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Running title: microbial inoculation improves mine soil remediation

Abstract

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

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

Keywords

Arsenic; Lead; phytoremediation; bioaugmentation; microbial consortium inoculation; metallicolous *Agrostis*

Highlights

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

1. Introduction

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

leading to the formation of technosols (Sheoran et al. 2010, Wong 2003). Such areas are generally characterized by low levels of macronutrients such as NPK (Sheoran et al. 2010), a lack of organic matter (Wong 2003), and a sandy texture, leading to a low ability to retain water (Sheoran et al. 2010). Therefore, the biological functionality of the soil is disturbed, and the growth of microorganisms and plants is impaired (Sheoran et al. 2010, Wong 2003). Due to the lack of vegetation, erosion on these areas is elevated, leading to a contamination of the surrounding areas due, for instance, to the transport of contaminated dusts by the wind. In addition, the underground water can be contaminated through leaching. Such spreading of the contamination can potentially be a threat to human health if the pollution enters the food chain through drinking water and crops. All these consequences were observed on mining sites such as the Pontgibaud site, a former French extraction mine site, located in the Massif-Central (France). This region is characterized by soils made of galena, arsenopyrite, and gray coppers, and a geochemical background showing the presence of Pb and arsenic (As). The mine district extracted argentiferous Galena until the end of the nineteenth century. It was the main mine producing silver (Ag) and Pb during the nineteenth century, and the largest metallic mine in France, extracting 50 000 tons of Pb and 100 tons of Ag. Previous studies on this site highlighted an acidic pH (pH 3-4), elevated concentrations in Pb and As and low organic matter and nutrient contents (Cottard 2010, Lebrun et al. 2017, 2018, Nandillon et al. 2019a, b, Thouin et al. 2019). This contaminated site is in need of remediation, to ameliorate its physical, chemical and biological parameters and regain functionality.

Compared to the conventional physical and chemical techniques that have been used over the last decades to treat metal(loid) polluted sites, bioremediation techniques using plants and/or microorganisms are cheaper and more environmentally friendly (Gong et al. 2018). Cristaldi et al. (2017) defined bioremediation as “the use of living organisms to clean up oil spills or remove other pollutants from soil, water or wastewater”. In the case of bioremediation of mine sites, coupling microbial bioremediation with phytoremediation is potentially a good option. Indeed, metal(loid)s cannot be

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

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

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

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

2. Materials and methods

2.1. Soil sampling and characterization

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

143 lead to 87000 m^3 of waste tailings highly contaminated with As (539 $mg.kg^{-1}$) and Pb (11453 $mg.kg^{-1}$)
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^2. Plants were grown for five weeks under greenhouse conditions: 20
161 ± 5 °C, 16 h photoperiod and 800 $\mu mol.m^{-1}.s^{-1}$ 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)

supplemented with 0.10 mg.L^{-1} sodium arsenate (Na_2HAsO_4) (As(III)) and 40 mg.L^{-1} nitrate lead ($Pb(NO_3)_3$) (Pb(II)). Both As(III) and Pb(II) concentrations were chosen based on the concentrations usually found in the soil pore waters of the Pontgibaud technosol (Lebrun et al. 2019, Nandillon et al. 2019). Such cultures were done in order to maintain a selective pressure and allow the selection of a microbial community adapted to the presence of As and Pb. Cultures were incubated under aerobic conditions at 28 °C and subjected to shaking (150 rpm). Five sub-cultures were carried out successively (every 2 days after checking the microbial growth using the absorbance value at 600 nm). Microorganisms that grew in the final sub-culture constituted the two consortia named Cons A (coming from soil A) and Cons C (coming from soil C).

2.3. Mesocosm pot experiment

2.3.1. Experimental design

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

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_3 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_3 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 $CaCl_2$ extractable As and Pb, corresponding to the plant's available amounts of
211 metal(loid)s, were measured by mixing a 0.01 M $CaCl_2$ solution with soil (1:10 solid:liquid ratio) and

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

2.3.5. Soil microbial activity

2.3.5.1 Soil enzyme activities linked to the C and P cycles

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

The β-glucosidase activity, related to the carbon cycle, was assessed by mixing 0.1 g soil with citrate phosphate buffer (0.15 M, pH 4-5) and 10 mM PNPG (4-nitrophenyl β-D glucopyranoside) as substrate. After two hours of incubation at 37 °C, the supernatant was recovered by centrifugation (14000 x g for 3 min), and 2 % Na_2CO_3 was added before an absorbance measurement at 410 nm. The activity was calculated using ε = 18400 $L.mol^{-1}.cm^{-1}$.

The hydrolytic activity, which represents the overall microbial activity, was assessed by the FDA (fluorescein diacetate) test. For this, 0.1 g of soil were mixed with potassium phosphate buffer (60 mM, 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 ε = 8000 $L.mol^{-1}.min^{-1}$.
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 µL 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-Wever index, $H' = - \Sigma p_i * \ln p_i$, with $p_i = Abs_i/\Sigma Abs$ and i representing the substrate
246 - Eveness, $E = H'/\ln 31$
247 - Richness = number of wells with $Abs_{590} > 0.25$
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 µm) at 3000 rpm. The microbial extract (supernatant) was inoculated into LB medium containing
254 100 $mg.L^{-1}$ 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 *S*oil 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^{TM} 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 ng.µL^{-1} and qPCR targeting 16S rRNA genes for bacterial biomass
274 quantification was performed using a CFX ConnectTM 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 µL and
277 contained 7.6 µL RNAse and DNAse free-water, 10 µL 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 $2.2*10^3$ to $2.2*10^7$ 16S rRNA gene copies. All samples were
280 analyzed in duplicates. Results were expressed as gene copies per g of soil.

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

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

2.4. Statistical analysis

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

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

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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ 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 $CaCl_2$ As and
339 Pb concentrations. *Agrostis* growth only affected the $CaCl_2$ 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 $CaCl_2$) while *Agrostis* growth tended to mainly increase the
344 availability of As and Pb ($CaCl_2$).
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 3rd 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 1However, 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, Eveness 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 *Lysisibacillus*, 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 β-diversity of soils and consortia was analyzed by clustering (Figure S4). This
428 clustering dendogram 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

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

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

4.1.1. Impacts of consortium inoculation on soil properties.

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

In our work, no impact on pH was observed for microbial inoculation when applied alone. Schoebitz and Vidal (2016) also observed that microbial consortium inoculation did not affect soil pH for inoculations performed on a slightly acidic sandy loam soil and a slightly acidic clay soil. However, other studies demonstrated that the inoculation of other types of soils with microorganisms can affect soil pH. For 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

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

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

4.1.3. Impact of microbial inoculation on plants

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

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

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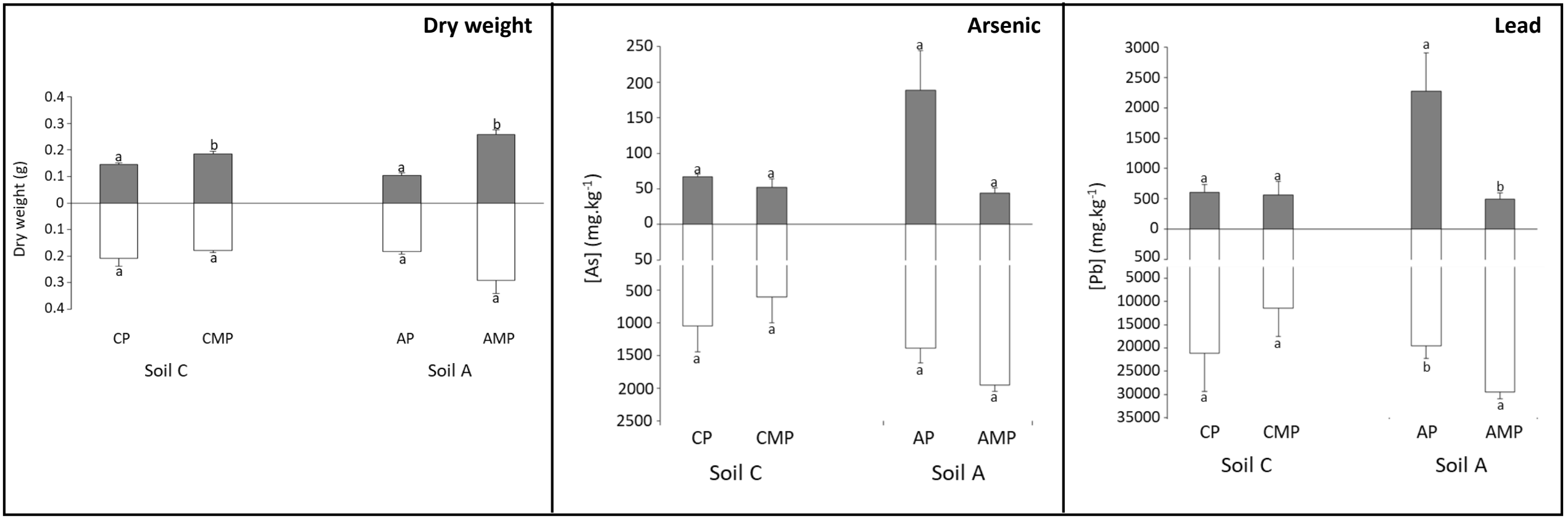


Figure 1: Dry weight (g), arsenic and lead concentrations (mg.kg^{-1}) of aerial (grey) and root (white) parts of *Agrostis capillaris* plants grown on the soils with or without consortium inoculation (M). Letters indicate difference between the treatments of each soil (soil C and soil A) ($p < 0.05$)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

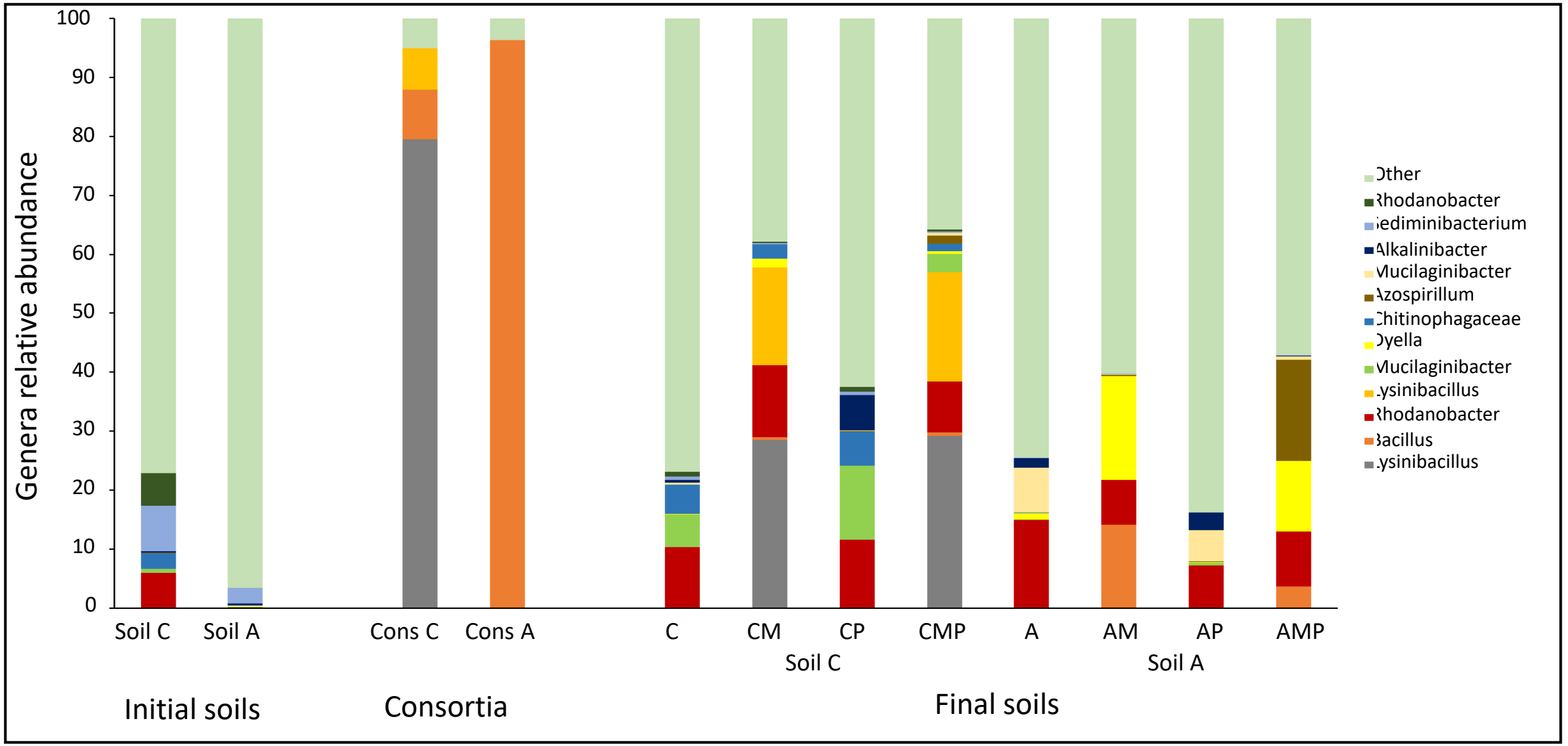


Figure 2: Relative abundance of the dominant genera (p ≥ 5 %) in the two initial soils (soil C and soil A), the two consortia (Cons C and Cons A) and in soil at the end of the experiment in soils C and A with or without consortium inoculation (M) and with or without *Agrostis* seedlings (P).

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 caracters) indicate significant difference ($p < 0.05$) (n = 3-4).

	WHC (%)	[As] ($mg.kg^{-1}$)	[Pb] ($mg.kg^{-1}$)
Soil C	31.43 ± 0.17 **a**	577 ± 61 **a**	12902 ± 1750 **a**
Soil A	31.33 ± 0.07 **a**	674 ± 113 **a**	8770 ± 1100 **a**

Table 2: Soil pore water physico-chemical properties (pH, electrical conductivity (EC) (µS.cm^{-1}), redox potential (Eh) (mV), metal(loid) (As and Pb) concentrations (mg.L^{-1}) and dissolved organic carbon (DOC) carbon (mg.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

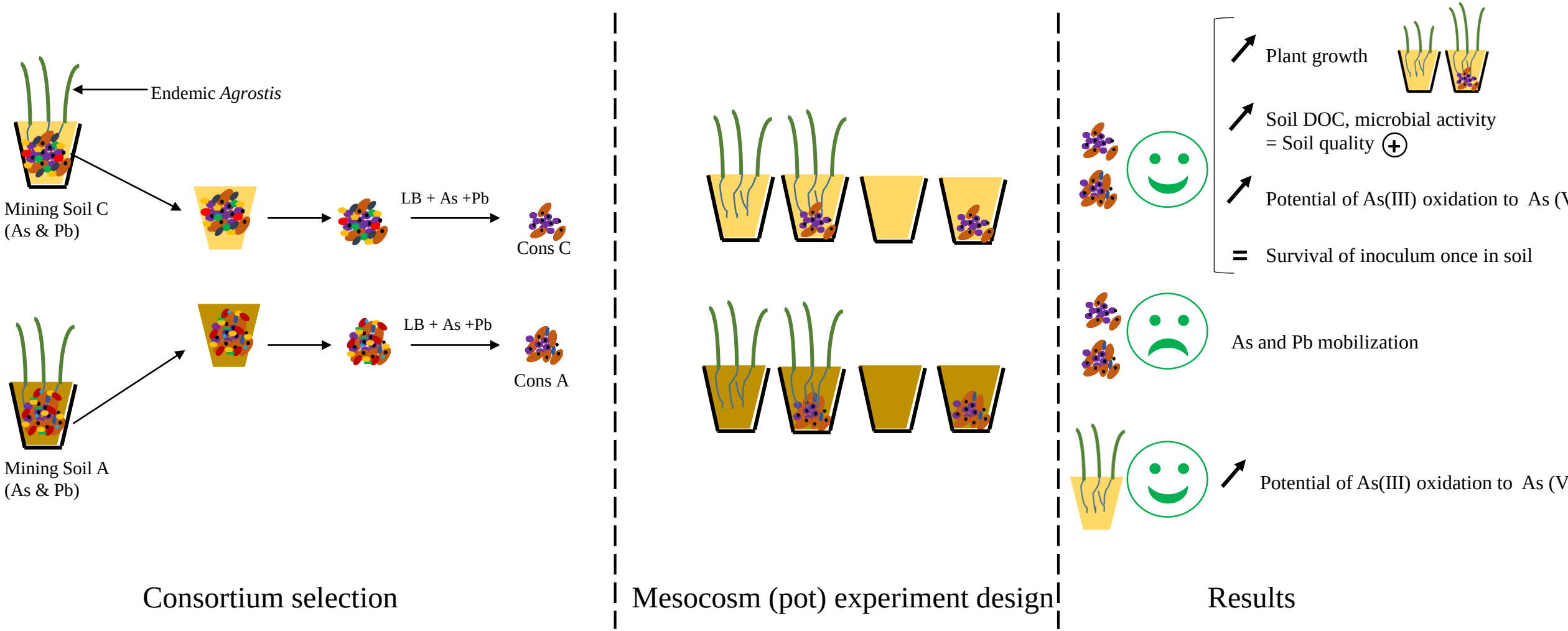
			pH	EC (µS.cm^{-1})	Eh (mV)	[As] (mg.L^{-1})	[Pb] (mg.L^{-1})	DOC (mg.L^{-1})
Soil C	T0		4.8 ± 0.1 b	338 ± 10 b	393 ± 14 a	0.30 ± 0.01 a	28.74 ± 2.38 b	ND
	T28	C	5.5 ± 0.3 ab	1050 ± 431 ab	268 ± 23 bc	0.06 ± 0.01 b	15.79 ± 1.28 b	23.6 ± 0.3 b
		CM	5.1 ± 0.2 ab	11347 ± 715 a	270 ± 13 bc	0.30 ± 0.16 a	50.62 ± 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 = eveness, 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^{-1} soil (log_{10})	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


Endemic *Agrostis*
Mining Soil C
(As & Pb)
LB + As +Pb
Cons C
Mining Soil A
(As & Pb)
LB + As +Pb
Cons A
Plant growth
Soil DOC, microbial activity
= Soil quality
Potential of As(III) oxidation to As (V
=
Survival of inoculum once in soil
As and Pb mobilization
Potential of As(III) oxidation to As (V
Consortium selection
Mesocosm (pot) experiment design
Results