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Effects of biochar, ochre and manure amendments associated to a metallophilous ecotype of *Agrostis capillaris* on As and Pb stabilization of a former mine technosol --Manuscript Draft--

Manuscript Number:	EGAH-D-19-00722R3	
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Abstract:	<p>Metal(loid)soil pollution is a major environmental and health issue, requiring these areas to be remediated, for example through phytoremediation processes. In order to allow proper plant establishment and growth, amendments must be applied to highly contaminated and poorly fertile soils. Amendments are diverse, but many studies have shown the beneficial effects of biochar, manure and ochre, although studies on their combined use are scarce. Moreover, no studies have evaluated the effect of these combined amendments on endemic plant growth. Endemic plants growing on contaminated soils showed higher tolerance towards pollutants compared to plants coming from unpolluted areas. Therefore, the aim of the present study was to evaluate both the effect of amendments (single or combined) on the physico-chemical properties of a former mining technosol, and the growth and metal(loid) accumulation ability of endemic <i>Agrostis capillaris</i> plants. This study revealed an improvement in the soil physico-chemical properties following the application of amendments, with combined amendments showing better results than the application of just one. On top of this, <i>Agrostis</i> plants performed better on the amended technosols, especially the ones receiving manure, due to its high nutrient content. Finally, based on soil properties, plant growth and the metal(loid) accumulation profile, the use of biochar combined with manure seems to be the most appropriate treatment. Indeed, this treatment showed an improvement in both soil fertility and plant growth. Moreover, <i>Agrostis</i> plants grown in these conditions were among those showing higher root metal(loid) concentration associated to a lower translocation toward aerial parts.</p>	
Response to Reviewers:	Dear Dr. Xunwen Chen (guest editor EGAH),	

We were pleased to learn your interest in the resubmission of our manuscript entitled “Effects of biochar, ochre and manure amendments associated to a metalicolous ecotype of *Agrostis capillaris* on As and Pb stabilization of a former mine technosol”.

The reviewers suggested some revisions to be done to the manuscript. We thank them for their work and for helping us to improve the quality of our paper. In this amended version, we have addressed their comments and have rewritten the text accordingly. Below is our item-by-item reply to each of their observations.

Having addressed all the issues raised by both reviewers, we hope that our work will be now considered suitable for publication in the *Environmental Geochemistry and Health*.

Looking forward to hearing from you.

Yours sincerely,

Dr. Sylvain BOURGERIE

Reviewer #1: All suggestions have been considered

Minor errors can be corrected at proof stage (for instance L205)

Following Reviewer #1 comment, the sentence was modified (line 217: “In addition, the supply of soluble organic matter and organic anions by the organic amendment...”).

Reviewer #3: In the revised ms, author explain the explanation of plant choose and the inner mechanism of metal absorption. However, the role of P on As leaching has already been reported. the adsorption of metall ions on biochar also have been demonstrated eles where. The core problem is the scientific reason on the mixture of these materilas for phytoremediation. however, authors did not explain clearly. Is there any data related to phosphrous or any other own information could be supported? if yes, please add these data, if not, please explain the advantage of the mixture on phytoremediation.

As suggested by Reviewer #3, some explanations were provided (lines 87-103) in introduction part. A reference (Liang et al. 2017) was added.



[Click here to view linked References](#)

1 Effects of biochar, ochre and manure amendments associated to a metalicolous ecotype of *Agrostis*
2 *capillaris* on As and Pb stabilization of a former mine technosol

3
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13

14

15 Abstract

16 Metal(loid)soil pollution is a major environmental and health issue, requiring these areas to be
17 remediated, for example through phytoremediation processes. In order to allow proper plant
18 establishment and growth, amendments must be applied to highly contaminated and poorly fertile
19 soils. Amendments are diverse, but many studies have shown the beneficial effects of biochar,
20 manure and ochre, although studies on their combined use are scarce. Moreover, no studies have
21 evaluated the effect of these combined amendments on endemic plant growth. Endemic plants
22 growing on contaminated soils showed higher tolerance towards pollutants compared to plants
23 coming from unpolluted areas. Therefore, the aim of the present study was to evaluate both the
24 effect of amendments (single or combined) on the physico-chemical properties of a former mining
25 technosol, and the growth and metal(loid) accumulation ability of endemic *Agrostis capillaris* plants.
26 This study revealed an improvement in the soil physico-chemical properties following the application
27 of amendments, with combined amendments showing better results than the application of just one.
28 On top of this, *Agrostis* plants performed better on the amended technosols, especially the ones
29 receiving manure, due to its high nutrient content. Finally, based on soil properties, plant growth and
30 the metal(loid) accumulation profile, the use of biochar combined with manure seems to be the most
31 appropriate treatment. Indeed, this treatment showed an improvement in both soil fertility and plant
32 growth. Moreover, *Agrostis* plants grown in these conditions were among those showing higher root
33 metal(loid) concentration associated to a lower translocation toward aerial parts.

34

35 Keywords: *Agrostis capillaris*; amendment; metal(loid)s; mine; stabilization

36

37 Introduction

38 Metals and metalloids are significant pollutants of soils. Indeed, there are more than three million
39 potentially polluted sites in Europe, of which about 250 000 are contaminated by metal(loid)s (Khalid
40 et al. 2016). This pollution is a big issue, as metal(loid)s, contrary to organic pollutants, cannot be
41 degraded, and thus accumulate in the soil. Moreover, the negative effects of the metal(loid) soil
42 pollution on both the environment and human health mean that metal(loid) polluted soils must be
43 remediated (Ali et al. 2013). Indeed, polluted soils often present a low biodiversity and a loss of
44 ecosystemic functions, and the lack of vegetation induces the transportation of polluted soil particles
45 towards the unpolluted surrounding area. Furthermore, metal(loid)s have detrimental effects on
46 human health. For instance, lead (Pb) can affect and damage many organs and systems in the body
47 as well as altering enzyme activities (Sharma and Agrawal 2005, Su et al. 2014). Arsenic (As) can affect
48 the reproduction, neurological and respiratory systems, among others (Mandal and Suzuki 2002).
49 Moreover, both are considered carcinogenic elements (Ahmad et al. 2015, Jarup 2003, Sharma and
50 Agrawal 2005).

51 Different remediation techniques, *i.e* physical, chemical and biological approaches, aiming to
52 diminish pollutant toxic effects can be employed. However, phytoremediation, defined as the use of

53 plants, and their associated microorganisms, to remediate and re-vegetate contaminated sites, is an
54 environment-friendly, energy efficient and cost-effective technique (Khalid et al. 2016). Several
55 processes of phytoremediation exist. Among them, phytostabilisation is more suitable for large and
56 highly contaminated areas. In this case, the pollutants are immobilized in the root area through (i)
57 absorption into the roots, (ii) adsorption onto the roots and/or (iii) precipitation in the rhizosphere
58 area (Cristaldi et al. 2017). This technique relies on the re-vegetation of the polluted area rather than
59 the real depollution of the soil, which can be difficult and time consuming when large amounts of
60 soils are contaminated. The re-vegetation of the polluted areas in the phytostabilisation process is
61 important because, in addition to the immobilization of the pollution to the rhizosphere, it will also
62 lessen the potential risk of contaminated soil spreading off-site by wind or water erosion (Gray et al.
63 2006). Such process has been studied for many years as the best option to reduce metal(loid)
64 spreading as well as minimize the entry of pollutants into the food chain (Wong 2003).
65 Moreover, in some cases, the extreme conditions (acidic pH, low nutrient content associated to high
66 pollution concentrations) of the polluted soils imply the need of amendments to allow plant growth.
67 Different amendments can be used, both organic and inorganic, although some have attracted more
68 attention than others over the last few years.
69 For instance, manure is commonly used as a fertilizer in agriculture. It is rich in organic materials and
70 essential nutrients (Kiran et al. 2017). Its application to soil, whether contaminated or not, allows an
71 improvement to the fertility and thus promotes plant growth (Walker et al. 2004). Moreover,
72 previous studies have demonstrated its ability to immobilize Pb (Tang et al. 2015), Cd, Cu, Zn (Huang
73 et al. 2018, Kiran et al. 2017) and Al (Whalen et al. 2000). However, its positive effects are usually
74 short-lasting and repeated application is necessary (Mosaddeghi et al. 2009).
75 Ochre, on the other hand, is not a typical agricultural amendment. Ochre is a by-product found in the
76 outflows of some mining systems (Olimah et al. 2015). It is a brown or yellowish-brown product, and
77 rich in Fe(III) oxyhydroxides (Abed et al. 2017, Olimah et al. 2015). For this reason, ochre is a very
78 valuable product for the remediation of arsenic, known to have an affinity for iron (Doi et al. 2005,
79 Olimah et al. 2015).
80 Finally, biochar is produced through the pyrolysis of biomass under conditions of limited oxygen
81 (Anawar et al. 2015). It is characterized by an alkaline pH, a large surface area and the presence of
82 functional groups on its surface, such as phenolic, carboxyl and hydroxyl (Cha et al. 2016, Lebrun et
83 al. 2018c). Such properties induce an immobilization of metallic pollutants, such as Pb, when added
84 to polluted soils (Lebrun et al. 2018a, b, Lomaglio et al. 2017). Moreover, biochar soil application
85 improves soil fertility and plant growth and tends to reduce metallic cation concentrations in plant
86 tissues (Chen et al. 2018).
87 Taken separately, all these amendments have benefits for contaminated soils but they also have
88 limits, especially for soils that have a poor fertility and contaminated by both metals and metalloids.
89 To overcome the first issue, the poor fertility, nutrient-rich organic amendments can be applied, such
90 as manure or compost. Biochar has a high capacity to immobilize cation metals and can be used to
91 overcome the second issue. Moreover, biochar and organic amendments can interact once applied
92 together in the soil (Liang et al. 2017): biochar increases the humification and properties of organic
93 amendments, whereas biochar properties are modified by the microorganisms present in the
94 manure. However, both amendments are not efficient regarding metalloids. Anions can even be
95 mobilized due to the organic matter and phosphates contained in manure, arsenic and P being
96 chemically similar and competing for the same sorption sites (Cao et al. 2003). Thus, it can be
97 necessary to add a third amendment, rich in iron, such as iron sulfate. The iron present will interact
98 with arsenic and immobilize it (Fresno et al. 2020). Such amendments demonstrated different effects
99 on soil properties, manure being mainly used for fertility improvement, biochar for metal
100 immobilization and ochre for metal(loid) immobilization. However, when soils are contaminated with
101 more than one element, especially a combination of metals and metalloids, it can be difficult to find
102 one amendment efficient for all the pollutants. Therefore, the complexity of multi-contaminated soils
103 can require it is necessary to apply the application of a combination of amendments. Several previous

104 studies showed that amendment combinations had better effects than the single application of the
105 same amendments (Beesley et al. 2010, Fresno et al. 2016, Oustriere et al. 2016).
106 Plant selection is an important criterion for phytoremediation success, and the use of endemic
107 species has shown better results in the past. For instance, *Agrostis capillaris* L., endemic species
108 found on a studied mine technosol, has been shown to be a good Pb phytostabilizer (Rodríguez-Seijo
109 et al. 2016). Many studies aimed at comparing metal-tolerant and non-tolerant ecotypes. All research
110 showed that the metal tolerant ecotype, collected on a polluted site, presented a higher metal(loid)
111 tolerance with a lower metal(loid) uptake and root-to-shoot translocation (Doubková and Sudová
112 2016, Sudová et al. 2008). This higher metal(loid) tolerance of populations grown on polluted sites
113 was associated to a long-term pollutant selection pressure (Sudová et al. 2008).
114 Nevertheless, even though manure, ochre and biochar have been much studied in polluted soil
115 remediation, combinations of the three have not yet been investigated. Moreover, to the best of our
116 knowledge, this is the first study associating a combination of amendments and *Agrostis capillaris*
117 from a metallicolous population. In this context, the aims of this study were to evaluate the effect of
118 the application of manure, ochre and biochar, alone or combined, on: (i) the physico-chemical
119 properties of a former mining site and (ii) *Agrostis capillaris* growth and metal(loid) stabilization
120 performance.

121

122 Material and methods

123 1. Site description

124 The mesocosm experiment was realized using soil collected from a former silver-lead extraction mine
125 site, located in Pontgibaud (Auvergne-Rhône-Alpes, France) (GPS coordinates 45° 47' 27" North and
126 2°49'38" East). This site has been described in previous studies (Lebrun et al. 2018a, b) and is mainly
127 contaminated by lead (11 453 mg.kg⁻¹) and arsenic (539 mg.kg⁻¹) (Lebrun et al. 2017).

128

129 2. Amendments

130 The commercial biochar was provided by La Carbonerie (Crissey, France). It was produced from the
131 slow pyrolysis at 500 °C of hardwood biomass (mixture of oak, beech and charm), followed by a
132 sieving to obtain a particle size between 0.5 and 1 mm.

133 The ochre amendment was collected at a former charcoal mine (Alès) in France, as described in
134 Thouin et al. (2019). Before its incorporation into the soil, it was dried and crushed to obtain a fine
135 powder.

136 Finally, cow manure was provided by a local farmer located in Pontgibaud (GPS coordinates 45° 47'
137 26" North and 2° 49' 44" East).

138 All three amendments were analyzed for pH, electrical conductivity (EC), redox potential and
139 NH₄NO₃-extractable As, Fe, K, P and Pb concentrations. pH and EC as well as redox potential of the
140 three amendments were determined using the same procedure as described in Lebrun et al. (2018a)
141 and measured using a multimeter (Metler-Toledo, Serveur excellence, Columbus, Ohio, USA).
142 Concentrations in NH₄NO₃-extractable metal(loid)s were determined after shaking a mixture of soil
143 and 1 mol.L⁻¹ NH₄NO₃ solution (2:5 solid:liquid ratio) at room temperature for two hours (Pueyo et
144 al. 2004), followed by ICP-AES (Inductively Coupled Plasma- Atomic Emission Spectroscopy) (ULTIMA,
145 HORIBA, Labcompare, San Francisco, USA) analyses. Amendment characteristics are given in Table 1.

146

147 3. Plant

148 *Agrostis capillaris* was chosen as the phytoremediator plant. It is a *Poaceae* characterized as a
149 facultative metallophyte. It has been found to grow spontaneously on contaminated soils (Austry et
150 al. 2013, Rodríguez-Seijo et al. 2016). Studies also showed its potential for the phytostabilization of
151 soils polluted by As (Austry et al. 2013) and Pb (Rodríguez-Seijo et al. 2016) and as presenting an
152 adaptive tolerance to contaminated sites (Austry et al. 2013). Moreover, *Agrostis capillaris* was
153 chosen because such plants have been found on the Pontgibaud polluted site and the use of seeds
154 collected from the site showed a potential for the phytoremediation of PG soil (Nandillon et al. 2019).

Field Code Changed

155 Similarly here, seeds of the metallicolous ecotype found on the site were collected to be used in this
156 study.

157 4. Experimental design

159 All three amendments were applied at 1 % (w/w), either alone or in combination, which gave eight
160 treatments in total: i) non-amended Pontgibaud technosol (P0%), ii) Pontgibaud + 1 % biochar (PB),
161 ii) Pontgibaud + 1 % ochre (PI), iii) Pontgibaud + 1 % biochar + 1 % ochre (PBI), iv) Pontgibaud + 1 %
162 manure (PM), vi) Pontgibaud + 1 % biochar + 1 % manure (PBM), vii) Pontgibaud + 1 % ochre + 1 %
163 manure (PIM) and viii) Pontgibaud + 1 % biochar + 1 % ochre + 1 % manure (PBIM). Ten pots were
164 prepared per treatment. The different mixtures were placed in 2 L pots and half the pots were sown
165 with *Agrostis capillaris* seeds (quantity equivalent to 2000 seeds.m⁻²) collected at Pontgibaud site.
166 The experiment lasted for 26 days in a phytotron (16h-8h photoperiod, 800 μmol.m⁻².s⁻¹ light
167 intensity, temperature 24 °C day and 21 °C night).

168 5. Soil analysis

170 The eight treatments were analyzed at the beginning of the experiment to determine soil NH₄NO₃-
171 extractable As, Fe, K, P and Pb concentrations. The procedure was the same as the one used for the
172 amendment characterization.

173 6. Soil pore water (SPW) sampling and analysis

175 SPWs were sampled in all pots twice over the length of the experiment: at the beginning, before seed
176 sowing (T0) and at the end, before plant harvest (TF). Sampling was performed using a Rhizon®
177 (model MOM, Rhizosphere Research Products, Wageningen, The Netherlands), as described in
178 Lebrun et al. (2017). SPWs were used directly to measure pH, EC and redox potential, using a
179 multimeter (Metler-Toledo, Serveur excellence, Columbus, Ohio, USA). SPWs were then acidified and
180 As, Fe, K, P and Pb concentrations were determined by ICP-AES.

181 7. Plant analysis

183 At the end of the experiment, *Agrostis* plants were harvested in each pot. Aerial and root parts were
184 separated, washed twice with tap water then distilled water, and dried (72 h at 60 °C) to determine
185 dry weight production per pot. Aerial and root biomasses were then submitted to acid digestion (0.2
186 g of plant material + 6 mL HNO₃ 70 % + 3 mL HCl 35 %) in a microwave (15 min increase up to 180 °C,
187 15 min hold at 180 °C, cooling) (Multiwave 5000, Anton Paar, Courtaboeuf, France), and plant As, Fe
188 and Pb concentrations were determined by ICP-AES, using standard solutions prepared from mother
189 solutions of As and Pb provided by Alfa Aesar-Fisher (Karlsruhe, Germany).

190 8. Statistical analyses

192 All data was analyzed using R software version 3.1.2 (R Development Core Team, 2009). Firstly, the
193 homogeneity and homoscedasticity of the data was evaluated using Shapiro and Bartlett tests
194 respectively. Next, Anova or Kruskal tests were performed, followed by a post hoc test to assess two-
195 by-two differences. In addition, for the SPW data, a "time effect" was assessed by comparing the
196 means at T0, TF-*Agrostis* and TF+*Agrostis* for each treatment: normality and homoscedasticity were
197 evaluated as previously described and mean comparison was realized using an Anova test for
198 parametric data or a Kruskal test for non-parametric data, followed by a post-hoc test, Tukey HSD or
199 Pairwise-Wilcox, respectively. Differences were considered significant at p < 0.05.
200 Finally, Pearson correlation coefficients were calculated between the different parameters
201 measured. Correlation was considered significant at p < 0.05.

202 Results and Discussion

203 1. Soil NH₄NO₃-extractable elements

205 Soil NH₄NO₃-extractable elements were analyzed at the beginning of the experiment in order to
206 investigate the phytoavailable fraction of metal(loid) in the soil. NH₄NO₃-extractable As

Field Code Changed

207 concentration was $2.29 \mu\text{g}\cdot\text{g}^{-1}$ on P0% and it only increased in two conditions (Table 2): the addition
208 of biochar and biochar + manure led to a 1.42 and 1.56-fold increases, respectively. In 2013,
209 Alvarenga et al. observed an increase in available As concentrations following compost amendment,
210 while Namgay et al. (2010) showed that biochar increased available As (from 12.7 to $14.9 \text{ mg}\cdot\text{kg}^{-1}$)
211 when applied at 1.5 % on a metal(loid) spiked soil. Both studies attributed this increase to a rise in
212 soil pH and organic matter, induced by amendment application. Moreover, Moreno-Jiménez et al.
213 (2016) found that the rise in As solubility following biochar amendments was likely to be related to
214 changes in available P. The same explanation was found in this study: NH_4NO_3 -extractable As
215 concentration was positively correlated with NH_4NO_3 -extractable P concentration ($R^2 = 0.51$, $p <$
216 0.001) (Table S1). With increasing P concentrations, As is displaced from sorption sites and thus
217 becomes more available (Moreno-Jiménez et al. 2016). In addition, the addition-supply of soluble
218 organic matter and organic anions by the organic amendment can lead to the formation of soluble
219 As complexes (Namyay et al. 2010).

220 The NH_4NO_3 -extractable Fe concentration was low for P0% ($0.02 \mu\text{g}\cdot\text{g}^{-1}$), and only increased following
221 the ochre amendment (27-fold) (Table 2), and can be explained by the NH_4NO_3 -extractable Fe
222 concentration of the ochre ($1.04 \mu\text{g}\cdot\text{g}^{-1}$) (Table 1). Such elevated Fe content in the ochre has been
223 previously observed by Abed et al. (2017) , Doi et al. (2005) and Thouin et al. (2019). However, one
224 surprising fact was that although NH_4NO_3 -extractable Fe concentration in manure was high (2.41
225 $\mu\text{g}\cdot\text{g}^{-1}$), no increase was observed following its addition to the Pontgibaud technosol, maybe due to
226 its low application rate.

227 NH_4NO_3 -extractable K concentration was $22.14 \mu\text{g}\cdot\text{g}^{-1}$ for P0%, and it increased with all treatments
228 containing manure (Table 2). This improvement can be directly related to the amendment
229 composition, as shown by the NH_4NO_3 extractable K concentrations of manure. Manure is a known
230 fertilizer characterized by elevated K concentrations and other nutrients that can be released into
231 the soil matrix, as shown in Celik et al. (2004), Mokolobate and Haynes (2002) and Materechera and
232 Mkhabela (2002).

233 NH_4NO_3 -extractable P concentration was low on P0% ($1.11 \mu\text{g}\cdot\text{g}^{-1}$) and increased with all treatments,
234 except manure alone (Table 2). Increases went from 1.8-fold for biochar to 3.0-fold for biochar +
235 manure. The amendments presented NH_4NO_3 -extractable P concentrations of 0.63, 250.28 and 8.40
236 $\mu\text{g}\cdot\text{g}^{-1}$ for ochre, manure and biochar, respectively (Table 1), which could explain the observed
237 increase in NH_4NO_3 -extractable P concentration following their addition to Pontgibaud. However, as
238 for Fe, contrary to the high concentrations of P found in manure, NH_4NO_3 -extractable P concentration
239 did not increase when manure was added alone to Pontgibaud.

240 NH_4NO_3 -extractable Pb concentration was $4198.27 \mu\text{g}\cdot\text{g}^{-1}$ for P0% (Table 2) and was observed to
241 increase following PB, PI and PBM treatments, and at the same rate (1.34-fold on average). Only the
242 combination of the three amendments (PBIM) decreased NH_4NO_3 -extractable Pb concentration (by
243 30 %). These results contradict previous studies, which showed that biochar, ochre and manure were
244 able to decrease the extractable fractions of cationic elements (Namyay et al. 2010, Walker et al.
245 2014). However, such an increase could be due to the increase in dissolved organic carbon following
246 these amendments, which mobilizes the Pb (Lee et al. 2009, Lebrun et al. 2019). Organic carbons can
247 decrease the sorption of Pb onto the soil surfaces, through competition with the free metal ions and
248 the formation of soluble complexes or by its preferential sorption on the soil surface over metallic
249 ions (Lee et al. 2009).

250

251 2. Soil pore water physico-chemical properties

252 SPW pH was determined at the beginning (T0) and at the end of the experiment, in both non-
253 vegetated (TF-*Agrostis*) and vegetated (TF+*Agrostis*) pots. At T0, SPW pH of P0% was acidic (pH 4.3)
254 (Table 3) and increased with all amendment treatments, from 1.0 (PB and PM) to 3.4 units (PBIM).
255 The same trends were observed for TF-*Agrostis* and TF+*Agrostis*, with acidic pH on P0% (pH 3.8 and
256 pH 3.9, respectively), which increased following treatments, between 0.8 (PB) and 3.2 units (PBIM)
257 for TF-*Agrostis* and between 0.9 (PB) and 3.0 units (PBIM) for TF+*Agrostis*. It can be noted that all
258 amendments, alone or combined, increased SPW pH compared to P0% at all sampling times, with

259 average rises between 1.1 (PB) and 3.2 units (PBIM). Finally, the results showed that SPW pH
260 increases were higher when amendments were combined than when they were applied alone,
261 showing neutral values compared to the slightly acidic values still observed with single amendment
262 application. Such increases in SPW pH following biochar, ochre and/or manure amendments have
263 previously been observed and attributed to the alkalinity of the three amendments (Marques et al.
264 2008, Olimah et al. 2015, Van Poucke et al. 2018), which induces a liming effect. Indeed, biochar,
265 ochre and manure presented alkaline pH values of 8.46, 8.29 and 9.52, respectively. However,
266 although manure presented the highest pH among the amendments, it did not induce the highest
267 increase in SPW pH when applied alone. This low increase compared to the high amendment pH was
268 also observed by Walker et al. (2004) when a cow manure with a pH of 9.09 was added to a
269 contaminated soil at a rate of 29 g.kg⁻¹ (1.12 units after 31 days). Such an effect could be explained
270 by the presence of more carboxyl groups and on biochar and redmud surfaces than on the manure
271 surface. These carboxyl groups can consume H⁺ (Lebrun et al. 2019).

272 The evolution of SPW pH with time was assessed, revealing that in almost all conditions (except PM
273 and PBM), SPW pH decreased with time, between 0.6 and 0.8 units, and with no effect of plant
274 growth. Such decreases with time have been observed previously (Hattab-Hambli et al. 2016,
275 Marseille et al. 2000), and explained by the oxidation/acidification of the substrates due to watering.

276

277 Non-amended Pontgibaud technosol presented a low EC at T0 (210 $\mu\text{S.cm}^{-1}$), which increased
278 following amendment application, except for with biochar alone (Table 3). SPW EC increases were
279 between 9.6 (PI) and 26.5-fold (PBIM). SPW EC increased only with PIM and PBIM, at TF-*Agrostis*,
280 compared to P0%. At TF+*Agrostis*, there was no effect with biochar alone and an increase with all
281 other treatments, between 2.5 (PI) and 6.6-fold (PBIM) for TF+*Agrostis*. Except for with biochar alone,
282 all treatments increased SPW EC compared to P0%, at all sampling times, with average increases
283 ranging from 2.9 (PI) to 7.9-fold (PBIM). As for SPW pH, amendment combinations tended to lead to
284 a greater increase in SPW EC than when applied alone. Such improvements are related to the EC of
285 the amendments (Walker et al. 2004): biochar 302 $\mu\text{S.cm}^{-1}$, ochre 7765 $\mu\text{S.cm}^{-1}$ and manure 9476
286 $\mu\text{S.cm}^{-1}$ (Table 1). Biochar alone did not have an effect on SPW EC. This could be related to biochar's
287 low EC compared to the two other amendments, and having similar EC values to that of the SPW P0%
288 at T0. It could also be related to the low application rate in this study. Indeed, in a previous study,
289 Lebrun et al. (2018a) used the same biochar and did not observe a SPW EC rise after biochar
290 application to a mine soil at T0 compared to P0%, although SPW EC increased with time, and was
291 higher than P0% at the end of the experiment. However, biochar application rates were 2% and 5%,
292 compared to the 1% used here. Furthermore, as observed by Lebrun et al. (2018a), SPW EC increased
293 after 26 days for the PB treatment, by 2.5-fold on average, with no plant effect, which could be due
294 to the biochar ageing. An increase was also observed in the P0% treatment, with a 3.2-fold rise on
295 average. However, all the other treatments observed a decrease in SPW EC with time. For PBI and
296 PBM, the decrease was only significant in non-vegetated pots while for PM it was significant in both
297 conditions, with no difference between TF-*Agrostis* and TF+*Agrostis*. Finally, for PIM and PBIM, the
298 decrease was higher in non-vegetated pots than in *Agrostis* vegetated pots. Such decreases can be
299 explained by a leaching of salts and/or root exudation/collection.

300 P0% SPW redox potential was 427 mV at T0 (Table 3) and all amendments caused it to decrease
301 between 6 % (PB) and 43 % (PIM). At TF, SPW redox potential of P0% was 439 and 421 mV, for non-
302 vegetated and vegetated conditions, respectively. Again, all amendments decreased SPW redox
303 potential between 10 % (PB) and 27 % (PIM and PBIM) for TF-*Agrostis*, and between 10 % (PB) and
304 23 % (PBI and PBIM) for TF+*Agrostis*. Contrary to pH and EC, all amendments decreased SPW redox
305 potential compared to P0% at all sampling times, and between 9 % (PB) and 31 % (PIM) on average.
306 Few changes in SPW redox potential were observed over time: SPW redox potential decreased by 4
307 % for TF-*Agrostis* compared to T0 on PBM while on PI, PIM and PBIM, it increased with time, between
308 1.1 and 1.4-fold, with no plant effect, following a reverse trend compared to pH. Indeed, redox
309 potential is known to have the opposite behavior to pH (Rinklebe et al. 2015).

310

311 3. Soil pore water element concentrations
312 At T0, SPW of P0% substrate presented a low As concentration (0.05 mg.L^{-1}) (Table 4), and there was
313 no immediate effect from amendment application, either alone or combined, which is consistent
314 with a previous study on biochar (Lebrun et al. 2018a). Similarly, amendment application had no
315 effect on SPW As concentrations, compared to P0%. However, for TF+*Agrostis*, SPW As concentration
316 increased by 2.3 times following the application of the three amendments together. The behavior of
317 SPW As concentrations for TF+*Agrostis* was linked to the amendment induced pH increase ($R^2 = 0.38$,
318 $p < 0.05$) (Table S1). Additionally, the increase in SPW As concentration with the PBIM treatment
319 could be related to the P concentration measured in the same treatment. Indeed, As and P are
320 chemically similar and thus compete for the same sorption site. Thus, with an increase in P
321 concentration, As mobility also increases (Fitz and Wenzel 2002, Cao et al. 2003). Furthermore, it
322 should be noted that contrary to what previous studies showed, ochre did not decrease As solubility.
323 Indeed, several studies showed that ochre was able to reduce soluble metal concentrations, and
324 particularly As, through sorption by Fe oxides (Abed et al. 2017, Doi et al. 2005, Olimah et al. 2015).
325 However, due to the input of other elements such as P, K but also Pb, by the amendments, the Fe
326 oxides could have been occupied by these elements and thus not be available for As sorption. Finally,
327 increases in SPW As concentration were observed with time for PBI, PBM, PIM and PBIM, with no
328 plant effect, except for PBM where the increase was only significant in the vegetated conditions.
329 SPW Fe concentrations were below detection limit for all treatments and at all times (Table 4).
330 At T0, SPW K concentrations was 14.83 mg.L^{-1} for P0% (Table 4) and increased with all amendments
331 (except for biochar and ochre alone), by between 1.1 (PBI) and 26.7-fold (PBIM). At TF, in the non-
332 vegetated condition, SPW K concentrations increased only with PM, PBM and PBIM. Moreover, in
333 the vegetated conditions, only the manure treatments increased SPW K concentrations. Such
334 increases in K may be explained by a supply of potassium provided by the different amendments,
335 especially manure, which showed the highest NH_4NO_3 -extractable K concentrations among the three
336 tested amendments (Table 1). Finally, SPW K concentrations were found to decrease with time for
337 PI, PM and PBIM with no plant effect, while the decreases for PBM and PIM were higher in the non-
338 vegetated pots. Such decreases can be attributed to both the leaching of K, and the effect of root
339 exudates in the case of PBM and PIM (Badri and Vivanco 2009, Erickson et al. 2005). Furthermore,
340 K's behavior can be related to the pH. Indeed, positive correlations were observed between the SPW
341 pH and SPW K concentrations at T0 ($R^2 = 0.35$, $p < 0.01$), TF-*Agrostis* ($R^2 = 0.41$, $p < 0.01$) and
342 TF+*Agrostis* ($R^2 = 0.39$, $p < 0.05$) (Table S1). This shows that SPW K concentrations tended to increase
343 with increasing SPW pH. This backs up observations made by Arienzo et al. (2009) who showed that
344 potassium availability is strongly related to pH, and is sustained for plants in neutral or slightly acidic
345 soils, which was the pH range observed after the incorporation of amendments into PG.
346 At T0, SPW P concentration was 0.14 mg.L^{-1} for P0% (Table 4) and decreased following biochar +
347 manure and biochar + ochre amendments by 71 % and 36 %, respectively, which could be due to P
348 sorption onto the amendments, especially biochar, and/or onto the soil's surface. On the contrary,
349 when ochre and manure were applied alone, SPW P concentrations increased by 1.2 and 1.4-fold,
350 which can be explained by a supply of P from manure and ochre. Indeed, ochre and manure especially
351 showed high extractable concentrations in K. Moreover, they have no structure, compared to
352 biochar; therefore their nutrients are easily leachable. At TF, in non-vegetated and vegetated
353 conditions, SPW P increased after adding the different amendments, except for with biochar. This
354 could be attributed to a P supply from the amendments, especially manure, which showed the
355 highest NH_4NO_3 -extractable P concentrations among the three amendments tested (Table 1). Finally,
356 SPW P concentrations decreased with time for P0%, PB and PI, without plant effect, which was
357 probably due to P leaching. On the contrary, SPW P concentration increased in PBI and in vegetated
358 PBM. This could have been due to leaching of the P from the amendments, as well as root exudates,
359 such as amino acids, carboxylic acids, sugars and flavonoids, which affect the behavior of nutrients
360 by modifying soil acidity, chelation, precipitation as well as altering the microbial activity and other
361 physical and chemical soil properties (Kidd et al. 2009).

362 P0% presented high SPW Pb concentrations at T0 (22.26 mg.L⁻¹) (Table 4), and decreased with all
363 amendments except manure alone. Decreases in SPW Pb concentration ranged from 30 % (PB) to 95
364 % depending on the amendments used (PBI, PIM and PBIM). For TF-*Agrostis*, SPW Pb concentrations
365 decreased for all amendments, except biochar alone, with decreases observed between 20 % (PB)
366 and 80 % (PIM). All amendments decreased SPW Pb concentrations for TF+*Agrostis*, between 24 %
367 (PM) and 80 % (PIM) compared to P0%. Such results are consistent with the sorption capacity of the
368 amendments. Indeed, Huang et al. (2018) showed that pig manure was able to reduce Cd, Cu, Pb and
369 Zn concentrations through precipitation onto phosphates and chelation with organic matter, while
370 Olimah et al. (2015) showed that ochre could adsorb As due to its high content of Fe(III)
371 oxyhydroxides. Moreover, Lebrun et al. (2018b) explained the reduction in SPW Pb concentrations
372 following biochar amendment to be partly due to its functional groups. Drops in SPW Pb
373 concentration can also be attributed to the increase in SPW pH induced by the application of
374 amendments (Kiran et al. 2017), as shown by the negative correlation observed between SPW pH
375 and SPW Pb concentrations at T0 ($R^2 = -0.91$, $p < 0.001$), for TF-*Agrostis* ($R^2 = -0.93$, $p < 0.001$) and
376 TF+*Agrostis* ($R^2 = -0.90$, $p < 0.001$) (Table S1). Regarding the evolution of SPW Pb with time, and
377 depending on the treatments tested, both increases and decreases were observed. For P0% and PB,
378 it decreased, with no plant effect, while for PM and PBM, the decrease was higher for plant growth,
379 which can be explained by both leaching, and ageing of the amendment, allowing higher sorption
380 and plant uptake. Finally, SPW Pb concentrations were observed to increase on PIM and PI, with and
381 without plants, respectively. On PBI and PBIM, SPW Pb concentrations increased in both conditions,
382 although it was higher without plants for PBI.

384 4. Plant growth and metal(loid) accumulation

385 *Agrostis capillaris* grown on P0% produced a low biomass: 0.48 g.pot⁻¹ and 0.92 g.pot⁻¹ for aerial and
386 root parts, respectively (Fig. 1). Regarding the aerial dry weight (DW), it only increased following the
387 manure amendments, with no difference between the four treatments containing manure. Root DW
388 increased for all amendments containing manure. This improvement in DW production was observed
389 previously, and can be explained by (1) a reduction in SPW As and Pb concentrations, (2) an
390 improvement in the soil physico-chemical properties (increases in pH and EC), and (3) the provision
391 of nutrients, especially K, by the amendments added, in particular manure (Jiang et al. 2014, Lebrun
392 et al. 2017, Walker et al. 2004). Indeed, manure was the only amendment leading to an increase in
393 aerial and root DW production compared to P0%, and the application of additional amendments did
394 not increase it any further. Manure treatments were also the ones presenting the highest NH₄NO₃-
395 extractable and SPW K and P concentrations. Moreover, K concentrations meet the concentrations
396 that are recommended for most of the plants to grow, *i.e.* 150-250 mg.kg⁻¹ (Rodriguez-Vila et al.
397 2017), only in the treatments containing manure. Therefore, the improvement of *Agrostis* growth in
398 this experiment was due to the fertilizing ability of manure, compared to biochar and redmud, which
399 are not fertilizers *per se*. These explanations were confirmed by the significant correlations found.
400 Aerial DW was positively correlated with NH₄NO₃-extractable K concentrations ($R^2 = 0.52$, $p < 0.001$)
401 and P concentrations ($R^2 = 0.73$, $p < 0.001$), SPW K concentrations at TF-*Agrostis* ($R^2 = 0.74$, $p < 0.001$)
402 and TF+*Agrostis* ($R^2 = 0.90$, $p < 0.001$) and SPW P concentrations at TF-*Agrostis* ($R^2 = 0.71$, $p < 0.001$)
403 and TF+*Agrostis* ($R^2 = 0.63$, $p < 0.001$) (Table 5). Root DW correlated positively with NH₄NO₃-
404 extractable K concentrations ($R^2 = 0.56$, $p < 0.001$) and P concentrations ($R^2 = 0.73$, $p < 0.001$), SPW
405 K concentrations at TF-*Agrostis* ($R^2 = 0.69$, $p < 0.001$) and TF+*Agrostis* ($R^2 = 0.76$, $p < 0.001$) and SPW
406 P concentrations at TF-*Agrostis* ($R^2 = 0.65$, $p < 0.001$) and TF+*Agrostis* ($R^2 = 0.56$, $p < 0.001$) (Table S1).

407
408 Aerial As concentration was 19.70 mg.kg⁻¹ in plants grown on P0% substrates (Fig. 2A) and decreased
409 only following PBM amendment, by 64 %. Such decreases contradict the observed increases in
410 NH₄NO₃-extractable As concentrations, but could be due to a dilution effect. This dilution effect was
411 consistent with the negative correlation between aerial DW and aerial As concentrations ($R^2 = -0.63$,
412 $p < 0.01$) (Table 5). When plants were grown on P0%, they accumulated 58.84 mg As kg⁻¹ in their
413 roots. Root As concentrations were found to increase with only with PBM. Such increment could be

414 explained by the affinity of As for organic matter, which can increase its availability to plants.
415 Moreover, a positive correlation was observed between root As concentrations and SPW P
416 concentrations for TF+*Agrostis* ($R^2 = 0.55$, $p < 0.001$) and NH_4NO_3 -extractable P concentrations ($R^2 =$
417 0.51 , $p < 0.001$) (Table S1). It could be caused by the fact that the supply of P from the amendments
418 increased the availability of As. This relation was previously observed by Gunes et al. (2009), where
419 they observed an increasing concentration of As in chickpea shoots when increasing concentrations
420 of As and P were applied to a clay loam soil. On the contrary, in the present case, the correlation
421 between aerial As concentrations and SPW P concentrations for TF+*Agrostis* was negative and lower
422 ($R^2 = -0.38$, $p < 0.05$) (Table S1). However, a negative correlation between rice grain As concentration
423 and available P was observed by Jiang et al. (2014). Similarly, this study showed a negative correlation
424 between aerial As concentration and NH_4NO_3 -extractable P concentrations ($R^2 = -0.62$, $p < 0.001$)
425 (Table S1). It can be hypothesized that P SPW concentrations, and its availability, increased As uptake
426 by the roots, although the transport system from roots to shoots was reduced. This can be explained
427 by the fact that, in the plants, As and P compete for the same transporters, as shown by Cao et al.
428 (2003) in *Pteris vittata* L.

429 Aerial Fe concentration was $193.73 \text{ mg.kg}^{-1}$ on P0% (Fig. 2B), and decreased following the
430 biochar+manure treatment, by 68 %. This could be due to a dilution effect, as plants grew better on
431 manure-amended technosols, and was also corroborated by the negative correlation between aerial
432 DW and aerial Fe concentrations ($R^2 = -0.63$, $p < 0.001$) (Table S1). On the contrary, root Fe
433 concentration, which was $515.11 \text{ mg.kg}^{-1}$ on P0%, increased with PBM, PIM and PBIM treatments.

434 Aerial Pb concentration was $257.16 \text{ mg.kg}^{-1}$ on P0% and decreased with all the amendments. This
435 decrease was contradictory to the increase in NH_4NO_3 -extractable Pb concentrations as well as the
436 study of Norini et al. 2019, which observed a decrease in Pb plant availability following the biochar
437 amendment. This could be due once again to a dilution effect. This was confirmed by the negative
438 correlation between aerial DW and aerial Pb concentrations ($R^2 = -0.71$, $p < 0.001$) (Table S1). Root
439 Pb concentrations was decreased with PI and PBI, whereas it increased following PBM treatment.

440 For all three metals and metalloids, As, Fe and Pb, the concentrations observed in *Agrostis* plants
441 were at levels considered normally toxic for plants, although *Agrostis* has been shown to tolerate
442 such high amounts (Rodríguez-Seijo et al. 2016). The concentrations were higher than the ones found
443 in previous studies (Dahmani-Muller et al. 2000), but the soil studied here presented higher pollution
444 concentrations.

445 In general, metal(loid) concentrations tended to decrease following amendment application,
446 especially in the aerial parts of plants grown on manure treated soils. This could have been the results
447 of the presence of higher contents in nutrients in these amendments. Abbas et al. (2018) showed
448 that a proper mineral nutrition of the plants could decrease plant Cd concentrations, due to the
449 competition between the essential elements and Cd. Since As and Pb are not essential nutrients, and
450 are even toxic to plants, plants tend to preferentially take up essential nutrients, especially when
451 they are in high concentrations. With more nutrients available in the soils amended with manure,
452 and other amendments to a lesser extent, they were preferentially taken up by *Agrostis capillaris*
453 compared to metal(loid)s.

454 Furthermore As, Fe and Pb concentrations in *Agrostis* plants were higher in the roots compared to
455 the aerial parts, which could be a tolerance mechanism (Rodríguez-Seijo et al. 2016). Indeed, roots
456 act as a trap organ for both metals and metalloids (Austruy et al. 2013), and the root cell wall, which
457 is negatively charged, can bind to pollutants with positive charges (Gautam and Agrawal 2017).

458 Finally, the metal(loid) concentrations showed a higher increase in the roots associated to a lower
459 translocation toward aerial parts in the PBM treatment, followed by PM and PIM.

460

461 Conclusion

462 In the present study, a phytoremediation mesocosm experiment was set up using a former mining
463 technosol amended with three different amendments, biochar, ochre and manure, applied alone or
464 combined. *Agrostis capillaris* plants were grown from seeds collected at the polluted site to test its
465 metal(loid) accumulation ability. The results showed that the different amendments, alone or

466 combined, improved soil conditions, which in turn allowed a better *Agrostis* plant growth, especially
467 with manure, due to its high nutrient supply. Based on this experiment, manure seems the best
468 amendment to ameliorate soil conditions and thus plant growth. However, it is a well-known fact
469 that the manure amendment has a short-term effect and would need to be re-applied regularly
470 (Mosaddeghi et al. 2009).. Therefore, when combined and added with ochre, and especially with
471 biochar, this will allow a longer term effect. Indeed, the SPW measurements showed that Pb
472 concentrations continued to decrease with time in PBM, contrary to PIM and PBIM treatments, which
473 encountered an increase in SPW Pb concentration with time. Moreover, the biochar + manure
474 treatment led to the lowest metal(loid) translocation to the aerial biomass. This study also showed
475 that *Agrostis*, metallicolous ecotype, seeds collected from the site could be used in a rehabilitation
476 process associated to the use of biochar and manure amendments. Indeed, the amendment
477 treatments will improve soil conditions (reduction of acidity and metal(loid) concentrations, supply
478 of nutrients) which will allow efficient *Agrostis* plant growth. Such treatments will reduce
479 contaminant spreading by soil erosion and water leaching.
480 However, the soil microbiota of the different substrates in particular, in both the bulk and the
481 rhizosphere, needs to be evaluated in order to assess the effect of amendments and *Agrostis* growth
482 on the soil microbial diversity and activity, as microorganisms are known to play an important role in
483 soil rehabilitation. Moreover, a test at the macrocosm level will be performed to confirm the present
484 results.

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489 References

- 491 Abbas, T., Rizwan, M., Ali, S., Adrees, M., Mahmood, A., Rehman, M. Z., Ibrahim, M., Arsad, M. &
492 Qayyum, M. F. (2018). Biochar application increased the growth and yield and reduced cadmium in
493 drought stressed wheat grown in an aged contaminated soil. *Ecotoxicology and environmental safety*,
494 148, 825-833.
- 495
496 Abed, S. N., Almuktar, S. A., & Scholz, M. (2017). Treatment of contaminated greywater using
497 pelletised mine water sludge. *Journal of environmental management*, 197, 10-23.
- 498
499 Ahmad, W., Najeeb, U. and Zia, M. H. (2015). Soil Contamination with Metals: Sources, Types and
500 Implications.
- 501
502 Ali, H., Khan, E. and Sajad, M. (2013). Phytoremediation of heavy metals—Concepts and applications.
503 *Chemosphere*, 91(7), 869-881.
- 504
505 Alvarenga, P., de Varennes, A. and Cunha-Queda, A. (2013). The Effect of Compost Treatments and
506 A Plant Cover with *Agrostis tenuis* on the Immobilization/Mobilization of Trace Elements in a Mine-
507 Contaminated Soil. *International Journal of Phytoremediation*, 16(2), 138-154.
- 508
509 Anawar, H., Akter, F., Solaiman, Z. and Strezov, V. (2015). Biochar: An Emerging Panacea for
510 Remediation of Soil Contaminants from Mining, Industry and Sewage Wastes. *Pedosphere*, 25(5),
511 654-665.
- 512
513 Arienzo, M., Christen, E. W., Quayle, W. and Kumar, A. (2009). A review of the fate of potassium in
514 the soil–plant system after land application of wastewaters. *Journal of Hazardous Materials*, 164(2-
515 3), 415-422.

516

517 Austruy, A., Wanat, N., Moussard, C., Vernay, P., Joussein, E., Ledoigt, G. and Hitmi, A. (2013).
518 Physiological impacts of soil pollution and arsenic uptake in three plant species: *Agrostis capillaris*,
519 *Solanum nigrum* and *Vicia faba*. *Ecotoxicology and Environmental Safety*, 90, 28-34.
520
521 Badri, D. V. and Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell &*
522 *Environment*, 32(6), 666-681.
523
524 Beesley, L., Moreno-Jiménez, E. & Gomez-Eyles, J. (2010). Effects of biochar and greenwaste compost
525 amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a
526 multi-element polluted soil. *Environmental Pollution*, 158(6), 2282-2287.
527
528 Cao, X., Ma, L. Q. and Shiralipour, A. (2003). Effects of compost and phosphate amendments on
529 arsenic mobility in soils and arsenic uptake by the hyperaccumulator, *Pteris vittata* L. *Environmental*
530 *Pollution*, 126(2), 157-167.
531
532 Celik, I., Ortas, I., and Kilic, S. (2004). Effects of compost, mycorrhiza, manure and fertilizer on some
533 physical properties of a Chromoxerert soil. *Soil and Tillage Research*, 78(1), 59-67.
534
535 Cha, J. S., Park, S. H., Jung, S. C., Ryu, C., Jeon, J. K., Shin, M. C. and Park, Y. K. (2016). Production and
536 utilization of biochar: a review. *Journal of Industrial and Engineering Chemistry*, 40, 1-15.
537
538 Chen, D., Liu, X., Bian, R., Cheng, K., Zhang, X., Zheng, J., Joseph, S., Crowley, D., Pan, G. and Li, L.
539 (2018). Effects of biochar on availability and plant uptake of heavy metals—A meta-analysis. *Journal*
540 *of Environmental Management*, 222, 76-85.
541
542 Cristaldi, A., Conti, G. O., Jho, E. H., Zuccarello, P., Grasso, A., Copat, C. and Ferrante, M. (2017).
543 Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. *Environmental*
544 *Technology & Innovation*, 8, 309-326.
545
546 Dahmani-Muller, H., Van Oort, F., Gelie, B. and Balabane, M. (2000). Strategies of heavy metal uptake
547 by three plant species growing near a metal smelter. *Environmental Pollution*, 109(2), 231-238.
548
549 Doi, M., Warren, G., & Hodson, M. E. (2005). A preliminary investigation into the use of ochre as a
550 remedial amendment in arsenic-contaminated soils. *Applied Geochemistry*, 20(12), 2207-2216.
551
552 Doubková, P. and Sudová, R. (2016). Limited impact of arbuscular mycorrhizal fungi on clones of
553 *Agrostis capillaris* with different heavy metal tolerance. *Applied Soil Ecology*, 99, 78-88.
554
555 Erickson, J. E., Cisar, J. L., Snyder, G. H., & Volin, J. C. (2005). Phosphorus and potassium leaching
556 under contrasting residential landscape models established on a sandy soil. *Crop science*, 45(2), 546-
557 552.
558
559 Fitz, W. and Wenzel, W. (2002). Arsenic transformations in the soil–rhizosphere–plant system:
560 fundamentals and potential application to phytoremediation. *Journal of Biotechnology*, 99(3), 259-
561 278.
562
563 Fresno, T., Moreno-Jiménez, E., & Peñalosa, J. M. (2016). Assessing the combination of iron sulfate
564 and organic materials as amendment for an arsenic and copper contaminated soil. A chemical and
565 ecotoxicological approach. *Chemosphere*, 165, 539-546.
566

567 Gautam, M. and Agrawal, M. (2017). Phytoremediation of metals using vetiver (*Chrysopogon*
568 *zizanioides* (L.) Roberty) grown under different levels of red mud in sludge amended soil. *Journal of*
569 *Geochemical Exploration*, 182, 218-227.

570

571 Gray, C. W., Dunham, S. J., Dennis, P. G., Zhao, F. J. and McGrath, S. P. (2006). Field evaluation of in
572 situ remediation of a heavy metal contaminated soil using lime and red-mud. *Environmental*
573 *Pollution*, 142(3), 530-539.

574

575 Gunes, A., Pilbeam, D. J. and Inal, A. (2009). Effect of arsenic-phosphorus interaction on arsenic-
576 induced oxidative stress in chickpea plants. *Plant and Soil*, 314(1-2), 211-220.

577

578 Hattab-Hambli, N., Motelica-Heino, M. and Mench, M. (2016). Aided phytoextraction of Cu, Pb, Zn,
579 and As in copper-contaminated soils with tobacco and sunflower in crop rotation: Mobility and
580 phytoavailability assessment. *Chemosphere*, 145, 543-550.

581

582 Huang, L. M., Yu, G. W., Zou, F. Z., Long, X. X. and Wu, Q. T. (2018). Shift of soil bacterial community
583 and decrease of metals bioavailability after immobilization of a multi-metal contaminated acidic soil
584 by inorganic-organic mixed amendments: A field study. *Applied Soil Ecology*, 130, 104-119.

585

586 Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, 68(1), 167-182.

587

588 Jiang, W., Hou, Q., Yang, Z., Zhong, C., Zheng, G., Yang, Z. and Li, J. (2014). Evaluation of potential
589 effects of soil available phosphorus on soil arsenic availability and paddy rice inorganic arsenic
590 content. *Environmental pollution*, 188, 159-165.

591

592 Khalid, S., Shahid, M., Niazi, N., Murtaza, B., Bibi, I. and Dumat, C. (2016). A comparison of
593 technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration*,
594 182, Part B, 247-268.

595

596 Kidd, P., Barceló, J., Bernal, M., Navari-Izzo, F., Poschenrieder, C., Shilev, S., Clemente, R. and
597 Monterroso, C. (2009). Trace element behaviour at the root-soil interface: Implications in
598 phytoremediation. *Environmental and Experimental Botany*, 67(1), 243-259.

599

600 Kiran, Y. K., Barkat, A., Cui, X. Q., Feng, Y., Pan, F. S., Tang, L. and Yang, X. E. (2017). Cow manure and
601 cow manure-derived biochar application as a soil amendment for reducing cadmium availability and
602 accumulation by *Brassica chinensis* L. in acidic red soil. *Journal of Integrative Agriculture*, 16(3), 725-
603 734.

604

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

608

609 Lebrun, M., Miard, F., Nandillon, R., Hattab-Hambli, N., Scippa, G. S., Bourgerie, S. and Morabito, D.
610 (2018a). Eco-restoration of a mine technosol according to biochar particle size and dose application:
611 study of soil physico-chemical properties and phytostabilization capacities of *Salix viminalis*. *Journal*
612 *of Soils and Sediments*, 18(6), 2188-2202.

613

614 Lebrun, M., Miard, F., Nandillon, R., Léger, J. C., Hattab-Hambli, N., Scippa, G. S., Bourgerie, S. and
615 Morabito, D. (2018b). Assisted phytostabilization of a multicontaminated mine technosol using
616 biochar amendment: Early stage evaluation of biochar feedstock and particle size effects on As and
617 Pb accumulation of two Salicaceae species (*Salix viminalis* and *Populus euramericana*). *Chemosphere*,
618 194, 316-326.

619
620 Lebrun, M., Miard, F., Renouard, S., Nandillon, R., Scippa, G. S., Morabito, D. and Bourgerie, S.
621 (2018c). Effect of Fe-functionalized biochar on toxicity of a technosol contaminated by Pb and As:
622 sorption and phytotoxicity tests. *Environmental Science and Pollution Research*, 25(33), 33678-
623 33690.

624
625 Lebrun, M., Miard, F., Nandillon, R., Scippa, G. S., Bourgerie, S. and Morabito, D. (2019). Biochar effect
626 associated with compost and iron to promote Pb and As soil stabilization and *Salix viminalis* L. growth.
627 *Chemosphere*, 222, 810-822.

628
629 Lee, S. H., Lee, J. S., Choi, Y. J. and Kim, J. G. (2009). In situ stabilization of cadmium-, lead-, and zinc-
630 contaminated soil using various amendments. *Chemosphere*, 77(8), 1069-1075.

631
632 [Liang, J., Yang, Z., Tang, L., Zeng, G., Yu, M., Li, X., Wu, H., Qian, Y., Li, X. and Luo, Y. \(2017\). Changes](#)
633 [in heavy metal mobility and availability from contaminated wetland soil remediated with combined](#)
634 [biochar-compost. *Chemosphere*, 181, 281-288.](#)

635
636 Lomaglio, T., Hattab-Hambli, N., Bret, A., Miard, F., Trupiano, D., Scippa, G. S., Motelica-Heino, M.,
637 Bourgerie, S. and Morabito, D. (2017). Effect of biochar amendments on the mobility and (bio)
638 availability of As, Sb and Pb in a contaminated mine technosol. *Journal of Geochemical Exploration*,
639 182, 138-148.

640
641 Mandal, B. K. and Suzuki, K. T. (2002). Arsenic round the world: a review. *Talanta*, 58(1), 201-235.

642
643 Marques, A. P., Oliveira, R. S., Rangel, A. O. and Castro, P. M. (2008). Application of manure and
644 compost to contaminated soils and its effect on zinc accumulation by *Solanum nigrum* inoculated
645 with arbuscular mycorrhizal fungi. *Environmental Pollution*, 151(3), 608-620.

646
647 Marseille, F., Tiffreau, C., Laboudigue, A. and Lecomte, P. (2000). Impact of vegetation on the mobility
648 and bioavailability of trace elements in a dredged sediment deposit: a greenhouse study. *Agronomie*,
649 20(5), 547-556.

650
651 Materechera, S. A. and Mkhabela, T. S. (2002). The effectiveness of lime, chicken manure and leaf
652 litter ash in ameliorating acidity in a soil previously under black wattle (*Acacia mearnsii*) plantation.
653 *Bioresource technology*, 85(1), 9-16.

654
655 Mokolobate, M. and Haynes, R. (2002). Comparative liming effect of four organic residues applied to
656 an acid soil. *Biology and Fertility of Soils*, 35(2), 79-85.

657
658 Mosaddeghi, M. R., Mahboubi, A. A., & Safadoust, A. (2009). Short-term effects of tillage and manure
659 on some soil physical properties and maize root growth in a sandy loam soil in western Iran. *Soil and*
660 *tillage research*, 104(1), 173-179.

661
662 Moreno-Jiménez, E., Fernández, J. M., Puschenreiter, M., Williams, P. N. and Plaza, C. (2016).
663 Availability and transfer to grain of As, Cd, Cu, Ni, Pb and Zn in a barley agri-system: Impact of biochar,
664 organic and mineral fertilizers. *Agriculture, Ecosystems & Environment*, 219, 171-178.

665
666 Namgay, T., Singh, B. and Singh, B. (2010). Influence of biochar application to soil on the availability
667 of As, Cd, Cu, Pb, and Zn to maize (*Zea mays* L.). *Aust. J. Soil Res.*, 48(7), 638-647.

668
669 Norini, M. P., Thouin, H., Miard, F., Battaglia-Brunet, F., Gautret, P., Guégan, R., Le Forestier, L.,
670 Morabito, D., Bourgerie, S., Motelica-Heino, M., 2019. Mobility of Pb, Zn, Ba, As and Cd toward soil

671 pore water and plants (willow and ryegrass) from a mine soil amended with biochar. *J. Env. Manage.*
672 232, 117-130.

673

674 Olimah, J. A., Shaw, L. J., & Hodson, M. E. (2015). Does ochre have the potential to be a remedial
675 treatment for As-contaminated soils?. *Environmental pollution*, 206, 150-158.

676

677 Oustriere, N., Marchand, L., Galland, W., Gabbon, L., Lottier, N., Motelica, M. & Mench, M. (2016).
678 Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and
679 phytotoxicity in a Cu-contaminated soil from a wood preservation site. *Science of The Total*
680 *Environment*, 566-567, 816-825.

681

682 Pueyo, M., López-Sánchez, J. and Rauret, G. (2004). Assessment of CaCl₂, NaNO₃ and NH₄NO₃
683 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils.
684 *Analytica Chimica Acta*, 504(2), 217-226.

685

686 R Development Core Team (2009) R: a language and environment for statistical computing. R
687 foundation for statistical Computing, Vienne, Austria

688

689 Rinklebe, J., Shaheen, S. M. and Frohne, T. (2016). Amendment of biochar reduces the release of toxic
690 elements under dynamic redox conditions in a contaminated floodplain soil. *Chemosphere*, 142, 41-
691 47.

692

693 Rodríguez-Seijo, A., Lago-Vila, M., Andrade, M. L. and Vega, F. A. (2016). Pb pollution in soils from a
694 trap shooting range and the phytoremediation ability of *Agrostis capillaris* L. *Environmental Science*
695 *and Pollution Research*, 23(2), 1312-1323.

696

697 Rodríguez-Vila, A., Forján, R., Guedes, R. S. and Covelo, E. F. (2017). Nutrient phytoavailability in a
698 mine soil amended with technosol and biochar and vegetated with *Brassica juncea*. *Journal of Soils*
699 *and Sediments*, 17(6), 1653-1661.

700

701 Sharma, R. K. and Agrawal, M. (2005). Biological effects of heavy metals: an overview. *Journal of*
702 *Environmental Biology*, 26(2), 301-313.

703

704 Su, C., Jiang, L. and Zhang, W. (2014). A review on heavy metal contamination in the soil worldwide:
705 situation, impact and remediation techniques. *Environmental Skeptics and Critics*, 3(2), 24-38.

706

707 Sudová, R., Doubková, P. and Vosátka, M. (2008). Mycorrhizal association of *Agrostis capillaris* and
708 *Glomus intraradices* under heavy metal stress: combination of plant clones and fungal isolates from
709 contaminated and uncontaminated substrates. *Applied Soil Ecology*, 40(1), 19-29.

710

711 Tang, X., Li, X., Liu, X., Hashmi, M., Xu, J. and Brookes, P. (2015). Effects of inorganic and organic
712 amendments on the uptake of lead and trace elements by *Brassica chinensis* grown in an acidic red
713 soil. *Chemosphere*, 119, 177-183.

714

715 Thouin, H., Norini, M. P., Le Forestier, L., Gautret, P., Motelica-Heino, M., Breeze, D., Gassaud, C. and
716 Battaglia-Brunet, F. (2019). Microcosm-scale biogeochemical stabilization of Pb, As, Ba and Zn in mine
717 tailings amended with manure and ochre. *Applied Geochemistry*, 111, 104438.

718

719 Van Poucke, R., Ainsworth, J., Maesele, M., Ok, Y. S., Meers, E. and Tack, F. M. G. (2018). Chemical
720 stabilization of Cd-contaminated soil using biochar. *Applied Geochemistry*, 88, 122-130.

721

- 722 Walker, D. J., Clemente, R. and Bernal, M. P. (2004). Contrasting effects of manure and compost on
723 soil pH, heavy metal availability and growth of *Chenopodium album* L. in a soil contaminated by
724 pyritic mine waste. *Chemosphere*, 57(3), 215-224.
725
- 726 Whalen, J. K., Chang, C., Clayton, G. W. and Carefoot, J. P. (2000). Cattle manure amendments can
727 increase the pH of acid soils. *Soil Science Society of America Journal*, 64(3), 962-966.
728
- 729 Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal
730 contaminated soils. *Chemosphere*, 50(6), 775-780.
731

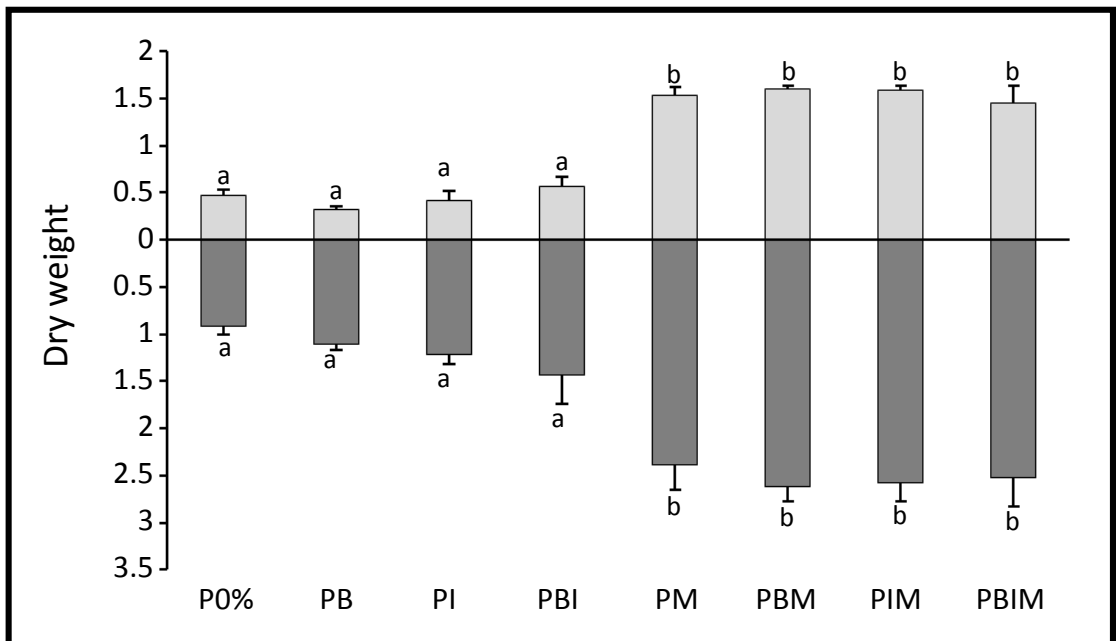


Fig 1: Dry weight (g.pot⁻¹) of the aerial (□) and root (■) parts of *Agrostis capillaris* plants grown on Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Letters indicate significant difference between treatments ($p < 0.05$) ($n = 5 \pm SE$).

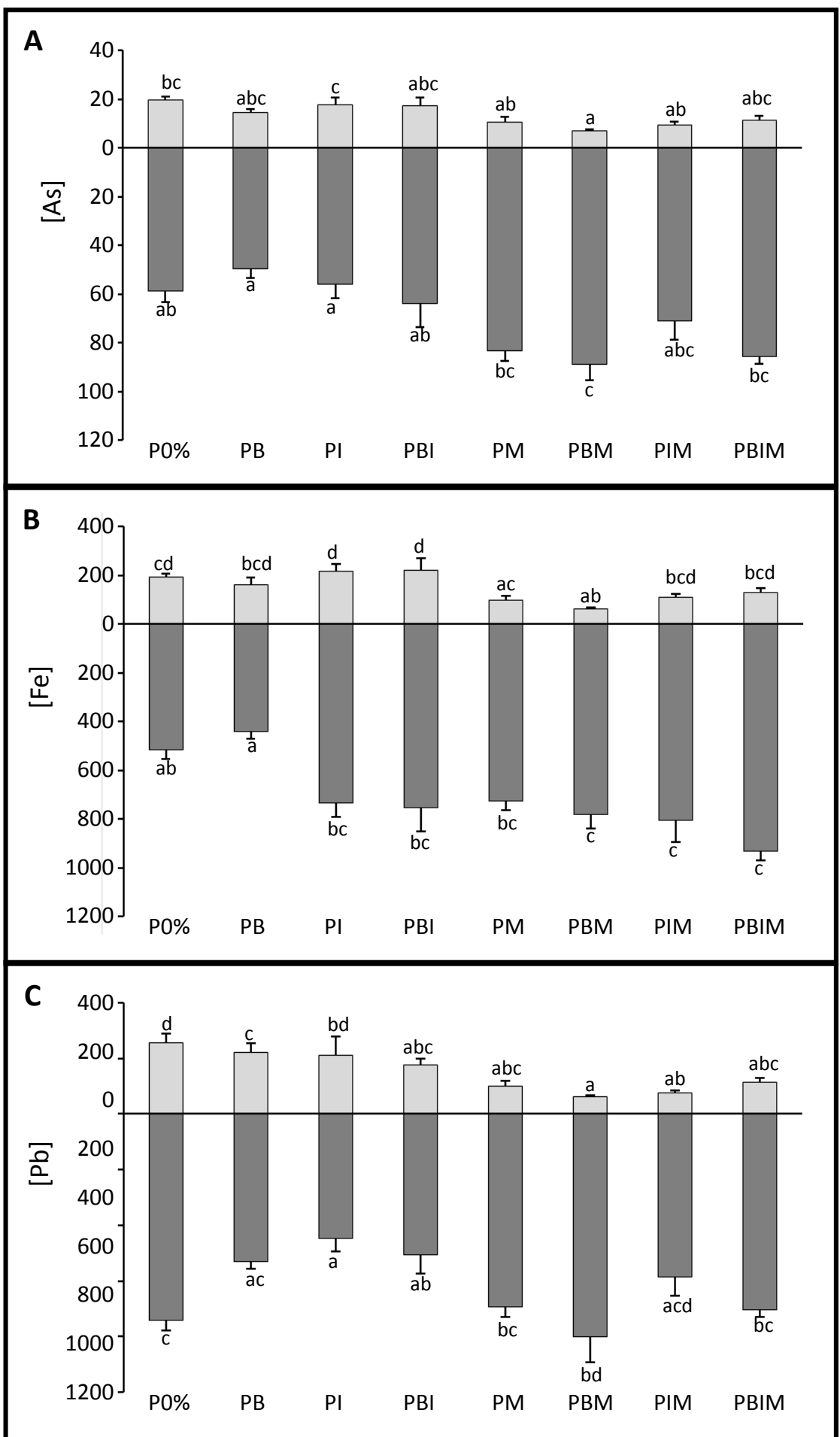


Fig 2: (A) As, (B) Fe and (C) Pb concentrations (mg.kg⁻¹) of the aerial (□) and root (■) parts of *Agrostis capillaris* plants grown on Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Letters indicate significant difference between treatments ($p < 0.05$) ($n = 5 \pm SE$).

Table 1: Amendments (biochar, ochre and manure) physico-chemical properties (pH, EC = electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$) and redox potential (mV)) and NH_4NO_3 -extractable element (As, Fe, K, P, Pb) concentrations ($\mu\text{g}\cdot\text{g}^{-1}$).

	Biochar	Ochre	Manure
Physico-chemical properties			
pH	8.46 ± 0.01	8.29 ± 0.03	9.52 ± 0.00
EC	302 ± 1	7765 ± 14	9476 ± 138
Redox potential	279.5 ± 6.5	217.4 ± 9.3	88.2 ± 0.9
NH_4NO_3 extractable concentrations			
As	0.90 ± 0.10	0.24 ± 0.09	0.75 ± 0.25
Fe	17.90 ± 5.10	1.04 ± 0.53	2.41 ± 0.20
K	752.30 ± 27.90	355.50 ± 0.53	16096.56 ± 65.49
P	8.40 ± 0.60	0.63 ± 0.07	250.28 ± 3.21
Pb	1.60 ± 5.10	0.31 ± 0.05	0.57 ± 0.06

Table 2: Soil NH₄NO₃-extractable element (As, Fe, K, P, Pb) concentrations (µg.g⁻¹) determined at the beginning of the experiment in Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Letters indicate significant difference between treatments (p < 0.05) (n = 5 ± SE). < DL = under detection limit (0.01 mg.L⁻¹)

	As	Fe	K	P	Pb
P0%	2.29 ± 0.25 ab	0.02 ± 0.02 a	22.14 ± 0.44 a	1.11 ± 0.14 a	4198.27 ± 138.94 bc
PB	3.25 ± 0.16 c	0.05 ± 0.04 a	25.76 ± 0.83 a	2.03 ± 0.11 b	5873.25 ± 288.53 d
PI	2.94 ± 0.21 bc	0.54 ± 0.09 b	24.56 ± 0.63 a	2.69 ± 0.12 c	5721.77 ± 123.12 d
PBI	3.23 ± 0.22 bc	0.13 ± 0.10 a	30.48 ± 0.63 a	3.24 ± 0.08 e	5215.40 ± 326.47 cd
PM	1.98 ± 0.19 a	< DL a	189.98 ± 8.85 c	1.42 ± 0.06 a	4005.37 ± 113.41 b
PBM	3.58 ± 0.35 c	< DL a	198.70 ± 9.85 c	3.35 ± 0.13 e	5706.37 ± 154.86 d
PIM	2.57 ± 0.26 abc	< DL a	165.12 ± 4.60 b	3.21 ± 0.09 de	3453.90 ± 397.50 ab
PBIM	2.30 ± 0.16 ab	< DL a	168.56 ± 6.58 bc	2.80 ± 0.10 cd	2962.18 ± 276.62 a

Table 3: Soil pore water physico-chemical properties (pH, electrical conductivity (EC) ($\mu\text{S.cm}^{-1}$), redox potential (mV)) determined at the beginning (T0) and at the end of the experiment in both non-vegetated (TF-*Agrostis*) and vegetated (TF+*Agrostis*) pots in Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Capital letters indicate significant difference between treatments for each sampling time whereas minuscule letters indicate significant difference T0, TF-*Agrostis* and TF+*Agrostis* for each treatment (=Time effect) ($p < 0.05$) ($n = 5-10 \pm \text{SE}$).

		pH		EC ($\mu\text{S.cm}^{-1}$)		Redox potential (mV)	
		Time effect		Time effect		Time effect	
P0%	T0	4.3 ± 0.1 A	b	210 ± 29 A	a	427 ± 7 F	a
	TF- <i>Agrostis</i>	3.8 ± 0.0 A	a	733 ± 53 A	b	439 ± 8 D	a
	TF+ <i>Agrostis</i>	3.9 ± 0.0 A	a	596 ± 75 A	b	421 ± 9 D	a
PB	T0	5.3 ± 0.2 B	b	255 ± 66 A	a	403 ± 6 E	a
	TF- <i>Agrostis</i>	4.6 ± 0.0 B	a	617 ± 30 A	b	396 ± 11 C	a
	TF+ <i>Agrostis</i>	4.8 ± 0.2 B	ab	661 ± 108 AB	b	378 ± 13 C	a
PI	T0	7.1 ± 0.1 D	b	2024 ± 59 B	c	318 ± 5 BC	a
	TF- <i>Agrostis</i>	6.3 ± 0.0 D	a	964 ± 77 AB	a	346 ± 7 AB	b
	TF+ <i>Agrostis</i>	6.3 ± 0.0 DE	a	1508 ± 196 BC	b	347 ± 4 AB	b
PBI	T0	7.2 ± 0.1 D	b	2137 ± 53 B	b	332 ± 2 C	a
	TF- <i>Agrostis</i>	6.6 ± 0.1 E	a	1417 ± 184 AB	a	324 ± 6 A	a
	TF+ <i>Agrostis</i>	6.6 ± 0.1 E	a	1895 ± 149 CD	b	323 ± 6 A	a
PM	T0	5.3 ± 0.2 B	a	2959 ± 140 C	b	383 ± 5 D	a
	TF- <i>Agrostis</i>	5.3 ± 0.1 C	a	1404 ± 211 AB	a	384 ± 5 C	a
	TF+ <i>Agrostis</i>	5.3 ± 0.1 C	a	1964 ± 82 CD	a	368 ± 6 BC	a
PBM	T0	6.1 ± 0.1 C	a	2923 ± 188 C	b	367 ± 3 D	b
	TF- <i>Agrostis</i>	6.3 ± 0.1 D	a	1379 ± 161 AB	a	352 ± 5 B	a
	TF+ <i>Agrostis</i>	6.1 ± 0.1 D	a	2387 ± 190 D	b	368 ± 4 BC	b
PIM	T0	7.5 ± 0.1 E	b	5451 ± 195 D	a	242 ± 5 A	a
	TF- <i>Agrostis</i>	6.8 ± 0.0 E	a	1778 ± 316 B	b	321 ± 7 A	b
	TF+ <i>Agrostis</i>	6.8 ± 0.0 E	a	3338 ± 352 E	c	330 ± 8 A	b
PBIM	T0	7.7 ± 0.0 E	b	5557 ± 130 D	c	307 ± 3 B	a
	TF- <i>Agrostis</i>	7.0 ± 0.1 E	a	2741 ± 453 C	a	321 ± 3 A	b
	TF+ <i>Agrostis</i>	6.9 ± 0.0 E	a	3927 ± 324 E	b	326 ± 2 A	b

Table 4: Soil pore water elements (As, Fe, K, P, Pb) concentrations (mg.L⁻¹) determined at the beginning (T0) and at the end of the experiment in both non-vegetated (TF-*Agrostis*) and vegetated (TF+*Agrostis*) pots in Pontgibaud technosol unamended (P0%) or amended with biochar (B), ochre (I) and manure (M), alone or combined. Capital letters indicate significant difference between treatments for each sampling time whereas minuscule letters indicate significant difference T0, TF-*Agrostis* and TF+*Agrostis* for each treatment (=Time effect) (p < 0.05) (n = 5-10 ± SE).

		[As] (mg.L ⁻¹)		[Fe] (mg.L ⁻¹)		[K] (mg.L ⁻¹)		[P] (mg.L ⁻¹)		[Pb] (mg.L ⁻¹)	
		Time effect		Time effect		Time effect		Time effect		Time effect	
P0%	T0	0.05 ± 0.02 A	a	0.00 ± 0.00 A	a	14.83 ± 5.07 A	a	0.14 ± 0.00 C	b	22.26 ± 0.62 E	b
	TF- <i>Agrostis</i>	0.07 ± 0.02 AB	a	0.00 ± 0.00 A	a	9.93 ± 0.66 A	a	0.04 ± 0.01 AB	a	6.78 ± 0.71 E	a
	TF+ <i>Agrostis</i>	0.04 ± 0.01 A	a	0.00 ± 0.00 A	a	12.31 ± 1.27 A	a	0.03 ± 0.01 A	a	9.19 ± 0.86 E	a
PB	T0	0.05 ± 0.01 A	a	0.00 ± 0.00 A	a	17.37 ± 4.11 AB	a	0.14 ± 0.00 C	b	15.51 ± 0.40 D	b
	TF- <i>Agrostis</i>	0.03 ± 0.00 A	a	0.00 ± 0.00 A	a	15.22 ± 1.03 A	a	0.02 ± 0.01 A	a	5.40 ± 0.18 D	a
	TF+ <i>Agrostis</i>	0.05 ± 0.01 AB	a	0.00 ± 0.00 A	a	15.60 ± 0.38 A	a	0.02 ± 0.00 A	a	5.62 ± 0.50 CD	a
PI	T0	0.08 ± 0.03 A	a	0.00 ± 0.00 A	a	11.90 ± 0.51 A	b	0.17 ± 0.01 D	b	1.91 ± 0.19 B	a
	TF- <i>Agrostis</i>	0.05 ± 0.01 AB	a	0.00 ± 0.00 A	a	8.72 ± 0.42 A	a	0.06 ± 0.01 BC	a	3.42 ± 0.19 BC	b
	TF+ <i>Agrostis</i>	0.03 ± 0.01 A	a	0.00 ± 0.00 A	a	6.80 ± 0.57 A	a	0.04 ± 0.01 A	a	2.52 ± 0.19 AB	a
PBI	T0	0.02 ± 0.01 A	a	0.04 ± 0.05 A	a	16.73 ± 0.65 B	a	0.04 ± 0.01 A	a	1.01 ± 0.12 A	a
	TF- <i>Agrostis</i>	0.07 ± 0.01 AB	b	0.00 ± 0.00 A	a	15.45 ± 1.39 A	a	0.08 ± 0.01 CD	b	2.85 ± 0.14 B	c
	TF+ <i>Agrostis</i>	0.04 ± 0.01 AB	b	0.00 ± 0.00 A	a	15.01 ± 0.21 A	a	0.09 ± 0.01 B	b	2.10 ± 0.33 A	b
PM	T0	0.07 ± 0.01 A	a	0.01 ± 0.01 A	a	305.15 ± 16.75 C	b	0.19 ± 0.00 D	b	24.89 ± 2.92 E	c
	TF- <i>Agrostis</i>	0.03 ± 0.00 AB	a	0.00 ± 0.00 A	a	145.50 ± 31.97 BC	a	0.07 ± 0.00 BC	a	4.32 ± 0.18 CD	a
	TF+ <i>Agrostis</i>	0.05 ± 0.01 AB	a	0.00 ± 0.00 A	a	223.09 ± 7.99 B	a	0.07 ± 0.01 AB	a	6.96 ± 0.54 D	b
PBM	T0	0.03 ± 0.00 A	a	0.00 ± 0.00 A	a	311.97 ± 19.04 CD	c	0.09 ± 0.00 B	a	10.24 ± 1.16 C	c
	TF- <i>Agrostis</i>	0.05 ± 0.00 AB	ab	0.00 ± 0.00 A	a	126.39 ± 24.48 BC	a	0.11 ± 0.01 D	ab	2.07 ± 0.19 AB	a
	TF+ <i>Agrostis</i>	0.07 ± 0.01 AB	b	0.00 ± 0.00 A	a	232.00 ± 22.91 B	b	0.11 ± 0.01 BC	b	4.26 ± 0.43 BC	b
PIM	T0	0.04 ± 0.00 A	a	0.00 ± 0.00 A	a	382.59 ± 10.51 DE	c	0.13 ± 0.01 C	a	1.06 ± 0.08 A	a
	TF- <i>Agrostis</i>	0.08 ± 0.01 B	b	0.00 ± 0.00 A	a	94.71 ± 23.00 AB	a	0.11 ± 0.01 D	a	1.35 ± 0.15 A	a
	TF+ <i>Agrostis</i>	0.06 ± 0.01 AB	b	0.00 ± 0.00 A	a	185.81 ± 20.41 B	b	0.11 ± 0.01 BC	a	1.85 ± 0.15 A	b
PBIM	T0	0.03 ± 0.00 A	a	0.00 ± 0.00 A	a	395.96 ± 8.01 E	b	0.13 ± 0.01 C	a	1.05 ± 0.04 A	a
	TF- <i>Agrostis</i>	0.08 ± 0.01 B	b	0.00 ± 0.00 A	a	191.11 ± 46.78 C	a	0.14 ± 0.01 E	a	1.46 ± 0.08 A	b
	TF+ <i>Agrostis</i>	0.09 ± 0.02 B	b	0.00 ± 0.00 A	a	238.21 ± 22.59 B	a	0.15 ± 0.02 C	a	2.05 ± 0.28 A	b

