
767 *assessment*, 191(7), 465.

768 Nandillon, R., Lebrun, M., Miard, F., Gaillard, M., Sabatier, S., Morabito, D., Bourgerie, S., 2019.  
769 Contrasted tolerance of *Agrostis capillaris* metallicolous and non-metallicolous ecotypes in the context of  
770 a mining technosol amended by biochar, compost and iron sulfate. *Environmental Geochemistry and*  
771 *Health*, 1-19.

772 Nicoară, A., Neagoe, A., Stancu, P., de Giudici, G., Langella, F., Sprocati, A. R., Iordache, V., Kothe, E.,  
773 2014. Coupled pot and lysimeter experiments assessing plant performance in microbially assisted  
774 phytoremediation. *Environmental Science and Pollution Research*, 21(11), 6905-6920.

775 Peña-Montenegro, T.D., Dussán, J., 2013. Genome sequence and description of the heavy metal tolerant  
776 bacterium *Lysinibacillus sphaericus* strain OT4b. 31. *Standards in genomic sciences*, 9(1), 42-56.

777 Poudel, P., Nepal, A., Magar, R.R., Rauniyar, P., Magar, L.B., 2019. Screening of potent arsenic resistant  
778 and plant growth promoting *Bacillus* species from the soil of Terai region of Nepal. *Tribhuvan University*  
779 *Journal of Microbiology*, 6, 1-9.

780 R Core Team (2017). R: A language and environment for statistical computing. R Foundation for  
781 Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

782 Rahman, A., Nahar, N., Nawani, N. N., Jass, J., Desale, P., Kapadnis, B. P., Hossain, K., Saha, A. K., Ghosh,  
783 S., Olsson, B., Mandal, A., 2014. Isolation and characterization of a *Lysinibacillus* strain B1-CDA showing  
784 potential for bioremediation of arsenics from contaminated water. *Journal of Environmental Science and*  
785 *Health, Part A*, 49(12), 1349-1360.

786 Rojas-Tapias, D.F., Bonilla, R., Dussán, J., 2014. Effect of inoculation and co-inoculation of *Acinetobacter*  
787 sp. RG30 and *Pseudomonas putida* GN04 on growth, fitness, and copper accumulation of maize (*Zea*  
788 *mays*). *Water, Air, & Soil Pollution*, 225(12), 2232.

789 Schoebitz, M., Vidal, G., 2016. Microbial consortium and pig slurry to improve chemical properties of  
790 degraded soil and nutrient plant uptake (Schoebitz and Vidal, 2016). *Journal of soil science and plant*  
791 *nutrition*, 16(1), 226-236.

792 Sheoran, V., Sheoran, A.S., Poonia, P., 2010. Soil reclamation of abandoned mine land by revegetation: a  
793 review. *International journal of soil, sediment and water*, 3(2), 13.

794 Stolte, J., Tesfai, M., Oygarden, L., Kvaerno, S., Keizer, J., Verheijen, F., Panagos, P., Ballabio, C. Hessel, R.,  
795 2016. *Soil threats in Europe: status, methods, drivers and effects on ecosystem services: deliverable 2.1*  
796 *RECARE project*. European Commission DG Joint Research Centre.

797 Thouin, H., Norini, M.-P., Le Forestier L., Gautret, P., Motelica-Heino M., Breeze, D., Gassaud, C.,  
798 Battaglia-Brunet, F., 2019. Microcosm-scale biogeochemical stabilization of Pb, As, Ba and Zn in mine  
799 tailings amended with manure and ochre. *Applied Geochemistry*, 104438.

800 Tirry, N., Joutey, N. T., Sayel, H., Kouchou, A., Bahafid, W., Asri, M., El Ghachtouli, N., 2018. Screening of  
801 plant growth promoting traits in heavy metals resistant bacteria: prospects in phytoremediation. *Journal*  
802 *of Genetic Engineering and Biotechnology*, 16(2), 613-619.

803 Touceda-González, M., Álvarez-López, V., Prieto-Fernández, Á., Rodríguez-Garrido, B., Trasar-Cepeda, C.,  
804 Mench, M., Puschenreiter, M., Quintela-Sabarís, C., Macías-García, F., Kidd, P., 2017. Aided  
805 phytostabilisation reduces metal toxicity, improves soil fertility and enhances microbial activity in Cu-rich  
806 mine tailings. *Journal of Environmental Management*, 186, 301-313.

807 Vareda, J.P., Valente, A. J., Durães, L., 2019. Assessment of heavy metal pollution from anthropogenic  
808 activities and remediation strategies: A review. *Journal of environmental management*, 246, 101-118.

809 Wong, M.H., 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated  
810 soils. *Chemosphere*, 50(6), 775-780.

811 Wu, S.C., Cheung, K.C., Luo, Y.M., Wong, M.H., 2006a. Effects of inoculation of plant growth-promoting  
812 rhizobacteria on metal uptake by *Brassica juncea*. *Environmental Pollution*, 140(1), 124-135.

813 Wu, S.C., Luo, Y.M., Cheung, K.C., Wong, M.H., 2006b. Influence of bacteria on Pb and Zn speciation,  
814 mobility and bioavailability in soil: a laboratory study. *Environmental Pollution*, 144(3), 765-773.

815 Younger, P.L., Coulton, R.H., Froggatt, E.C., 2005. The contribution of science to risk-based decision-  
816 making: Lessons from the development of full-scale treatment measures for acidic mine waters at Wheal  
817 Jane, UK. *Science of the Total Environment*, 338, 137-154.

818 Yu, X., Liu, X., Zhu, T.H., Liu, G.H., Mao, C., 2012. Co-inoculation with phosphate-solubilizing and  
819 nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and  
820 nutrient uptake by walnut. *European Journal of Soil Biology*, 50, 112-117.

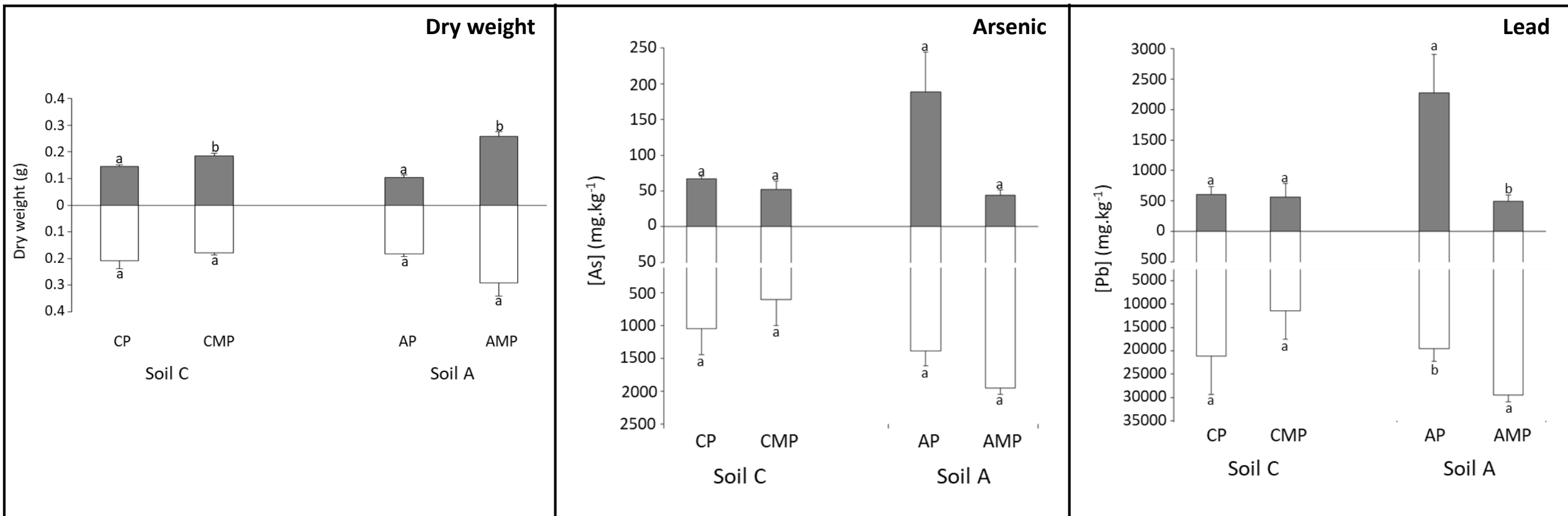


Figure 1: Dry weight (g), arsenic and lead concentrations (mg.kg<sup>-1</sup>) of aerial (grey) and root (white) parts of *Agrostis capillaris* plants grown on the soils with or without consortium inoculation (M). Letters indicate difference between the treatments of each soil (soil C and soil A) ( $p < 0.05$ ) ( $n = 4$ ).

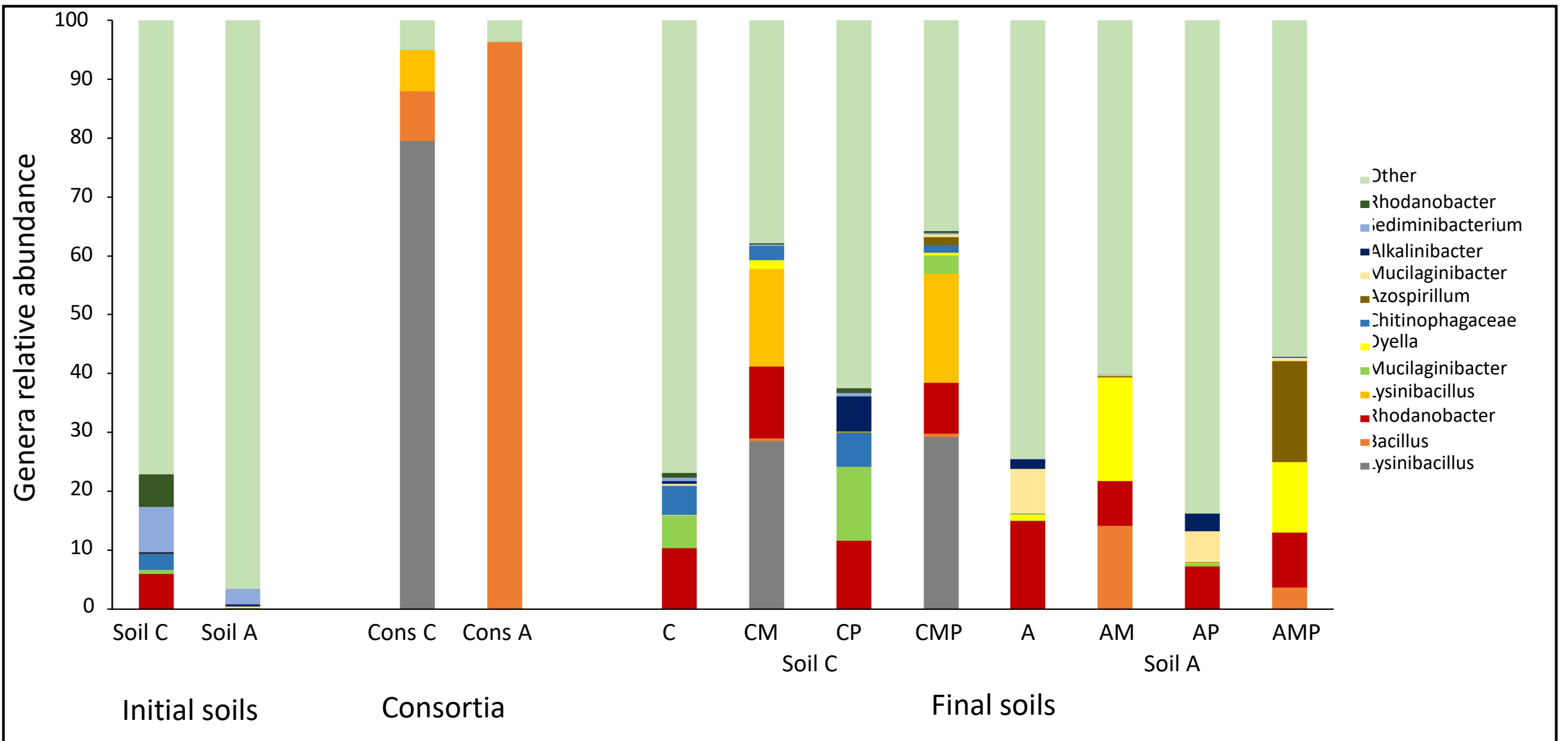


Figure 2: Relative abundance of the dominant genera ( $p \geq 5\%$ ) in the two initial soils (soil C and soil A), the two consortia (Cons C and Cons A) and in soil at the end of the experiment in soils C and A with or without consortium inoculation (M) and with or without *Agrostis* seedlings (P).

Table 1: Soil properties before seed sowing.

WHC = water holding capacity (%). Soil C = soil collected before microbial inoculation on unvegetated Pontgibaud site (= control soil); soil A = soil collected before microbial inoculation on Pontgibaud site vegetated by *Agrostis capillaris*. Letters (bold characters) indicate significant difference ( $p < 0.05$ ) ( $n = 3-4$ ).

	WHC (%)	[As] (mg.kg <sup>-1</sup> )	[Pb] (mg.kg <sup>-1</sup> )
Soil C	31.43 ± 0.17 <b>a</b>	577 ± 61 <b>a</b>	12902 ± 1750 <b>a</b>
Soil A	31.33 ± 0.07 <b>a</b>	674 ± 113 <b>a</b>	8770 ± 1100 <b>a</b>

Table 2: Soil pore water physico-chemical properties (pH, electrical conductivity (EC) ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), redox potential (Eh) (mV), metal(loid) (As and Pb) concentrations ( $\text{mg}\cdot\text{L}^{-1}$ ) and dissolved organic carbon (DOC) carbon ( $\text{mg}\cdot\text{L}^{-1}$ ) collected at the beginning (T0) and at the end (T28) of the pot experiment in the soils (C and A) with or without consortium inoculation (M) and with or without *Agrostis* seedings (P). Letters indicate difference between the four treatments for each soil (soil C and soil A) ( $p < 0.05$ ) (n = 4-8). ND: not determined

			pH	EC ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	Eh (mV)	[As] ( $\text{mg}\cdot\text{L}^{-1}$ )	[Pb] ( $\text{mg}\cdot\text{L}^{-1}$ )	DOC ( $\text{mg}\cdot\text{L}^{-1}$ )
Soil C	T0		4.8 ± 0.1 b	338 ± 10 b	393 ± 14 a	0.30 ± 0.01 a	28.74 ± 2.38 b	ND
	T28	C	5.5 ± 0.3 ab	1050 ± 431 ab	268 ± 23 bc	0.06 ± 0.01 b	15.79 ± 1.28 b	23.6 ± 0.3 b
		CM	5.1 ± 0.2 ab	11347 ± 715 a	270 ± 13 bc	0.30 ± 0.16 a	50.62 ± 9.68 a	29.3 ± 1.1 a
		CP	5.0 ± 0.2 ab	614 ± 81 ab	337 ± 14 ab	0.09 ± 0.02 ab	24.29 ± 4.88 b	9.4 ± 0.3 d
		CMP	5.6 ± 0.2 a	5101 ± 233 a	231 ± 17 c	0.34 ± 0.04 ab	31.27 ± 7.11 ab	20.2 ± 0.2 c
Soil A	T0		4.9 ± 0.1 a	273 ± 10 b	366 ± 11 a	0.30 ± 0.00 b	19.33 ± 1.25 c	ND
	T28	A	5.3 ± 0.2 a	1215 ± 116 ab	319 ± 21 ab	0.31 ± 0.00 ab	40.51 ± 3.43 c	15.2 ± 0.3 b
		AM	4.8 ± 0.2 a	9696 ± 936 a	289 ± 16 b	0.48 ± 0.06 a	125.55 ± 19.82 a	25.9 ± 0.7 a
		AP	5.3 ± 0.1 a	705 ± 109 ab	336 ± 5 ab	0.33 ± 0.00 ab	34.30 ± 3.33 c	10.3 ± 0.1 c
		AMP	5.0 ± 0.1 a	5567 ± 126 a	295 ± 5 b	0.55 ± 0.08 a	71.52 ± 2.87 b	27.1 ± 0.9 a

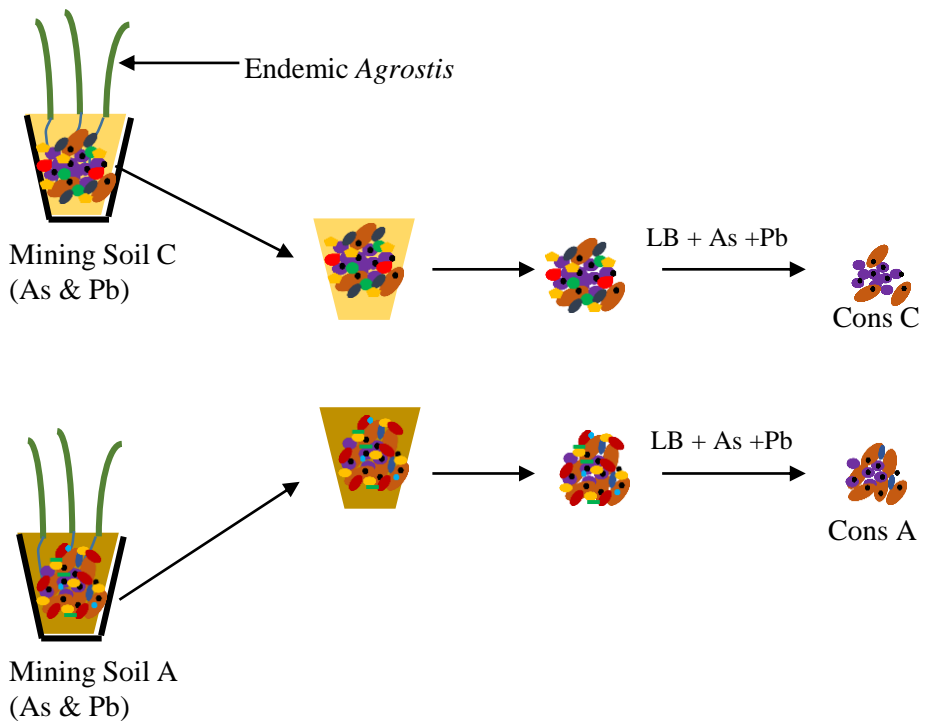


Table 3: Biolog microbial activity of the soils (C and A) with or without consortium inoculation (M) and with or without *Agrostis* seedings (P). Soils were collected at the end of the pots experiment (28 days). Microbial activity was assessed using Biolog parameters (AWCD = average well color development, H = Shannon Weaver diversity index, E = evenness, Richness = number of well with an absorbance < 0.25) and the capacity to oxidize As (III) into As (V) (in this case, « - » means no As(III) oxidation, « + », « ++ » and « +++ » mean weak, medium and high As(III)-oxidising activity, respectively). Letters indicate difference between the four treatments of each soil (soil C and soil A) ( $p < 0.05$ ) ( $n = 3$ ).

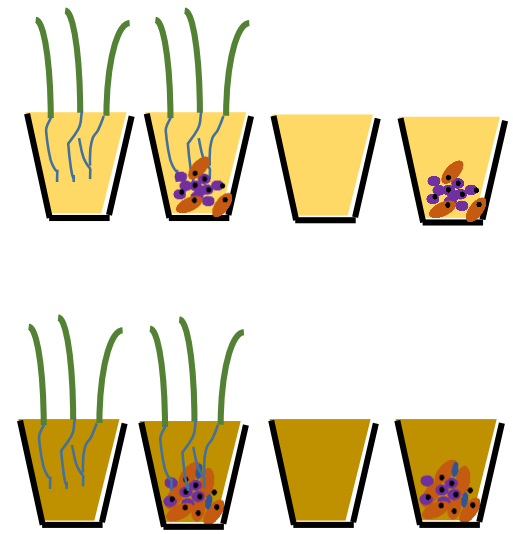
		AWCD	H	E	Richness	AsIII --> AsV
Soil C	C	0.64 ± 0.03 c	3.00 ± 0.03 b	0.87 ± 0.01 b	21 ± 0 b	-
	CM	1.15 ± 0.11 a	3.17 ± 0.03 a	0.92 ± 0.01 a	26 ± 1 a	+
	CP	0.79 ± 0.02 bc	3.07 ± 0.03 ab	0.89 ± 0.01 ab	24 ± 0 ab	-
	CMP	0.95 ± 0.04 ab	3.13 ± 0.01 a	0.91 ± 0.00 a	24 ± 1 ab	++
Soil A	A	0.70 ± 0.02 bc	2.94 ± 0.05 b	0.86 ± 0.01 b	20 ± 1 b	+
	AM	1.07 ± 0.03 a	3.18 ± 0.02 a	0.93 ± 0.01 a	25 ± 1 a	++
	AP	0.57 ± 0.05 c	2.92 ± 0.04 b	0.85 ± 0.01 b	19 ± 1 b	++
	AMP	0.81 ± 0.05 b	3.07 ± 0.03 ab	0.89 ± 0.01 ab	23 ± 1 ab	+++

Table 4: Bacterial biomass, represented by the number of 16S rRNA gene copies per gram of soil, and soil bacterial community diversity, represented by the alpha diversity index, of the initial soils (C and A), the consortia (Cons C and A) isolated from these soil and the final soils (C and A) with or without consortium inoculation (M) and with or without *Agrostis* seedlings (P).

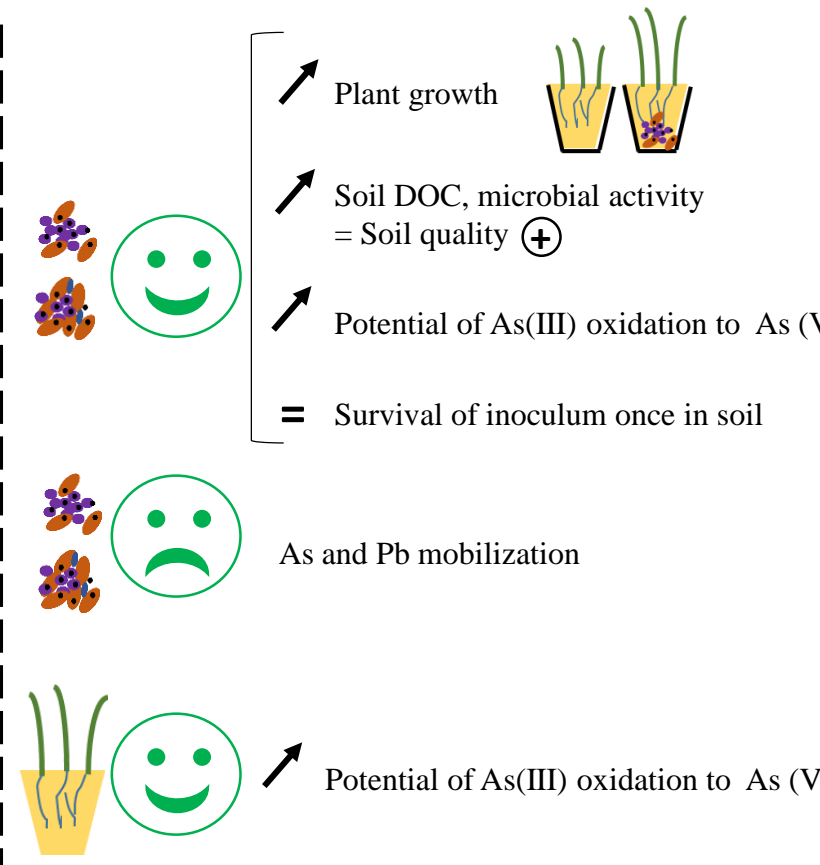
		Code	16S copy.g <sup>-1</sup> soil (log <sub>10</sub> )	Observed OTUs	Chao1	Shannon	InvSimpson
Initial soils	Soil C			644	697	4.88	49
	Soil A			708	730	5.65	156
Consortia obtained	Cons C			31	36	0.83	2
	Cons A			20	26	0.24	1
Final soils	Soil C	C	8.12 ± 0.02	649	683	4.57	34
	Soil C	CM	8.95 ± 0.02	502	590	2.87	7
	Soil C	CP	8.07 ± 0.01	654	702	4.36	23
	Soil C	CMP	8.75 ± 0.00	578	639	3.22	8
	Soil A	A	8.37 ± 0.02	724	751	4.42	22
	Soil A	AM	8.50 ± 0.00	706	756	3.80	14
	Soil A	AP	8.06 ± 0.02	764	809	5.25	62
	Soil A	AMP	8.07 ± 0.01	750	772	4.31	17



Consortium selection



Mesocosm (pot) experiment design



Results