

1 **Phytomanagement with grassy species, compost and dolomitic**
2 **limestone rehabilitates a meadow at a wood preservation site**

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23 **Abstract**

24 Brownfield surface is expanding in Europe, but as often abandoned or underused, these
25 areas become refuge for microbial, faunal and floral biodiversity. **However, brownfield**
26 **sites are generally contaminated, likely posing severe environmental risks. At a former**
27 **wood preservation site contaminated with Cu, we evaluated the efficiency of compost**
28 **and dolomitic limestone incorporation into the soil, followed by revegetation with Cu-**
29 **tolerant grassy species, as a phytomanagement option to increase vegetation cover and**
30 **plant diversity while reducing pollutant linkages. 7 years of phytomanagement**
31 **enhanced natural revegetation through the improvement of soil physicochemical**
32 **properties, particularly with compost-based amendments.** The compost incorporation
33 increased soil Cu solubility; however, no increment in Cu availability and a reduction in
34 Cu-induced phytotoxicity were observed with the compost. The improved soil nutrient
35 availability and the soil phytotoxicity mitigation in compost-amended soils facilitated
36 over the 7 years the growth of beneficial plant colonists, including leguminous species,
37 which can potentially promote essential soil functions. Soil treatments did not affect Cu
38 uptake and translocation by plants and shoot Cu levels indicated no risk for the food
39 chain. **Overall, a long-term phytomanagement combining an initial amendment of**
40 **compost and dolomitic limestone with the cultivation of Cu-tolerant grassy populations**
41 **can ameliorate such Cu-contaminated soils, by mitigating risks induced by Cu excess,**
42 **ultimately allowing the development of a meadow that can provide ecological and**
43 **economic benefits in terms of ecosystem services.**

44

45

46 **Keywords:** *Agrostis capillaris*; *Agrostis gigantea*; metal pollution; organic
47 amendments; phytotoxicity; phytoremediation

48 **1. Introduction**

49 Since the 1970's, a vast land anthropization in Europe has led to the depletion of entire
50 natural and semi-natural habitats and, ultimately, to a loss of biodiversity (Desrousseau
51 et al., 2019). In parallel, the increasing brownfield surface area, often abandoned or
52 underused, has eventually become refuge for numerous fauna or flora species (Connop
53 et al., 2016). Among them, current and former wood preservation sites are generally
54 large areas that present Cu-contaminated soils derived from the use of Cu-based salts as
55 wood preservatives and patches of mixed soil contamination including polycyclic
56 aromatic hydrocarbons (PAH) (Mench et al., 2018; Frick et al., 2019). These sites are
57 likely secondary sources of Cu contamination with potential deleterious effects on
58 ecosystems and human health. Dispersion of metal(loid)s in these sites can be prevented
59 by phytomanagement based on the implementation of a vegetation cover assisted with
60 soil amendments (Lagomarsino et al., 2011; Marchand et al., 2011; Kidd et al., 2015;
61 Frick et al., 2019).

62 Selection of plant species is crucial for the long-term success of
63 phytomanagement of metal-contaminated soils. An appropriate mixed stand of plant
64 species tolerant to metal excess and other adverse environmental conditions prevailing
65 in these sites, i.e. low soil organic matter (SOM), poor soil structure and texture, low
66 water holding capacity and nutrient contents, disturbed soil microbial communities, etc.,
67 would be needed for effective revegetation on the site, while keeping metal
68 translocation to aerial parts as low as possible to avoid food chain contamination
69 (Gómez-Sagasti et al., 2012; Burges et al., 2016).

70 In this respect, grassy species are known colonisers of metal-contaminated soils
71 that can establish themselves and develop an extensive root system due to their ability
72 to overcome the restraints of growth under the extreme ecological circumstances from

73 these soils (Bleeker et al., 2002). At a former wood preservation site (hereinafter
74 referred to as “Biogeco site”, St. Médard d’Eyrans, France), Cu-tolerant populations of
75 *Agrostis capillaris* and other grassy species have demonstrated their fitness and a Cu-
76 excluder phenotype, i.e. limiting Cu translocation to aerial plant parts, useful for
77 phytomanaging Cu-contaminated soils, particularly in combination with soil
78 amendments able to minimize Cu exposure (Bes et al., 2010; Hego et al., 2014). This
79 demonstrates also the importance of directing efforts to the conservation of the unique
80 biodiversity existing in these polluted sites (Garbisu et al., 2020).

81 The application of mineral and organic amendments can facilitate the
82 establishment of a plant cover by improving the *in situ* stabilization of metals and
83 reducing their mobility and bioavailability in soil, via adsorption, sorption and/or
84 precipitation processes (Hattab et al., 2014; Tiberg et al., 2016), but also by adding
85 nutrients for plant growth, increasing SOM content, raising soil pH and water holding
86 capacity, and enhancing soil biological activity (Alvarenga et al., 2009; Burges et al.,
87 2020; Menzies Plier et al., 2020). Bes and Mench (2008) have tested several inorganic
88 and organic amendments for reducing available soil Cu and its accumulation in plants
89 using potted Cu-contaminated soils from the Biogeco site. At this site, compost and
90 dolomitic limestone, alone and combined, have demonstrated their efficiency to
91 facilitate revegetation with various bioenergy crops, e.g. sunflower, tobacco, poplar and
92 willow short-rotation coppice (Kolbas et al., 2011, 2020; Marchand et al., 2011; Hattab
93 et al., 2014; Hattab-Hambli et al., 2016; Oustriere et al., 2016; Quintela-Sabaris et al.,
94 2017; Mench et al., 2018; Xue et al., 2018).

95 Here, we hypothesized that the combination of organic amendment (e.g.
96 compost) with dolomitic limestone followed by cultivation of a mixed stand of grassy
97 species from Cu-contaminated sites could be one relevant feasible phytomanagement

98 option to promote soil properties and natural revegetation and increase biodiversity,
99 while reducing Cu-induced environmental risks. This will eventually lead to the
100 rehabilitation of the soil ecosystem, enhancing its multiple ecological functions and
101 maximizing the ecological and economic benefits provided by soil ecosystem services.

102 This study aimed at assessing in the field the effectiveness of compost and
103 dolomitic limestone incorporation into the soil, alone and combined, and the use of Cu-
104 tolerant grassy species for initiating a meadow as a potential phytomanaging option for
105 Cu-contaminated soils.

106

107 **2. Materials and methods**

108 *2.1 Site and field trial*

109 The studied area is located in a wood preservation site at Saint-Médard d'Eyrans,
110 Gironde, SW France (N 44°43.353', W 000°30.938') with a temperate Atlantic climate
111 (variable mean rainfall and temperature; in 2012: 841 mm, 13.8°C). Wood preservatives
112 have been successively used for over a century, i.e. initially creosote and thereafter
113 various Cu-salts, mainly Cu sulphates (Mench et al., 2018). The site consists in about 10
114 ha of derelict areas with patches of natural attenuation and plant communities
115 dominated by *Agrostis capillaris* L., *Rumex acetosella* L., *Senecio inaequidens* D.C.,
116 *Populus nigra* L., *Salix caprea* L., and *Cytisus scoparius* L. (Bes et al., 2010). Only 2 ha
117 remain with a limited activity today. Anthropogenic topsoils are developed on an
118 alluvial sandy soil (Fluvisol – Eutric Gleysols, World Reference Base for soil
119 resources). A site survey indicated a high spatial variability (65-2600 mg Cu kg⁻¹) of
120 total topsoil Cu, whereas As, Zn, Cr and other metal(loid)s were at their background
121 levels (Mench and Bes, 2009; Bes et al., 2010). Some polycyclic aromatic hydrocarbons
122 (PAH) reached high concentrations at sub-sites (in mg kg⁻¹ soil dry weight - DW):

123 fluoranthene (1.9), indeno[1,2,3-cd]pyrene (0.95), benzo[g,h,i]perylene (0.8), and
124 benzo[b]fluoranthene (0.8) (Mench and Bes, 2009). More data on PAH in Jones et al.
125 (2016). Soil texture is sandy, i.e. 85.8% sand, 5.9% clay, and 8.3% silt, with 1.6%
126 SOM, C/N 17.2, soil pH 7, and a low cation exchange capacity (CEC, 3.5 cmol kg⁻¹)
127 (Mench and Bes, 2009). For detailed description on site history, soil characterization,
128 and zoning of soil ecotoxicity, see Mench and Bes (2009), Bes et al. (2010) and Kolbas
129 et al. (2020). Plant communities were characterized in Bes et al. (2010).

130 The field trial (hereinafter referred to as “the PG sub-site”, PG meaning Grassy
131 Plots) consists in 4 blocks containing 4 plots each (1m x 3m). In April 2006, the 0 – 50
132 cm depth soil layer was loosened with a tiller, the integrity of the sub-soil being
133 preserved. Then, four soil treatments were applied to the plots (n = 4) following a
134 randomized block design with a 0.5 m space between plots: (1) DL: dolomitic limestone
135 (0.2%, w/w); (2) OM: compost made from poultry manure and pine bark chips (5%,
136 w/w); (3) OMDL: the combination of compost and dolomitic limestone; and (4) UNT:
137 untreated soil. The amendments were incorporated into the topsoil (0-25 cm) and mixed
138 with a stainless steel spade. The origin and composition of the soil amendments are
139 detailed in Lagomarsino et al. (2011). In November 2006, five grassy species belonging
140 to the *Poaceae* family were selected, based on their Cu-tolerant ecotype, and
141 transplanted in all plots: *Agrostis capillaris* L. (AC), *Agrostis gigantea* Roth (AG),
142 *Deschampsia cespitosa* (L.) P. Beauv. (DC), *Sporobolus indicus* (L.) R. Br. (SI) and
143 *Vulpia myuros* (L.) C.C. Gmel (VM). Seeds of AG were collected at a Cu/Ni
144 contaminated site in Sudbury, Canada (Bagatto and Shorthouse, 1999), while seeds of
145 DC came from a contaminated site in Katowice, Poland (Dr. R. Kucharski et al, IETU,
146 Katowice). Seeds of Cu-tolerant AC, SI and VM populations were collected from
147 mature plants on the Biogeco site (summer 2006). The seeds were germinated on an

148 uncontaminated sandy soil and 3-month-old plantlets were transplanted to the plots.
149 Plants were weekly watered using individual plastic reservoirs (1.2 L) during the first
150 summer to avoid early mortality due to drought effect. In the spring of 2008, mortality
151 and shoot biomass of the transplanted plant species were monitored after a 2-year
152 growth period, and thereafter shoot ionomes were determined as described below.

153

154 *2.2 Soil and plant parameters*

155 In early 2012, six topsoil samples (0-25 cm) were randomly collected within each plot
156 and in an uncontaminated kitchen garden (CTRL) with the similar soil type located 17
157 km away of the site, Gradignan, France, using a steel spade, and combined to form a
158 composite sample (2 kg fresh weight). Soil samples were air-dried and sieved to 2 mm
159 prior to analysis. In parallel, fresh aliquots of topsoil samples for each plot were
160 transferred to 1 L plastic pots where Rhizon moisture samplers (Eijkelkamp, the
161 Netherlands), **type MOM (used to collect soil pore water for analysis of macro and**
162 **micro elements)**, were inserted (45° angle) and maintained one month at 75% of the
163 water holding capacity. Subsequently, soil pore water was sampled (two times 10 mL, 5
164 days apart). Fresh aliquots of topsoil samples were also used for the phytotoxicity assay
165 described in section 2.3. All soil analysis, including total metal(loid) concentrations,
166 were performed at the INRA Laboratoire d'Analyses des Sols (LAS), Arras (France),
167 using standard methods (INRA LAS, 2019). Soil pH was measured according to the
168 French norm (Afnor X31-103, 1994). The negative of the base 10 logarithm of the
169 molar concentration of free Cu ions in the solution (pCu) was computed based on the
170 equation proposed by Sauvé (2003):

$$171 \quad \text{pCu}^{2+} = 3.20 + 1.47 \cdot \text{pH} - 1.84 \cdot \log_{10}(\text{Total soil Cu})$$

172 where $p\text{Cu}^{2+}$ is free Cu activity and Total soil Cu corresponds to the total Cu
173 concentration in the topsoil (in mg Cu kg^{-1}). Soil pore water solution was analysed for
174 pH (Hanna instruments, pH 210, combined electrode Ag/AgCl - 34), electrical
175 conductivity, i.e. EC, (WTW Multiline P4 metre, Germany) and element concentrations
176 using ICP-AES (Varian Liberty 200).

177 Diffusive fluxes of Cu were measured in soil samples using diffusive gradients
178 in thin films (DGT) fitted with chelex-100-resin-impregnated gels, in triplicate. Soil
179 subsamples were incubated at 25°C for 24 h for equilibration, subsequently placed on
180 the top of DGT sample devices, and kept in plastic boxes with water-saturated
181 atmosphere at 25°C for a period of 24 h. Then, DGT devices were retrieved and opened,
182 and the Chelex resins were eluted with 1 mL of 1M HNO_3 following Zhang et al.
183 (2001). Soil solutions were obtained after centrifugation of soil slurries. Cu
184 concentration for both soil solution (Cu_{soil}) and resin gel eluates were analysed using
185 inductively coupled plasma-mass spectrometry (ICP-MS; Elan 9000 DRc, Perkin
186 Elmer). Time-averaged concentrations of Cu at the DGT-soil interface (DGT-Cu) and R
187 (ratio between DGT-Cu and Cu_{soil}) were calculated according to Ernstberger et al.
188 (2002).

189 In 2013, the occupancy of the plant cover and plant species richness were
190 assessed per plot. Then, for each plot, aerial parts of all existing plants, except tree
191 species, were harvested, washed thoroughly with deionized water, and oven-dried at
192 65°C for 48 h to calculate dry weights. For determining shoot ionome, samples were
193 ground (<1.0 mm particle size, Fritsch Pulverisette 19) and subsamples (0.5 g **dry**
194 **weight**) were wet-digested under microwaves (CEM Marsxpress 1200W) with 5mL
195 suprapure 14M HNO_3 , 2mL 30% (v/v) H_2O_2 not stabilized by phosphates and 1mL

196 MilliQ water, according to Bes and Mench (2008). Elements in digests were determined
197 by ICP-AES (Varian Liberty 200).

198

199 *2.3 Phytotoxicity bioassay*

200 A Plantox test using dwarf beans (*Phaseolus vulgaris*, cv. Mangetout) was performed to
201 evaluate soil phytotoxicity, as described in Bes and Mench (2008). Beans were sown in
202 potted (1.3 L) fresh soil samples (4 plants per pot, 4 pots per treatment). Plants were
203 grown under controlled conditions (14h/10h light/dark cycle, 25/22°C day/night
204 temperature, 65% relative humidity, and a photosynthetic photon flux of 150 $\mu\text{mol m}^{-2}$
205 s^{-1}) in a growth chamber and were watered daily with deionised water at 75% water
206 holding capacity. Fifteen days after sowing, plants were harvested and fresh weights of
207 the primary leaves and total shoots were determined for each plant.

208

209 *2.4 Statistical analysis*

210 The effect of soil treatments on soil physicochemical properties, plant properties and
211 dwarf beans biomass was assessed by means of one-way analysis of variance (ANOVA)
212 using R (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria). When
213 significant differences occurred between soil treatments, multiple comparisons of mean
214 values were made using post-hoc Tukey's HSD test. Linear Pearson regression was
215 used to assess significant correlations between soil physicochemical properties.
216 Multivariate analyses were performed by means of redundancy analysis (RDA) and
217 variation partitioning analysis, using Canoco 5 (ter Braak and Šmilauer, 2012), to
218 further evaluate the influence of soil treatments on the biomass, species richness and
219 occupancy of the plant vegetation cover through changes in soil properties.

220

221 **3. Results**

222 *3.1 Soil physicochemical and phytotoxicity parameters*

223 Both compost treatments, *i.e.* OM and OMDL, significantly increased the soil CEC and
224 contents of organic C, SOM and total N (Table 1). Levels of these soil properties in the
225 DL plots were similar to those of the UNT plots. The DL and OMDL treatments slightly
226 increased pH values in both soil and soil pore water, albeit differences were not
227 significant as compared to the UNT soil.

228 Regarding the concentration and availability of Cu in soil, the soil treatments did
229 not influence total soil Cu and free Cu activity (pCu) (Table 1). Total Cu concentration
230 in the soil pore water (SPW-Cu) of the OM and OMDL soils, however, was roughly 2-
231 fold higher than that of the UNT soils. Cu concentration measured with DGT (DGT-Cu)
232 showed no significant influence of the soil treatments, whereas R values, **which indicate**
233 **the Cu resupply capacity from the solid phase to the soil solution**, were lower in the
234 OMDL soils. Pearson correlation values between soil physicochemical parameters
235 indicated a positive relationship between SPW-Cu and soil properties improved by the
236 OM and OMDL treatments (Supplementary table 1): values of SPW-Cu were more
237 influenced by increasing levels of organic C (R = 0.67; p<0.01), total N (R = 0.68;
238 p<0.01) and CEC (R = 0.62; p<0.01) in soil than by the total soil Cu.

239 The phytotoxicity bioassay showed, based on both primary leaf and shoot
240 biomass (Figure 1), that all studied soil treatments promoted the growth and
241 development of dwarf beans, as compared to beans cultivated in the UNT soils.
242 Nonetheless, beans growth peaked in the OMDL soils, indicating that this soil treatment
243 resulted in the highest mitigation of Cu-induced phytotoxicity in year 6.

244

245 3.2 Plant properties

246 After a 2-year growth period, the survival of the transplanted plant species was
247 monitored, showing that all plant species managed to establish in the plots, except *S.*
248 *indicus* that did not survive (Figure 2). Remaining plant species established in the field
249 trial with varying success, with *A. gigantea* and *V. myuros* presenting the lowest
250 mortality, followed by *D. caespitosa*, and, lastly, *A. capillaris*. However, **identification**
251 **of plant species in the field trial in year 7 (Table 2)** indicates that changes in the
252 population dynamics of the transplanted plants through the following years have
253 occurred: *V. myuros* and *D. caespitosa* disappeared from all the plots whereas *A.*
254 *gigantea* and *A. capillaris* were still present. In year 7, plots have been colonised by
255 other herbaceous species, among which the dominant species were *Rumex acetosella*
256 and members of the *Asteraceae*, i.e. *Senecio inaequidens*, *Hypochaeris radicata*,
257 *Omalotheca sylvatica*, and *Fabaceae* families, i.e. *Ornithopus perpusillus*, *Medicago*
258 *arabica*, and *Vicia sativa*. Colonising species also included shrubs and woody species,
259 i.e. *Cytisus scoparius*, *Populus nigra* and *Quercus robur*.

260 **Differences in the presence and occurrence of the identified plant species among**
261 **soil treatments were also registered (Table 2).** Generally, soil treatments increased the
262 number of plant species, particularly in the OM and OMDL plots, as compared to the
263 UNT ones. This is corroborated by the estimations of plant species richness (Table 3),
264 which were significantly higher in the OMDL plots. Total shoot biomass and the
265 occupancy percentage of the plant cover were also higher in the compost-amended plots
266 (OM and OMDL), although these differences were not statistically significant. **The**
267 **similarity in species composition of plant communities between different plots was**
268 **higher among amended plots (Supplementary table 2). The DL, OM and OMDL plots**
269 **sheltered plant species not present in the UNT plots, i.e. *Erigeron sumatrensis* (also**

270 known as *Conyza albida*), *Cirsium vulgare* and *Crepis biennis* (Table 2). Other species,
271 e.g. *Daucus carota*, *Ornithopus perpusillus*, *Medicago sativa* and *Crepis praemorsa*,
272 grew only in the OM and OMDL soils. Figure 3 demonstrates the effect of compost-
273 based amendments on the growth and development of the plant cover through the
274 improvement of soil properties. The RDA shows soil parameters related to soil organic
275 matter and nutrient contents being positively correlated with plant parameters, and
276 species richness in particular, while soil parameters indicating the Cu contamination
277 level showed little influence.

278 Regarding the shoot ionomes (Table 4), no significant effect of the soil
279 treatments was identified for the majority of elements. Although not statistically
280 significant, values of shoot Cu concentration showed a decreasing trend in the OMDL
281 plots. Moreover, values of shoot Cu concentration in year 7 were considerably lower
282 than those found in year 2 in the transplanted species (Supplementary table 3). Values
283 of shoot P concentration, on the other hand, were significantly higher in both compost-
284 amended plots (OM and OMDL). Shoot Mg, K, and B concentrations showed
285 increasing levels in amended plots, but these differences were not significant.

286

287 **4. Discussion**

288 *4.1 Effect of soil treatments on Cu availability and phytotoxicity*

289 The incorporation of organic amendments into metal(loid)-contaminated soils can
290 improve soil physicochemical properties, e.g. by reducing metal availability,
291 incorporating organic matter and available nutrients, and modifying pH and other
292 physicochemical parameters, and can promote soil microbial properties (Alvarenga et
293 al., 2009; Epelde et al., 2009; Kidd et al., 2015; Mench et al., 2018; Burges et al., 2020).
294 Here, both soil treatments containing compost, i.e. OM and OMDL, increased the levels

295 of several soil properties, particularly those related to organic matter and nutrient
296 contents, corroborating the lasting positive effect of this compost after 6 years of
297 phytomanagement.

298 The incorporation of soil amendments with the same chemical composition
299 (OM, DL and OMDL) in other phytomanaged field trials at this site has generally
300 resulted in an overall decrease in the available soil Cu fraction assessed by single
301 chemical extractions (notably salt solutions, e.g. 1 M NH_4NO_3) (Kolbas et al., 2011;
302 Hattab-Hambli et al., 2016; Mench et al., 2018). In these studies, total Cu
303 concentrations in soil pore water can however increase in the compost-amended soils,
304 but the percentage of free Cu ions was low (i.e. < 5%). Here, Cu availability in soil
305 estimated in terms of total Cu concentration in the soil pore water (SPW-Cu) was
306 significantly higher in compost-amended plots, owing to the higher organic matter
307 content in topsoil (Table 1) and its biogeochemical cycle (mineralisation /
308 humification). This input of degradable organic matter may come from the decay of
309 senescent plant shoots and rhizodeposition, containing cellulose, lignin and other
310 polysaccharides, and from the compost incorporated into the OM and OMDL soils. In
311 comparison, in a short-rotation coppice with poplar and willows at this site, Xue et al.
312 (2018) reported an ongoing C decomposition 6 years after the application of similar soil
313 amendments, suggesting the slow organic matter decomposition of this compost and
314 carbon inputs via the leaf and herbaceous litter. The (bio)degradation of the organic
315 matter may not only release metals bound to the remaining compost and soil organic
316 matter but also result in a flush of dissolved organic matter that is known to promote Cu
317 solubilisation, increasing total Cu concentration in the soil pore water (Brandt et al.,
318 2010; Burges et al., 2015).

319 Total metal concentration in the soil pore water is believed to mirror root
320 exposure to metals (Sauvé, 2003; Hattab et al., 2014). However, soluble metals include
321 metals bound to colloids and organic ligands, that are not all directly bioavailable, and
322 estimations of soluble Cu do not account for the depletion at the root-soil interface and
323 depletion-induced resupply from the solid phase. DGT is a passive sampling method
324 that excludes strongly bound, organically complexed metals and colloidal species,
325 measuring the labile metal fraction in soil that can be taken up by biota and,
326 accordingly, can contribute to toxicity (Zhang et al., 2001; Ernstberger et al., 2002;
327 Paller et al., 2019). Here, DGT-Cu values showed no influence of the soil treatments
328 (Table 1), suggesting that much of the soluble Cu in compost-amended soils was
329 associated with organic ligands that are unable to dissociate and, hence, was not
330 available. This was similar in the OMDL plots under a sunflower – tobacco rotation in
331 another field trial (Supplementary table 4, Mench et al., 2018). In contrast, R values,
332 indicating the depletion extent of soil solution concentration at the DGT interface
333 (Ernstberger et al., 2002), decreased in compost-amended soils, notably in the OMDL
334 plots under grassy crops (Table 1) and high yielding crops (sunflower / tobacco) as well
335 (Supplementary table 4). Accordingly, despite the increment of soluble Cu, Cu
336 bioavailability in soil was limited, as well as the Cu resupply capacity from the solid-
337 phase to the soil solution, with the incorporation of compost and the subsequent
338 increase in DOM.

339 In any case, the potential Cu toxicity must be assessed not only through the
340 interpretation of soil physicochemical parameters, but also by relating such data with
341 the ecotoxicological responses in bioassays. Soil phytotoxicity bioassays are frequently
342 used to assess the influence of soil organic amendments on the mitigation of metal-
343 induced phytotoxicity (Bes and Mench, 2008; Marchand et al., 2011; Galende et al.,

2014; Kumpiene et al., 2014; Lacalle et al., 2018). Although this often results from a reduction in metal availability, Marchand et al. (2011) reported a decrease in phytotoxicity in the leachates of compost-amended soils, despite finding no changes in free Cu. Here, the ecotoxicity bioassay using dwarf beans showed that soil treatments, and OMDL in particular, allowed a higher shoot biomass, indicating a better mitigation of Cu-induced soil phytotoxicity in these soils amended with compost and dolomitic limestone in combination (Figure 1). This suggests that the compost may have contributed with forms of dissolved organic matter that complex with Cu, e.g. fulvic acids, resulting in much of the soluble Cu not being available and not contributing to Cu phytotoxicity. Increase in soil CEC, total soil N, and soil organic matter (and likely water holding capacity) (Table 1), would also contribute to promote the growth of bean plants.

356

357 *4.2 Effect of soil treatments on the development of the plant cover*

358 Here we focused on the potential of this mixed stand of 5 grassy species, *A. capillaris*
359 and *V. myuros* populations being present and their seeds collected at this site, to initiate
360 a meadow, as well as the potential beneficial influence of soil treatments on its
361 development. Despite successful colonisation of most of the transplanted plant species
362 in the first 2 years, *D. caespitosa* and *V. myuros* disappeared from the plots in the
363 following years after repeated heat waves and long periods of drought in the summer
364 (Table 2). It is worth mentioning that sampling time has proved to be a relevant factor in
365 explaining differences in plant diversity and composition in the site (Bes et al., 2010).
366 *Vulpia myuros* has a short annual life cycle in spring and tends to disappear in summer,
367 possibly indicating a seasonal absence at the sampling time here (end of summer) rather
368 than a complete loss of this specie. Both *A. capillaris* and *A. gigantea*, however, were

369 widely present in the plots in year 7, being likely more resilient to progressive climate
370 change conditions. *Agrostis capillaris*, in particular, was a dominant species across all
371 the plots. This goes in line with previous findings that demonstrated the fitness and the
372 excluder phenotype of the Cu-tolerant populations of *A. capillaris* from this site (Bes et
373 al., 2010; Hego et al., 2014). Then, as a typical succession, there was a progressive
374 colonization in the plots with other plant species (Table 2), dominated mainly by
375 members of the *Poaceae* and *Asteraceae* families, which are plant groups commonly
376 found in areas contaminated with industrial waste (Desjardins et al., 2014), followed by
377 *R. acetosella* (*Polygonaceae*), which is also known to include Cu-tolerant ecotypes
378 (Bagatto and Shorthouse, 1999). This plant succession likely occurred from natural
379 spreading and germination of seeds from plant species from the site, the majority of
380 colonising species being already present in this site before this study (Bes et al. 2010),
381 and the surroundings. Heavy colonization by *C. scoparius* has made it necessary to
382 carry out an annual cut to maintain the grassland option. Nevertheless, the colonisation
383 by trees, i.e. *Q. robur* and *P. nigra*, over the period of 7 years could be gradually turning
384 this grassland into a mixed woody-herbaceous system.

385 The application of soil amendments, and compost-based amendments in
386 particular, facilitated the colonisation of a higher number of species, resulting in an
387 increase in plant diversity (Table 2; Table 3). This spontaneous vegetation promoted by
388 soil amendments, represented by members of the *Asteraceae* and *Fabaceae* families,
389 and *Daucus carota* (*Apiaceae*), corresponded mostly to herbaceous species with an
390 annual life cycle, usual colonisers in contaminated sites as annual species respond more
391 quickly to environmental changes (Rich et al., 2008; Shutcha et al., 2015). Untreated
392 soils, however, remained colonised mainly by perennial grasses (Supplementary table
393 5), which are often regarded as pioneer species in highly contaminated sites due to their

394 tolerance to metal-induced stress (Bagatto and Shorthouse, 1999; Bes et al., 2010).
395 Interestingly, the majority of the new colonising species, i.e. not previously identified in
396 this site (Bes et al., 2010), belonged to the *Asteraceae* family. Compost-amended plots
397 sheltered fodder species from the N-fixing *Fabaceae* family, i.e. *M. arabica*, *O.*
398 *perpusillus* and *V. sativa*, that in this site were known to grow in the less contaminated
399 areas (Bes et al., 2010). Other studies in this site have also reported that the
400 incorporation of greenwaste compost, in combination with dolomitic limestone,
401 promoted the growth of leguminous species, i.e. white clover, following a reduction in
402 soil Cu bioavailability (Mench et al., 2018). This was accompanied by structural
403 changes in soil microbial communities, reflected in increments of N-fixing bacterial
404 groups, such as *Rhizobiales* or *Rhodospirillales* (Burges et al., 2020). Considering that
405 *Fabaceae* members tend to grow in less phytotoxic soils (Wang et al., 2004; Shu et al.,
406 2005), our results suggest that the compost incorporation may have prompted better soil
407 conditions for the growth and development of these plant species (Figure 3).

408 Limited nutrient availability can be a factor for increasing phytotoxicity (Bes
409 and Mench, 2008), which explains that Cu-induced phytotoxicity reduction here, along
410 with plant species diversity, was paralleled with increasing levels of soil organic matter
411 and nutrients (Table 1; Figure 3), and shoot P concentration in plants (Table 4).
412 Moreover, despite both compost-based treatments equally improved soil properties, the
413 OMDL treatment allowed both the most pronounced Cu-induced toxicity reduction and
414 the highest plant species richness (Figure 1; Table 3). This suggests an additional
415 synergic effect, potentially due to the nutrients supplied by the incorporation of
416 dolomitic limestone that are not sufficiently present in the compost alone, i.e. content in
417 Ca, Mg, etc. Overall, the application of compost, notably in combination with dolomitic
418 limestone, not only promoted plant growth and development of this grassland, in line

419 with previous findings (Bes and Mench, 2008; Marchand et al., 2011; Hattab-Hambli et
420 al., 2016; Mench et al., 2018), but it also allowed a plant diversity that can potentially
421 enhance soil key functions, such as N cycling and SOM accumulation, contributing to C
422 sequestration and improving ecosystem services (Teixeira et al., 2015).

423 No influence of the soil amendments was identified on the uptake and
424 accumulation of Cu by plants (Table 4). However, the shoot Cu concentrations found
425 here in any of the plots did not exceed the upper critical threshold levels, i.e. the lowest
426 tissue concentration of an element above which the plant yield is reduced by 10%,
427 reported by Macnicol and Beckett (1985) for *Agrostis* spp. and other grassy species (30-
428 35 mg Cu kg⁻¹ DW). Comparing with plants grown in plots with similar topsoil Cu
429 levels from this site, our values of shoot Cu concentration were similar to those obtained
430 by Mench et al. (2018) in sunflower in amended soils (26 mg Cu kg⁻¹) and considerably
431 lower than those obtained in year 2 in the transplanted plant species, including *Agrostis*
432 (59-137 mg Cu kg⁻¹; Supplementary table 3). This could indicate that the plants
433 composing the grassland are able to control the uptake and translocation of Cu, even in
434 the absence of amendments, limiting the entrance of Cu into the food chain. Moreover,
435 shoot Cu values here were slightly above the minimum average value of 10 mg Cu kg⁻¹
436 considered adequate in pasture for both plant nutrition and animal health for New
437 Zealand conditions (Longhurst et al., 2004). European Union directives do not have any
438 particular restrictions for Cu in animal fodder, while guidelines in Switzerland state a
439 limit of 40 mg Cu kg⁻¹ in fodder for cattle (Directive, 2002). Therefore, the shoot Cu
440 concentrations here fell within the optimum range for maintaining both food chain
441 safety and an adequate plant nutritional status, suggesting also the suitability of this
442 grassland as a grazing system. However as some PAH are present in the soils at this site

443 (Jones et al., 2016), exposure through soil ingestion by herbivores during grazing should
444 be investigated.

445

446 **5. Conclusions**

447 Soil amendments containing a compost made of pine bark chips and poultry manure
448 improved soil physicochemical properties, including an increment in soil organic matter
449 and nutrient content, at a Cu-contaminated site phytomanaged with grassy species, 6
450 years after their application. The compost incorporation increased Cu solubility.
451 However, most of this soluble Cu was likely complexed with **dissolved organic matter**
452 and, hence, was not available, as reflected in the reduced Cu resupply capacity from the
453 solid-phase to the soil solution and the mitigation of Cu-induced phytotoxicity. After 7
454 years of phytomanagement, soil amendments, and notably compost combined with
455 dolomitic limestone, increased plant diversity, facilitating the colonization of several
456 plant species. The improvement in nutrient availability and the reduction in Cu-induced
457 toxicity with the incorporation of compost contributed to promote the colonization of
458 species from the *Fabaceae* family involved in N-fixation. Shoot Cu concentrations
459 remained adequate regarding the food chain safety and plant nutrient status of this
460 grassland. Results demonstrate the persistent beneficial influence to combine compost
461 and dolomitic limestone on the growth and development of this grassland, promoting
462 the diversity of plant species that enhances soil key functions and provides ecological
463 benefits. Further monitoring, including also soil microbial communities and plant cover,
464 is needed to assess the effectiveness of these soil treatments and grassland system in the
465 longer-term, as a relevant long-term phytomanagement option for such Cu-
466 contaminated soils.

467

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478

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658 **Table 1.** Effect of soil treatments on physico-chemical properties of soil and soil pore water
659 in year 6 (Mean values \pm standard deviation). Values followed by different letters are
660 significantly different ($P < 0.05$) according to Tukey's test. No letter in a row indicates no
661 significant difference. **UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL:**
662 **compost and dolomitic limestone; CTRL: uncontaminated control soil Eutric gleysol from a**
663 **kitchen garden nearby (17 km) the site, Gironde, France (Mench et al. 2018).**

	UNT	DL	OM	OMDL	CTRL
pH	7.3 \pm 0.8	7.7 \pm 0.2	7.3 \pm 0.2	7.6 \pm 0	7.5
CEC (cmol kg ⁻¹)	2.92 \pm 0.8 ^b	3.17 \pm 0.13 ^b	4.84 \pm 0.79 ^a	5.64 \pm 0.58 ^a	16.1
SOM (g kg ⁻¹)	11.9 \pm 2.5 ^b	11.6 \pm 1.4 ^b	26.9 \pm 7.2 ^a	29.2 \pm 6.8 ^a	71.4
Organic C (g kg ⁻¹)	6.8 \pm 1.5 ^b	6.7 \pm 0.8 ^b	15.6 \pm 4.2 ^a	16.9 \pm 3.9 ^a	41.3
Total N (g kg ⁻¹)	0.44 \pm 0.06 ^b	0.45 \pm 0.06 ^b	0.91 \pm 0.22 ^a	0.99 \pm 0.21 ^a	2.91
C/N	15.5 \pm 1.4 ^a	15.1 \pm 0.8 ^a	17 \pm 0.8 ^a	17.1 \pm 0.4 ^a	14.2
SPW-pH	7.1 \pm 0.6	7.4 \pm 0.1	7.1 \pm 0.2	7.4 \pm 0.1	
SPW-CE (μ S cm ⁻¹)	495 \pm 139	584 \pm 108	401 \pm 76	538 \pm 59	
SPW-Cu (μ g L ⁻¹)	310 \pm 138 ^b	223 \pm 36 ^b	627 \pm 150 ^a	698 \pm 195 ^a	49 \pm 14
Total Cu (mg kg ⁻¹)	848 \pm 160	844 \pm 147	846 \pm 194	1028 \pm 132	22
DGT-Cu (μ g L ⁻¹)	424 \pm 298	410 \pm 247	494 \pm 274	658 \pm 423	16
R ratio	0.36 \pm 0.04 ^a	0.33 \pm 0.09 ^{ab}	0.26 \pm 0.05 ^{ab}	0.22 \pm 0.06 ^b	0.13
pCu	8.8 \pm 0.7	9.2 \pm 0.5	8.5 \pm 0.2	8.9 \pm 0.1	

664 SOM: soil organic matter; SPW-pH: pH in the soil pore water; SPW-CE: soil electrical
665 conductivity in the soil pore water; SPW-Cu: Cu concentration in the soil pore water; **DGT-**
666 **Cu: Cu concentration using DGT; R ratio: ratio between DGT-Cu and Cu concentration in the**
667 **soil solution; pCu: - Log10 [potential concentration of free Cu ions].**

669 **Table 2.** Identified plant species and their occurrence (n.: number of plots per soil treatment where that plant species is present) in year 7. **UNT:**
 670 **untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.**

UNT		DL		OM		OMDL	
Species	n.	Species	n.	Species	n.	Species	n.
<i>Rumex acetosella</i>	4	<i>Rumex acetosella</i>	4	<i>Cytisus scoparius</i>	4	<i>Cytisus scoparius</i>	4
<i>Agrostis capillaris</i>	4	<i>Agrostis capillaris</i>	4	<i>Rumex acetosella</i>	4	<i>Rumex acetosella</i>	4
<i>Quercus robur</i>	2	<i>Cytisus scoparius</i>	3	<i>Agrostis capillaris</i>	4	<i>Agrostis capillaris</i>	4
<i>Cytisus scoparius</i>	2	<i>Erigeron sumatrensis</i> ²	3	<i>Populus nigra</i>	3	<i>Erigeron sumatrensis</i> ²	4
<i>Senecio inaequidens</i>	2	<i>Senecio inaequidens</i>	2	<i>Senecio inaequidens</i>	3	<i>Quercus robur</i>	3
<i>Gnaphalium sylvaticum</i> ¹	2	<i>Agrostis gigantea</i>	2	<i>Erigeron sumatrensis</i> ²	3	<i>Senecio inaequidens</i>	3
<i>Populus nigra</i>	1	<i>Crepis</i> sp.	2	<i>Quercus robur</i>	2	<i>Daucus carota</i>	3
<i>Agrostis gigantea</i>	1	<i>Cirsium vulgare</i>	1	<i>Vicia sativa</i>	2	<i>Cirsium vulgare</i>	3
<i>Hypochaeris radicata</i>	1	<i>Hypochaeris radicata</i>	1	<i>Crepis</i> sp.	2	<i>Crepis</i> sp.	3
<i>Crepis</i> sp.	1	<i>Gnaphalium sylvaticum</i> ¹	1	<i>Ornithopus perpusillus</i>	2	<i>Ornithopus perpusillus</i>	2
<i>Asteraceae</i> sp.	1	<i>Asteraceae</i> sp.	1	<i>Agrostis gigantea</i>	1	<i>Populus nigra</i>	1
		<i>Crepis biennis</i>	1	<i>Daucus carota</i>	1	<i>Agrostis gigantea</i>	1
		<i>Pilosella tardans</i> ³	1	<i>Cirsium vulgare</i>	1	<i>Hypochaeris radicata</i>	1
				<i>Hypochaeris radicata</i>	1	<i>Gnaphalium sylvaticum</i> ¹	1
				<i>Gnaphalium sylvaticum</i> ¹	1	<i>Medicago arabica</i>	1
				<i>Medicago arabica</i>	1	<i>Crepis praemorsa</i>	1
				<i>Crepis praemorsa</i>	1	<i>Crepis biennis</i>	1
				<i>Crepis biennis</i>	1	<i>Conyza canadiensis</i>	1
						<i>Hypericum perforatum</i>	1

671 1, **also known as** (a.k.a.) *Omalotheca sylvatica*; 2, a.k.a. *Conyza albida*; 3, a.k.a. *Hieracium tardans*.

672 **Table 3.** Effect of soil treatments on plant parameters in year 7 (Mean values \pm standard
 673 deviation). Values followed by different letters are significantly different ($P < 0.05$)
 674 according to Tukey's test. No letter in a row indicates no significant difference. **UNT:**
 675 **untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic**
 676 **limestone.**

	UNT	DL	OM	OMDL
Total shoot biomass (g DW plot⁻¹)	219 \pm 152	165 \pm 91	371 \pm 164	361 \pm 204
Species richness	5.3 \pm 2.2 ^b	6.8 \pm 1.7 ^{ab}	9.3 \pm 1.7 ^{ab}	10.8 \pm 2.9 ^a
Plant cover occupancy (%)	46 \pm 32	59 \pm 42	68 \pm 27	76 \pm 28

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680 **Table 4.** Effect of the soil treatments on the shoot ionomes of plants in year 7 (Mean
681 values \pm standard deviation, in mg kg DW⁻¹). Values followed by different letters are
682 significantly different (P<0.05) according to Tukey's test. No letter in a row indicates
683 no significant difference. **UNT: untreated soil; DL: dolomitic limestone; OM: compost;**
684 **OMDL: compost and dolomitic limestone.**

	UNT	DL	OM	OMDL
Al	76 \pm 59	44 \pm 8	34 \pm 12	39 \pm 15
B	7.3 \pm 5.1	8.8 \pm 3.5	10.5 \pm 1.9	11.2 \pm 3.4
Ca	5575 \pm 1543	5826 \pm 1678	5310 \pm 614	5519 \pm 439
Cu	26 \pm 15	23 \pm 10	26 \pm 4	18 \pm 7
Fe	99 \pm 58	76 \pm 12	67 \pm 7	70 \pm 23
Mg	881 \pm 380	1432 \pm 387	1236 \pm 179	1262 \pm 81
Mn	152 \pm 67	141 \pm 18	111 \pm 20	144 \pm 40
P	992 \pm 289 ^b	1006 \pm 176 ^b	1695 \pm 64 ^a	1491 \pm 212 ^a
K	4304 \pm 238	5700 \pm 2327	5203 \pm 1007	6841 \pm 1714
Na	141 \pm 65	159 \pm 116	199 \pm 111	298 \pm 102
Zn	68 \pm 35	48 \pm 14	49 \pm 19	43 \pm 13

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688 **Supplementary table 1.** Pearson's correlation values between physicochemical parameters of soil and soil pore water in year 6.

	Tot Cu	SPW-Cu	DGT-Cu	R	pCu	CEC	OC	SOM	pH	Tot N
SPW-Cu	N.S.									
DGT-Cu	N.S.	0.53*								
R	N.S.	N.S.	N.S.							
pCu	N.S.	-0.53*	N.S.	N.S.						
CEC	0.51*	0.62**	N.S.	-0.62**	N.S.					
OC	N.S.	0.67**	N.S.	N.S.	N.S.	0.95***				
SOM	N.S.	0.67**	N.S.	N.S.	N.S.	0.95***	0.99***			
pH	N.S.	N.S.	N.S.	N.S.	0.94***	N.S.	N.S.	N.S.		
Tot N	N.S.	0.68**	N.S.	N.S.	N.S.	0.94***	0.99***	0.99***	N.S.	
C/N	N.S.	N.S.	N.S.	N.S.	N.S.	0.79***	0.79***	0.79***	N.S.	N.S.

689 Tot Cu: total soil Cu; SPW-Cu: Cu concentration in the soil pore water; DGT-Cu: Cu concentration using DGT; **R: ratio between DGT-Cu and**690 **Cu concentration in the soil solution**; pCu: - Log₁₀ [potential concentration of free Cu ions]; CEC: cationic exchange capacity of soil; OC: soil

691 organic C; SOM: soil organic matter; Tot N: total soil N.

692 **Supplementary table 2.** Sørensen index values showing the similarity coefficient in
693 plant species composition between plots in year 7 (Mean values \pm standard deviation).
694 UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and
695 dolomitic limestone.

UNT-DL	0.52 \pm 0.14
UNT-OM	0.49 \pm 0.13
UNT-OMDL	0.47 \pm 0.14
DL-OM	0.55 \pm 0.13
DL-OMDL	0.56 \pm 0.12
OM-OMDL	0.63 \pm 0.11

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698 **Supplementary table 3.** Shoot ionomes (mg kg⁻¹) in year 2 depending on soil treatment and plant species (Mean values ± standard deviation).

699 **UNT: untreated soil; DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.**

	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
<i>Agrostis gigantea</i>										
UNT	454 ± 254	4400 ± 978	91 ± 66	742 ± 402	5559 ± 1677	1287 ± 341	217 ± 195	141 ± 29	828 ± 502	42 ± 3
DL	354 ± 22	6359 ± 2050	59 ± 11	632 ± 238	8708 ± 2712	1582 ± 352	96 ± 18	152 ± 14	1000 ± 252	36 ± 10
OM	515 ± 41	3844 ± 345	65 ± 22	974 ± 271	7116 ± 249	1204 ± 202	190 ± 104	194 ± 57	992 ± 40	38 ± 5
OMDL	554 ± 343	5524 ± 1144	83 ± 57	785 ± 336	7553 ± 1760	1641 ± 127	130 ± 9	163 ± 8	1124 ± 65	46 ± 1
<i>Agrostis capillaris</i>										
UNT	616 ± 60	4548 ± 1237	137 ± 1	1018 ± 46	9298 ± 3359	915 ± 141	102 ± 28	178 ± 103	1248 ± 288	54 ± 30
DL	nm	nm	nm	nm	nm	nm	nm	nm	nm	nm
OM	634	2840	104	994	9220	766	172	210	1352	64
OMDL	678 ± 54	4950 ± 1287	98 ± 34	1036 ± 156	7710 ± 1485	903 ± 117	300 ± 261	380 ± 212	1295 ± 16	56 ± 9
<i>Deschampsia caespitosa</i>										
UNT	626 ± 178	3693 ± 951	71 ± 23	486 ± 132	5359 ± 853	1518 ± 36	51 ± 19	98 ± 2	575 ± 3	25 ± 6
DL	647 ± 9	5267 ± 877	65 ± 9	519 ± 24	5944 ± 80	2366 ± 11	80 ± 30	76 ± 11	788 ± 124	41 ± 15
OM	632 ± 252	3310 ± 1174	50 ± 19	504 ± 192	6690 ± 1457	1913 ± 180	63 ± 12	76 ± 0	2353 ± 1056	43 ± 9
OMDL	1004 ± 6	3643 ± 145	100 ± 18	783 ± 4	6749 ± 837	2671 ± 411	66 ± 4	99 ± 17	2702 ± 144	58 ± 9
<i>Vulpia myuros</i>										
UNT	1488 ± 84	2232 ± 365	499 ± 83	978 ± 42	1095 ± 35	576 ± 1	47 ± 9	191 ± 1	923 ± 92	36 ± 6
DL	689 ± 128	3879 ± 1379	150 ± 5	553 ± 48	1322 ± 438	663 ± 251	35 ± 13	191 ± 4	860 ± 152	28 ± 3
OM	837 ± 606	2130 ± 184	159 ± 123	515 ± 295	1097 ± 25	596 ± 57	49 ± 10	202 ± 15	1051 ± 202	39 ± 6
OMDL	646 ± 344	2410 ± 88	151 ± 123	472 ± 110	1223 ± 58	638 ± 71	33 ± 3	220 ± 6	1169 ± 70	43 ± 6

700 1, n = 1; 2, n = 2; 3, n = 3; nm, not measured.

701 **Supplementary table 4.** Soil and soil pore water physicochemical properties in year 9
 702 from a field trial phytomanaged with compost and dolomitic limestone (OMDL) and a
 703 sunflower-tobacco rotation crop (Mean values \pm standard deviation; n = 1 for UNT).
 704 (Mench et al. 2018; Kolbas et al. 2020). **UNT: untreated soil; OMDL: compost and**
 705 **dolomitic limestone.**

	UNT	OMDL
pH	6.3	7.4 \pm 0.2
CEC (cmol kg ⁻¹)	3.1	7.0 \pm 3.3
SOM (g kg ⁻¹)	17	33 \pm 14
Organic C (g kg ⁻¹)	10	19 \pm 8
Total N (g kg ⁻¹)	0.7	1.4 \pm 0.6
C/N	14	14 \pm 0.3
SPW-pH	5.4	6.9 \pm 0.2
SPW-Cu (μ g L ⁻¹)	547	752 \pm 68
Total Cu (mg kg ⁻¹)	760	842 \pm 69
DGT-Cu (μ g L ⁻¹)	269 \pm 9	293 \pm 79
R ratio	0.50 \pm 0.01	0.27 \pm 0.02
pCu	5.4	6.4 \pm 0.2

706 **CEC: cationic exchange capacity of soil; SOM: soil organic matter; SPW-pH: pH**
 707 **values in the soil pore water; SPW-Cu: Cu concentration in the soil pore water; DGT-**
 708 **Cu: Cu concentration using DGT; R: ratio between DGT-Cu and Cu concentration in**
 709 **the soil solution; pCu: - Log₁₀ [potential concentration of free Cu ions];**

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711 **Supplementary table 5.** Additional information on the plant species found in year 7.
 712 For species number, values were total number of plant species per treatment. For plant
 713 type, plant life cycle and reproduction method, values were percentages based on the
 714 total number of plant species per treatment. ND: not determined. **UNT: untreated soil;**
 715 **DL: dolomitic limestone; OM: compost; OMDL: compost and dolomitic limestone.**

	UNT	DL	OM	OMDL
Species number	11	13	18	19
Plant type				
Tree	18.2	0	11.1	10.5
Schrub	9.1	7.7	5.6	5.3
Creep	0	0	0	0
Herbaceous	63.6	84.6	77.8	84.2
ND	9.1	7.7	5.6	0
Life cycle				
Annual	0	23.1	38.9	36.8
Perennial	81.8	53.8	55.6	57.9
ND	18.2	23.1	5.6	5.3
Reproduction method				
Woody species ¹	18.2	0	11.1	10.5
Seeds	27.3	46.2	55.6	57.9
Tubers	0	0	0	0
Bulbs	0	0	0	0
Rhizomes	27.3	23.1	22.2	21.1
Runners	9.1	7.7	5.6	5.3
Tuft ²	0	0	0	0
Rosettes ³	0	0	0	0
ND	18.2	23.1	5.6	5.3

716 ¹ Woody species represent all perennial species without reproduction by tuber, bulb,
 717 rhizome, runner, tiller, and rosette like trees and shrubs

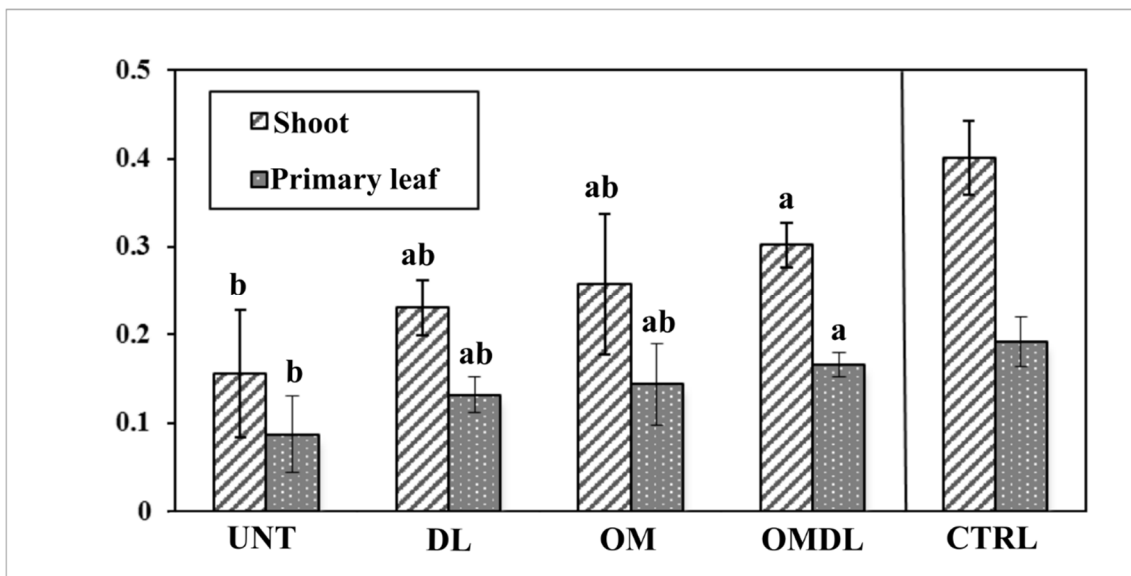
718 ² adventive or secondary tufts (**collection of grasses growing together at the base**) that
 719 separate from the parent to form genetically-identical offspring

720 ³ adventive or secondary rosettes (**circular cluster of leaves growing from the base of a**
 721 **stem**) that separate from the parent to form genetically-identical offspring

722

723 **Figure captions**

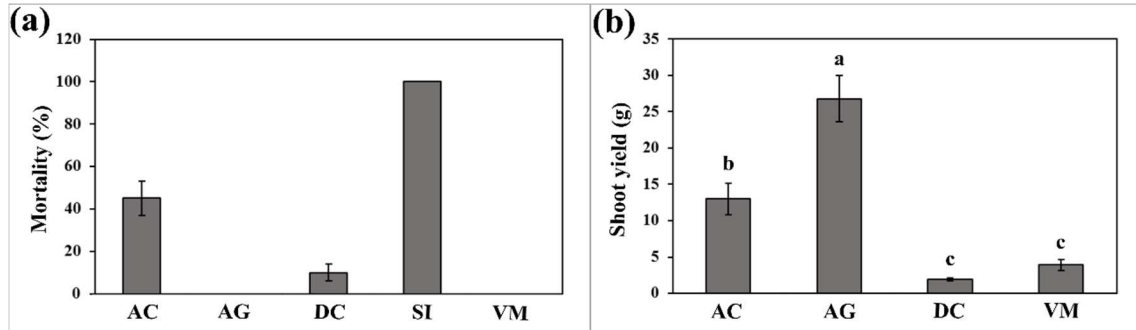
724 **Figure 1.** Effect of soil treatments on the shoot and primary leaf biomass of dwarf
725 beans (g DW plant⁻¹). Mean values (n = 16) ± SD. Treatments with different letters are
726 significantly (P <0.05) different according to **Tuckey's HSD-test**. **DL: dolomitic**
727 **limestone; OM: compost amendment; OMDL: compost amendment with dolomitic**
728 **limestone; UNT: unamended; CTRL: Uncontaminated soil Eutric gleysol from a**
729 **kitchen garden nearby (17 km) the site, Gironde, France (Mench et al. 2018).**



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732 **Figure 2.** Percentage of mortality (a) and total shoot biomass (g DW plot⁻¹) (b) of the
733 **transplanted plant species in the field trial in year 2.** AC: *A. capillaris*; AG: *A. gigantea*;
734 DC: *D. caespitosa*; SI: *S. indicus*; VM: *V. myuros*.

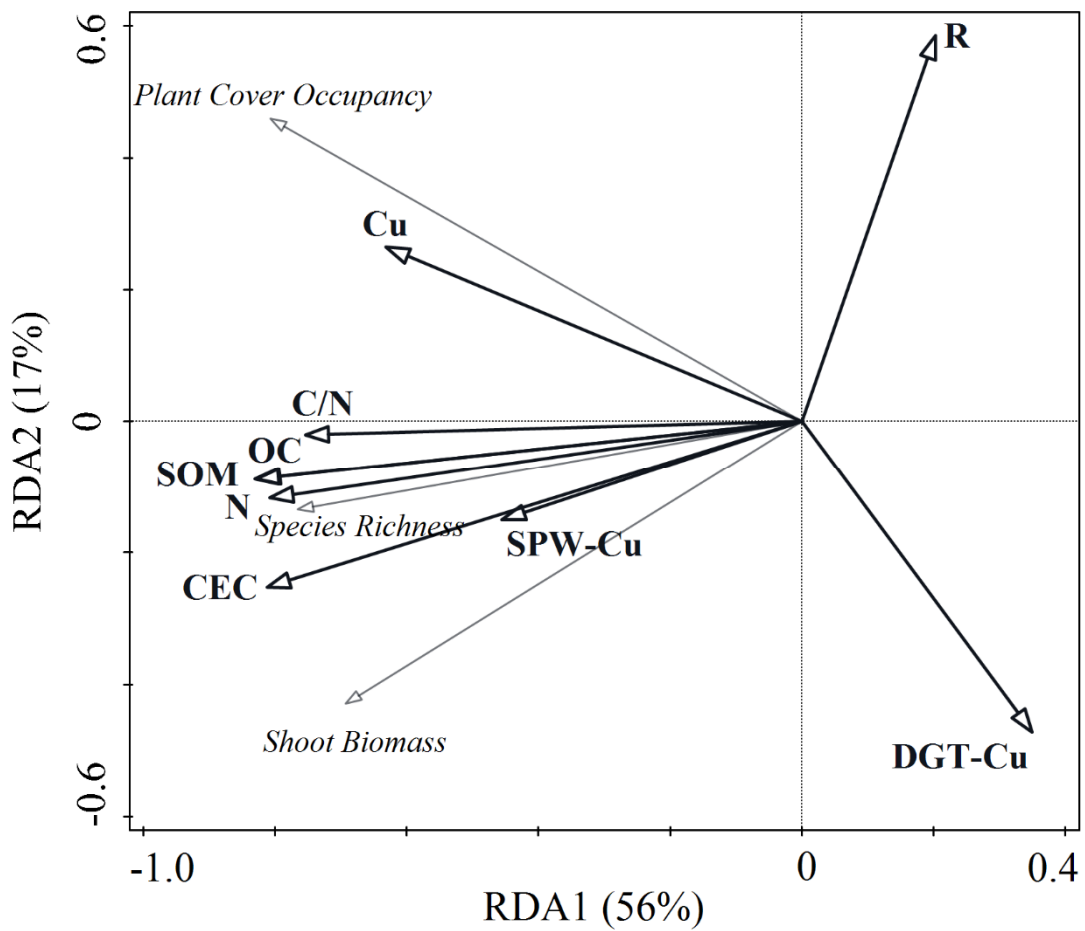


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738 **Figure 3.** Biplot of redundancy analysis (RDA) showing the variation of plant
 739 parameters in year 7 explained by most relevant soil physicochemical properties (bold
 740 arrows) ($F = 2.5$; $P < 0.05$). RDA1 and RDA2 account for 56% and 17% of the total
 741 variance, respectively. Cu: total soil Cu; SPW-Cu: Cu concentration in the soil pore
 742 water; DGT-Cu: Cu concentration using DGT; **R: ratio between DGT-Cu and Cu**
 743 **concentration in the soil solution**; CEC: cationic exchange capacity of soil; OC: soil
 744 organic C; SOM: soil organic matter; N: total soil N.



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