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1 **Contrasting effects of siderophores pyoverdine and desferrioxamine B on the**  
2 **mobility of iron, aluminum, and copper in Cu-contaminated soils**

3

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## **Highlights**

DFOB supply did not increase the mobility of Cu in soil whereas Pvd did

DFOB and Pvd mobilized metals in soil mainly through a ligand-controlled mechanism

The soil Cu contamination level altered the metal mobilization efficiency of DFOB and Pvd

DFOB and Pvd efficiency in mobilizing Fe and Al decreased over time

Pvd degradation partly contributed to reducing Pvd mobilization efficiency over time

26 **Abstract**

27 Siderophores are biogenic metallophores that can play significant roles in the dynamics  
28 of a range of metals, including Cu, in the soil. Understanding the impact of siderophores  
29 on the mobility and the availability of metals in soil is required to optimize the  
30 efficiency of soil remediation processes such as phytoextraction. This study compared  
31 the ability of siderophores desferrioxamine B (DFOB) and pyoverdine (Pvd) to  
32 mobilize metals in a series of Cu-contaminated soils, and investigated the extent their  
33 metal mobilization efficiency changed over time and with the level of Cu contamination  
34 of the soil. Siderophores were supplied (or not) to Cu-contaminated soils and metal  
35 mobility was assessed through their total concentration in 0.005 M CaCl<sub>2</sub> extract.  
36 DFOB selectively mobilized Fe and Al while Pvd also mobilized Cu and Ni, Co, V and  
37 As but to a lesser degree. The 1:1 relationship between DFOB in the CaCl<sub>2</sub> extract and  
38 Fe+Al mobilized from the solid phase suggests that DFOB mobilized metals by ligand-  
39 controlled dissolution. The accumulation of Cu in soil enhanced the adsorption of  
40 DFOB and Pvd at the surface of soil constituents and the mobilization of Fe to the  
41 detriment of Al by the two siderophores. The metal mobilization efficiency of DFOB  
42 and to a lesser extent of Pvd decreased over 22 days. According to <sup>15</sup>N-Pvd analyses,  
43 Pvd degradation at least partly contributed to the progressive reduction in the metal  
44 mobilization efficiency of Pvd. The processes behind these results and the relevance of  
45 these results for manipulating the availability of Cu (and Fe) in soil are discussed.

46

47 **Keywords**

48 Ligand-promoted mobilization, metals, vineyard soils, phytoextraction, speciation, <sup>15</sup>N  
49 labeling

50

## 51 **1. Introduction**

52 Copper is a major component of crop protection methods against fungi and bacteria as  
53 it is the only active substance allowed in organic farming that has both a strong biocidal  
54 effect and a wide range of action (Andrivon et al., 2017). Copper is used to prevent a  
55 variety of crop diseases including mildew, some fungal diseases and most bacterial  
56 diseases, particularly on grapevines, and on fruit and vegetable crops (Andrivon et al.,  
57 2017). The two main crop diseases, in terms of planted area and economic importance,  
58 for which Cu is used are downy mildew of vine caused by the oomycete *Plasmopara*  
59 *viticola* and apple scab caused by the ascomycete *Venturia inaequalis* (Andrivon et al.,  
60 2017). The soil of many vineyards and orchards is contaminated by Cu due to the long-  
61 term use of Cu-based fungicides such as Bordeaux mixture. Copper contamination is  
62 particularly high in old vineyards (Komarek et al., 2010; Mackie et al., 2012) and in old  
63 orchards (Zhou et al., 2011; Wang et al., 2015) where the concentration of Cu in the  
64 topsoil (0-20 cm) can reach several hundred mg kg<sup>-1</sup> soil.

65 Soil Cu contamination in vineyard and orchard soils, although moderate compared to  
66 that of Cu-polluted soils located near Cu mines (Zotti et al., 2014), Cu smelting factories  
67 (Wang et al., 2014) or at wood preservation sites (Kolbas et al., 2020), has consequences  
68 for the functioning and the sustainability of these ecosystems since it has chronic effects  
69 on the dynamics of soil populations. Karimi et al. (2021) performed a meta-analysis of  
70 Cu ecotoxicity and reported that Cu harms soil microorganisms at concentrations above  
71 200 kg Cu ha<sup>-1</sup> (Cu of 67 mg kg<sup>-1</sup> soil), which is currently the level found in many  
72 vineyard and orchard topsoils. Excess Cu reduces microbial activity (Soler-Rovira et  
73 al., 2013) and biodiversity (Viti et al., 2008) in vineyard topsoils, and reduces microbial  
74 biomass and C mineralization rates in apple orchard topsoils (Wang et al., 2009). Ways  
75 to reduce the Cu contamination of vineyard and orchard soils are thus needed along

76 with the use of other substances than Cu to protect vines and fruit trees against bacterial  
77 and fungal diseases.

78 Phytoextraction progressively reduces the concentration of metals in soil by  
79 accumulating metals in harvestable plant parts (Bert, 2013). Copper phytoextraction is  
80 still in the experimental stage as yields do not yet reduce soil Cu contamination  
81 sufficiently. The limited phytoavailability of Cu in vineyard or orchard soils (e.g.  
82 compared to Ni in serpentine soils) and its preferential accumulation in the roots of  
83 most Cu-extracting plants makes phytoextraction of even 1 kg Cu ha<sup>-1</sup> year<sup>-1</sup> difficult.

84 One way to increase Cu phytoavailability in the soil without causing Cu leaching to  
85 groundwater is to inoculate the rhizosphere of the Cu-accumulating plant with  
86 siderophore producing bacteria (SPB). Siderophores are biogenic metallophores  
87 released by some plants (*Poaceous* species), soil fungi (e.g. *Aspergillus*) and soil  
88 bacteria (e.g. *Streptomyces*, *Pseudomonads*) to guarantee their iron nutrition under iron  
89 starvation. Although siderophores are generally considered as biological iron uptake  
90 agents, they can form stable complexes (Braud et al., 2010) and play a significant role  
91 in the biogeochemical cycling of a range of metals, including Cu (Kraemer et al., 2015).

92 Previous studies (Cornu et al., 2014; 2019) revealed for instance, that the mixed  
93 catecholate and hydroxamate siderophore pyoverdine (Pvd) enhanced the mobility (i.e.  
94 the solid-solution transfer) of Cu in vineyard soils. However, the *in situ* deployment of  
95 bioaugmentation-assisted phytoextraction for Cu requires a better understanding of the  
96 processes used by siderophores to mobilize Cu in soil, in order to identify the conditions  
97 (type of siderophore, soil characteristics, etc.) needed to optimize their efficiency.

98 Batch experiments showed that mineral Fe-bearing phases (Fe oxyhydroxides, clays)  
99 dissolve almost exclusively via a ligand-controlled dissolution mechanism in presence  
100 of siderophores (Cheah et al., 2003; Kraemer, 2004; Akafia et al., 2014). This means

101 that the siderophore-promoted mobilization of Fe primarily relies on Fe complexation  
102 by siderophores, in solution and at the mineral surface, which increases both the  
103 solubility and the dissolution rate of mineral Fe-bearing phases. However, this  
104 theoretical model does not necessarily apply to Cu, because Cu and Fe do not have the  
105 same geochemistry, or to soils, because minerals in soils are associated with solid  
106 organic matter, and metals and metallophores (other than siderophores) are present in  
107 large numbers.

108 The aim of the present study was to better understand the processes used by  
109 siderophores to mobilize metals in soils. The first objective was to investigate the  
110 relationship between the complexation and the mobilization of metals in soil by  
111 comparing the efficiency of Cu mobilization of two siderophores with contrasting  
112 stability constants for Cu(II): desferrioxamine B (DFOB) and Pvd. The second  
113 objective was to test whether the level of soil contamination by Cu affects the efficiency  
114 of DFOB and Pvd in mobilizing metals in soil. The third objective was to assess the  
115 duration of the siderophore effect on the mobility of metals in the soil by monitoring  
116 changes in the metal mobilization efficiency of DFOB and Pvd over time.

117

## 118 **2. Material and methods**

119 The study was based on three experiments, each one designed to address a specific  
120 objective. Experiment 1 was designed to evaluate the effect of a supply of DFOB on  
121 the mobility of metals in a series of 14 Cu-contaminated soils. Experiment 2 was  
122 designed to test whether the level of Cu accumulation in soil is likely to alter the metal  
123 mobilization efficiency of DFOB and Pvd. This experiment was based on soil L whose  
124 original concentration of Cu ( $100 \text{ mg Cu kg}^{-1}$ ) was artificially increased by spiking.  
125 Experiment 3 was designed to assess whether the metal mobilization efficiency of



126 DFOB and Pvd persists over time. In this experiment, the solid-liquid partitioning of  
127 metals was compared 1, 8 and 22 days after siderophore was added to soils K and L.

128

## 129 2.1 Soils

130 Fourteen Cu-contaminated topsoils (0-20 cm) were used. Soils A to K are vineyard soils  
131 collected in 2012 in the AOC (i.e. registered designation of origin) zone of Pessac-  
132 Léognan (immediately south of Bordeaux, France). Soil L is a former vineyard soil  
133 collected in 2015 in Mauguio (10 km east of Montpellier, France), in which symptoms  
134 of Cu phytotoxicity were observed in durum wheat (Michaud et al., 2007). Soil M is a  
135 crop soil collected in the Pierrelaye-Bessancourt plain (24 km northwest of Paris,  
136 France). According to Bourrenane et al. (2002), soils in this plain are contaminated with  
137 trace metals (Cd, Cu, Pb, and Zn) because they were irrigated with wastewater between  
138 1899 and 2000 and amended with smut compost and urban sludge in the mid-1960s.  
139 Soil N was collected in 2017 at a former wood preservation site in St Médard d'Eyrans  
140 (20 km south of Bordeaux, France) (for more details see Mench et al., 2018). Soil  
141 samples were air-dried and sieved to 2 mm before analysis. Table 1 summarizes the  
142 results of the soil analyses conducted by a soil-testing laboratory (LAS, INRA Arras,  
143 France), using standard methods.

144 As detailed in Cornu et al. (2019), the vineyard soils A to K have a coarse texture, a  
145 rather low organic matter (OM) content, and a low cation exchange capacity (CEC).  
146 Their total Cu contents range from 100 to 174 mg kg<sup>-1</sup> and EDTA-extractable Cu  
147 contents (Cu<sub>EDTA</sub>) range from 53 to 132 mg kg<sup>-1</sup>. Soil L is characterized by a clay loam  
148 texture, high carbonate content and a low EDTA-extractable Cu content. Soil M is  
149 characterized by a coarse texture, a high OM content (> 5%), and high total and EDTA-  
150 extractable contents of Cu (336 and 223 mg kg<sup>-1</sup>, respectively). Soil N is characterized

151 by a coarse texture, low OM content and low CEC, and by very high total and EDTA-  
152 extractable contents of Cu (905 and 515 mg kg<sup>-1</sup>, respectively). The soil samples also  
153 differ in their pH, ranging from 5.9 in soil A to 8.6 in soil J. Eight out of the 14 samples  
154 are carbonate soils (soils F to M), the six others (soils A to E and soil N) are not. For  
155 the purposes of Exp. 2, soil L was spiked with Cu (added as CuSO<sub>4</sub>) one year prior to  
156 being used for the present study. The four concentrations of Cu compared in Exp. 2  
157 were 100 (original value), 300, 850 and 1,600 mg Cu kg<sup>-1</sup> soil DW.

158

## 159 2.2 Desferrioxamine B

160 DFOB was purchased as mesylate salt (C<sub>25</sub>H<sub>48</sub>N<sub>6</sub>O<sub>8</sub>·CH<sub>4</sub>O<sub>3</sub>S, CAS Number: 138-14-7,  
161 M= 656.79 g mol<sup>-1</sup>) from Merck. DFOB stock solution (1 mM) was prepared 24 h prior  
162 to use by dissolving the DFOB mesylate salt in ultra-pure water. The concentration of  
163 DFOB in the soil extraction solution (see section 2.5) was assessed by converting all  
164 the DFOB present in solution to DFOB-Fe(III), and by measuring the absorbance of the  
165 DFOB-Fe(III) complex at 439 nm (Helios Epsilon, Thermo Spectronic), according to  
166 the protocol of Cheah et al. (2003). Briefly, a 2 mL-aliquot of extraction solution was  
167 acidified to pH < 2 by addition of 4 μL of 70% perchloric acid. Then, 66 μL of 9.4 mM  
168 Fe(ClO<sub>4</sub>)<sub>3</sub> solution was added to obtain an Fe concentration of 302 μM. The molar  
169 extinction coefficient (ε) of the DFOB-Fe(III) complex was the same in the extraction  
170 solutions as in 0.005 M CaCl<sub>2</sub> and was 2650 ± 50 L mol<sup>-1</sup> cm<sup>-1</sup>.

171

## 172 2.3 Pyoverdine

173 *Pseudomonas fluorescens* (ATCC 13525) was used to produce Pvd using the procedure  
174 described in Cornu et al. (2019). Briefly, bacteria were grown for seven days at 25 °C  
175 under shaking (200 rpm) in a Dworking & Foster (DF) medium with no iron, in fed-

176 batch mode. After seven days, the culture medium was centrifuged (7,650 g) and the  
177 Pvd-containing supernatant was filtered and adjusted to pH 6 before being purified in a  
178 two-step procedure. First, the Pvd-containing supernatant was put in contact with a  
179 hydrophobic polyaromatic resin (Amberlite<sup>®</sup> XAD-4) for 24 h at 4 °C. After each  
180 contact (n= 4), the resin was recovered by filtration and the Pvd was eluted from the  
181 resin with 100% methanol and concentrated by evaporation. The Pvd concentrate was  
182 then loaded onto a C18 column (Lichroprep<sup>®</sup> RP-18), eluted with methanol/H<sub>2</sub>O 70/30  
183 (v/v) and concentrated by evaporation prior to storage at -20 °C. As detailed in Cornu  
184 et al. (2019), three major Pvd isoforms were identified by HILIC-ESI-MS in purified  
185 Pvd, with a molecular weight of 1,159.52, 1,160.50 and 1,189.52 Da. The semi-  
186 structural formulae of the three isoforms are shown in suppl. Fig. F1. For Exp. 3, <sup>15</sup>N-  
187 labeled Pvd was produced by growing bacteria in a DF medium in which the nitrogen  
188 source was changed to <sup>15</sup>N-labeled ammonium sulfate (98 atom % <sup>15</sup>N, Merck). Delta  
189 <sup>15</sup>N analyses (EA-IRMS, see section 2.6) showed that the vast majority of the nitrogen  
190 atoms of the <sup>15</sup>N-labeled Pvd were <sup>15</sup>N. Pvd stock solution (1 mM) was prepared 24 h  
191 prior to use by dissolving lyophilized Pvd in ultra-pure water. The concentration of Pvd  
192 in the soil extraction solution (see section 2.5) was assessed by measuring the difference  
193 in absorbance at 380 nm (Helios Epsilon, Thermo Spectronic) between the Pvd-treated  
194 and the control in each soil (for more details, see Cornu et al., 2019). At 380 nm, Pvd  
195 absorbance is not sensitive to changes in pH (Moll et al., 2008) or to changes in the  
196 metals with which it is associated (Braud et al., 2010).

197

## 198 2.4 Experimental design

199 Disks of dry soil (5 g, 3 mm thick, 32 mm in diameter) were placed in plastic lids,  
200 rewetted to 80% WHC and equilibrated for two weeks at 20 °C before being used for

201 the assay. This delay avoids working during the microbial flush that usually follows soil  
202 rewetting and may strongly influence the dynamics of metals in soils (Cornu et al.,  
203 2007). In Exp. 1, six disks were prepared for each soil (n= 14). Three soil disks were  
204 supplied with desferrioxamine B (DFOB) and the other three with ultra-pure water  
205 (control), and equilibrated for one day at 20 °C before extraction. In Exp. 2, nine disks  
206 were prepared for each soil Cu concentration (n= 4). Three soil disks were supplied  
207 with DFOB, three others with pyoverdine (Pvd) and the last three with ultra-pure water  
208 (control), and equilibrated for one day at 20 °C before extraction. In Exp. 3, 27 disks  
209 were prepared for each soil (n= 2). Nine soil disks were supplied with DFOB, nine  
210 others with Pvd and the last nine with ultra-pure water (control), and equilibrated for  
211 one, eight, or 22 days at 20 °C before extraction. In all three experiments, DFOB and  
212 Pvd were supplied at a concentration of 200  $\mu\text{mol kg}^{-1}$  soil DW by adding 1 mL from a  
213 1 mM stock solution to the surface of soil disks.

214

## 215 2.5 Soil extraction and analyses of the extraction solution

216 Soil extraction was performed as follows: 5 g of wet soil were shaken with 9 mL of  
217 0.005 M  $\text{CaCl}_2$ . All the suspensions were shaken for 2 h at 20 °C, centrifuged at 5,000  
218 g for 10 min and the supernatant was filtered through 0.2 mm cellulose acetate filters  
219 (Cornu et al., 2014; 2019). After measurement of the pH and the concentration of DFOB  
220 and/or Pvd (see below), 10 mL of the extraction solution were acidified with 2%  $\text{HNO}_3$   
221 (v/v) and stored at 4 °C until further analysis. In Exp. 3, a 2 mL subsample of the  
222 extraction solution was stored non-acidified for  $\text{A}^{254}$ , DOC, total N and delta  $^{15}\text{N}$   
223 analyses. The pH and the concentrations of DFOB and Pvd were measured straight after  
224 soil extraction. pH was measured using a combined microelectrode (E16M331,  
225 Radiometer Analytical); the concentrations of DFOB and Pvd were measured using the

226 protocols described in section 2.2 and 2.3, respectively. Total concentrations of Al, Fe,  
227 Mn, Cu, As, Ni, Co, Cr and V were determined by ICP-MS (7700x, Agilent  
228 Technologies) at the central analytical facility of the University of the Basque Country  
229 (UPV/EHU, Bilbao, Spain) from a subsample acidified with 2% HNO<sub>3</sub>. The absorbance  
230 of dissolved organic matter at 254 nm (A<sup>254</sup>) was measured by UV-VIS spectrometry  
231 (Cary 1 Bio, Varian, quartz cells) with a path length of 1 cm. The total concentration of  
232 N and the ratio of N isotopes (expressed as delta <sup>15</sup>N) were determined at the *PLATeau*  
233 *d'Isotopie de Normandie* (PLATIN', Caen, France). Tin capsules filled with 15 mg of  
234 Chromosorb<sup>®</sup> (inert powder) were used to encapsulate 100 µL of extraction solution.  
235 The capsules were placed in an oven at 40 °C for 12 h to dry the sample. The capsules  
236 were sealed with tweezers before the sample was analyzed by an elemental analyzer  
237 (EA3000, EuroVector) linked to a continuous flow isotope mass spectrometer (IRMS,  
238 Isoprime, GV Instruments).

239

## 240 2.6 Geochemical modeling of the extraction solution

241 Metal speciation in the extraction solution in the presence of Pvd and dissolved organic  
242 matter (DOM) was predicted using ORCHESTRA software, version 2021 (Meeussen  
243 2003). Input data for the model were pH, Pvd, DOM, anions (NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>, Cl<sup>-</sup>)  
244 and total dissolved Ca, Fe, Al, Cu, and Ni. The concentrations of NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and PO<sub>4</sub><sup>3-</sup>  
245 (fixed at 0.88, 0.1 and 0.02 mM, respectively) were assessed based on the literature,  
246 and the concentration of Cl<sup>-</sup> was adjusted to equilibrate the charge balance between  
247 anions and cations. Thermodynamic constants for Pvd complexation to H<sup>+</sup>, Fe<sup>3+</sup>, Al<sup>3+</sup>,  
248 Cu<sup>2+</sup>, Ni<sup>2+</sup> and Ca<sup>2+</sup> were taken from the literature (Albrecht-Gary et al., 1994; Cornu  
249 et al., 2014; Ferret et al., 2015) or estimated (Suppl. Table T1 lists the stability constants  
250 used for Pvd-Al and Pvd-Ca) and were added to the default MinteqV4 database. For

251 the calculation of speciation, DOM was described as fulvic acid, and metal binding to  
252 DOM was described using the NICA-Donnan model using the corresponding generic  
253 parameters (Kinniburgh et al., 1999; Milne et al., 2003; Koopal et al., 2005). The  
254 concentration of fulvic acid in the extraction solution was assessed from  $A^{254}$  values  
255 using the equation described in Amery et al. (2008), and considering a specific UV-  
256 absorbance at 254 nm of standard fulvic acid ( $SUVA_{FA}$ ) equal to  $55 \text{ L g}^{-1} \text{ cm}^{-1}$   
257 (Benedetti et al., 2002). Activity coefficients were calculated using the Davies equation.  
258 Equilibrium reactions are described taking the equilibrium with atmospheric  $\text{CO}_2$  into  
259 account.

260

## 261 2.7 Statistical analyses

262 Two-way ANOVA was performed on the parameters monitored in the extraction  
263 solution to assess the significance of treatment effects (soil, time, siderophore supply)  
264 and their interaction. Tukey's HSD tests were performed at 5% to identify the  
265 parameters monitored in the extraction solution that were significantly affected by the  
266 supply of siderophore (DFOB, Pvd), the concentration of Cu in the soil and/or the  
267 length of incubation. Linear regressions were performed to quantify the relationships  
268 between the amount of siderophore (DFOB, Pvd) in the extraction solution and the  
269 amount of metals mobilized from the solid phase. The software used for statistical  
270 analysis was SYSTAT 11 Edition 2004 (SPSS Inc., 233S. Wacker Drive, Chicago, USA).

271

## 272 **3. Results and Discussion**

### 273 3.1 DFOB selectively promoted the mobility of Fe and Al in soil

274 Experiment 1 revealed that adding DFOB increased the mobility of Fe and Al in a wide  
275 range of soils and over a wide range of soil pH ( $5.9 < \text{pH}_{\text{water}} < 8.6$ ). The concentrations

276 of total Fe (Fig. 1a) and total Al (Fig. 1b) in the CaCl<sub>2</sub> extract were always higher in  
277 soils supplied with DFOB than in control soils. The DFOB-promoted mobilization of  
278 Fe and Al was observed in both carbonate (F-M) and non-carbonate (A-E, N) soils. This  
279 is a crucial point since siderophores are produced only when Fe availability is low  
280 (Visca et al., 2007), which is usually the case in soil at alkaline pH. Experiment 1 also  
281 showed that Fe and Al were the only two metals (among the eight monitored + As)  
282 whose mobility in soil was affected by the addition of DFOB. This finding was  
283 evidenced by two-way ANOVA (suppl. Table T2), which showed that the effect of  
284 DFOB was highly significant ( $p < 0.001$ ) for the concentrations of Fe and Al whereas  
285 it was not ( $p > 0.05$ ) for the concentrations of Mn, Cu, Ni, As, Co, Cr and V, in the  
286 CaCl<sub>2</sub> extract. Notably, the addition of DFOB did not increase Cu mobility in soil. The  
287 concentration of total Cu (Fig. 1c) in the CaCl<sub>2</sub> extract was on average the same in  
288 DFOB-treated and in control soils, in 12 of the 14 Cu-contaminated soils tested in the  
289 present study. In this respect, DFOB contrasts with Pvd, which has been shown to bind  
290 and mobilize Cu in series of Cu-contaminated soils (Tansupo et al., 2008; Cornu et al.,  
291 2019) (see sections 3.2 and 3.3 for more details on this comparison). The targeted effect  
292 of DFOB on Fe and Al is in agreement with the results of previous studies that  
293 investigated the effect of DFOB on the mobility of metals in soil (Neubauer et al., 2000;  
294 Zhong et al., 2013; Cornu et al., 2017). The high affinity of DFOB for Fe(III) ( $\log \beta =$   
295 31.9) and Al(III) ( $\log \beta = 25.5$ ) may explain why DFOB failed to affect the mobility of  
296 divalent metals like Cu(II) in Fe-rich and Al-rich matrices such as soil. However, it does  
297 not explain why DFOB failed to increase the mobility of Mn(III) for which DFOB  
298 exhibits a higher affinity ( $\log \beta = 29.9$ ) than for Al(III). One possible explanation is that  
299 Mn(III) was less available for complexation than Fe(III) and Al(III) in these soils due  
300 to the low concentration of Mn-bearing phases compared to Fe- and Al-bearing phases

301 (Table 1). This result contrasts with the results of a study by Akafia et al. (2014) in  
302 which the addition of DFOB enhanced the release of Mn from a purified Mn(III)  
303 oxyhydroxide mineral, thereby underlining the difficulty in predicting the effect of  
304 siderophores in soil in batch dissolution experiments.

305 Figure 2a shows the relationship between the amount of DFOB in the CaCl<sub>2</sub> extract and  
306 the amount of Fe+Al released from the solid phase in DFOB-treated soils. The slope  
307 equal to one (1.01) and the non-significant intercept of the regression line show that the  
308 two variables follow a 1:1 relationship. This 1:1 relationship suggests that Fe and Al  
309 were predominantly mobilized by ligand-controlled dissolution (also called  
310 complexolysis) and as 1:1 M-DFOB complexes in DFOB-treated soils. It recalls the  
311 1:1 relationship observed for Pvd in almost the same series of soil (Cornu et al., 2019)  
312 and confirms that the siderophore-promoted mobilization of metals in soil primarily  
313 relies on their complexation by the siderophore (either in pore water or at the surface  
314 of metal-bearing phases). For Fe, this result is in line with the results of batch  
315 experiments showing that Fe oxyhydroxydes dissolve exclusively via a ligand-  
316 controlled mechanism in presence of siderophores (Kraemer, 2004). The 1:1  
317 stoichiometry between the amount of DFOB in the CaCl<sub>2</sub> extract and the amount of  
318 Fe+Al released from the solid phase was observed even in soils A, B and N, in which  
319 the addition of DFOB slightly lowered the pH of the CaCl<sub>2</sub> extract (suppl. Fig. F2).  
320 This suggests that, in these soils, the amount of Fe+Al released by proton-controlled  
321 dissolution (also called acidolysis), if any, was negligible compared to the amount  
322 released by ligand-controlled dissolution in presence of DFOB.

323 In ligand-controlled dissolution, the pool of metallophores irreversibly sorbed onto the  
324 solid phase represents a loss of metallophores from the solution that reduces their effect  
325 on metal mobilization (Kraemer, 2004). Hence, the extent to which siderophores



326 increase the mobility of metals in soil closely depends on their partitioning between the  
327 solid and the liquid phase. The amount of DFOB measured in the  $\text{CaCl}_2$  extract was  
328 used to calculate the solid-liquid partitioning coefficient ( $K_d$ ) of DFOB by difference  
329 with the amount of DFOB supplied. The  $K_d$  value of DFOB ranged from 1.8 to 24 L  
330  $\text{kg}^{-1}$  among soils, which means that between 47% to 92% of the DFOB supplied was  
331 bound to the solid phase after 24 h. The strong affinity of DFOB for soil constituents  
332 underlines the fact that siderophores can strongly adsorb to soil colloids and particles  
333 (Ahmed and Holmstrom, 2014; Harrington et al., 2015; Rai et al., 2020). As detailed in  
334 Rai et al. (2020), both DFOB and DFOB-metal complexes may (i) specifically adsorb  
335 to metal oxyhydroxide surfaces by forming covalent bonds between hydroxamic acid  
336 moieties and surface cations, (ii) adsorb to negatively charged surfaces by electrostatic  
337 bonds since they are cationic species at  $\text{pH} < 8$ , and (iii) be associated with solid organic  
338 matter by hydrophobic interactions. As expected, the relationship between the  $K_d$  of  
339 DFOB and the amount of metals mobilized by DFOB was close and negative (suppl.  
340 Fig. F3). The linear regression coefficients obtained between the  $K_d$  of DFOB and the  
341 physico-chemical characteristics of soils are shown in suppl. Table T3. The fact that the  
342  $K_d$  of DFOB was closely and positively correlated with the clay content ( $r = 0.77$ ,  $p =$   
343  $0.001$ ) and to the CEC ( $r = 0.79$ ,  $p = 0.001$ ) suggests that DFOB was mainly bound to  
344 the fine soil fraction and through ion-exchange mechanisms. Therefore, DFOB is  
345 hypothesized to most affect the mobility of Fe and Al in soils with a very coarse texture  
346 like soils A and N. The negative relationship between the clay content and the amount  
347 of metals mobilized by DFOB ( $r = -0.73$ ,  $p = 0.003$ ) supports this hypothesis and showed  
348 that adding DFOB (at  $200 \mu\text{mol kg}^{-1}$ ) to a soil containing 5% clay (soil A) led to the  
349 mobilization of 13 times more metals (Fe+Al) than in a soil containing 23% clay (soil  
350 H).

351 DFOB speciation in solution was calculated for each soil from the difference in Al and  
352 Fe concentrations measured in the CaCl<sub>2</sub> extract in the DFOB treatment and in the  
353 control. Figure 2b shows that DFOB was mainly associated with Fe in soils A, B, C, E,  
354 H, M, N and with Al in soil L, while in the six other soils, DFOB was associated with  
355 the two metals in similar proportions. This suggests rather selective DFOB-promoted  
356 dissolution of Fe-bearing phases in soils A, B, C, E, H, M, N and of Al-bearing phases  
357 in soil L. One possible hypothesis is that DFOB associated with either Fe or Al in the  
358 CaCl<sub>2</sub> extract, depending on which was more accessible for complexation. Under this  
359 hypothesis, Al was more accessible for complexation than Fe in soil L, which may be  
360 consistent with the high concentration of Al oxide in this soil (Table 1). However, the  
361 absence of a clear relationship between the ratio of Fe oxide to Al oxide in soil and the  
362 ratio of DFOB-Fe to DFOB-Al in the CaCl<sub>2</sub> extract (suppl. Fig. F4) suggests that other  
363 factors than the mineral composition of the soil determine the extent to which a metal-  
364 bearing phase is prone to DFOB-promoted dissolution. As reviewed in Kraemer et al.  
365 (2015), the rate of ligand-controlled dissolution is influenced by environmental factors  
366 that may change the speciation of the ligand (in this case DFOB) in pore water and at  
367 the surface of metal-bearing phases, including pH, metal ion surface coverage (see  
368 section 3.2 below), and surfactant adsorption, as well as the presence of other organic  
369 molecules such as humic substances or low molecular weight organic acids (LMWOA).  
370

### 371 3.2 Relationship between the level of soil Cu contamination and the metal mobilization 372 efficiency of DFOB and Pvd

373 Experiment 2 showed how the addition of DFOB and Pvd affected the mobility of  
374 metals in soil L, whose Cu concentration was artificially increased by spiking. Soil L  
375 was chosen because it is a calcareous soil in which symptoms of Cu-induced Fe

376 deficiency (interveinal chlorosis) were observed in durum wheat (Michaud et al., 2007).  
377 It is therefore a soil in which the link between the level of Cu contamination and Fe  
378 dynamics needed to be examined in more detail.

379 The results obtained in the non-spiked treatment (Cu100) confirmed that DFOB and  
380 Pvd have contrasting effects on the mobility of metals in soil (Fig. 3). First, they differ  
381 in the spectrum of metals they mobilize. DFOB only mobilized the trivalent metals  
382 Fe(III) and Al(III) (see section 3.1) whereas Pvd also mobilized the divalent metals  
383 Cu(II), Ni(II) and Co(II) as well as V and As (suppl. Table T4). For Cu, this difference  
384 can be explained by the presence of a catecholate function within the chelating group  
385 of Pvd that gives this siderophore a higher affinity for Cu(II) ( $\log \beta = 20.1$ ; Cornu et al.,  
386 2014) than DFOB ( $\log \beta = 14.6$ ; Kraemer et al., 2015). However, this explanation is not  
387 valid for Ni, for which Pvd ( $\log \beta = 10.9$ ; Ferret et al., 2015) and DFOB ( $\log \beta = 11.8$ ;  
388 Kraemer et al., 2015) exhibit almost the same affinity, and probably not for Co, V and  
389 As, either. Second, DFOB and Pvd differ in the total amount of metals they mobilize.  
390 DFOB mobilized on average 4 fold more metals than Pvd at Cu100 (Fig. 3). This is  
391 probably because less DFOB ( $K_d = 15 \text{ L kg}^{-1}$ ) than Pvd ( $K_d = 88 \text{ L kg}^{-1}$ ) was bound to  
392 the solid phase in this soil after 24 h, for reasons that may be linked to the large size  
393 and the high polarity of Pvd (Boiteau et al., 2020).

394 Figure 3 shows that the amount of DFOB and to a lesser extent that of Pvd in the  $\text{CaCl}_2$   
395 extract gradually decreased with an increase in the concentration of Cu in the soil. The  
396 amount of DFOB in the  $\text{CaCl}_2$  extract decreased from 118 nmoles at Cu100 to 30  
397 nmoles at Cu1600 (Fig. 3a), which means that the  $K_d$  of DFOB increased from 15 to 64  
398  $\text{L kg}^{-1}$  when the concentration of Cu in soil increased from 100 to 1600  $\text{mg kg}^{-1}$ .  
399 Likewise, the  $K_d$  of Pvd increased from 88 to 121  $\text{L kg}^{-1}$  when the concentration of Cu  
400 in soil increased from 100 to 1600  $\text{mg kg}^{-1}$ . By which process(es) can the accumulation

401 of Cu in soil promote the adsorption of DFOB and Pvd at the surface of soil constituents?

402 The most likely hypothesis is that the process is the same for the two siderophores and  
403 that  $\text{Cu}^{2+}$  ions serve as cation bridges linking negative sites on soil constituents and an  
404 anionic or polar functional group on siderophores. The hypothesis is based on the fact  
405 that at pH above 8, which is the case of soil L (Table 1), DFOB and Pvd (as well as  
406 their metal-complexes) are neutral and negatively-charged species, respectively, and  
407 most metal-bearing phases (including metal oxyhydroxides) have a negative surface  
408 charge. In addition, the high solubility of both DFOB and Pvd in water as well as their  
409 large polar surface area (206 Å and 636 Å, respectively) suggest that they both contain  
410 polar functional groups. Bridging with multivalent cations has been proposed as a  
411 possible adsorption mechanism for siderophores to humic substances (Harrington et al.,  
412 2015; Boiteau et al., 2020) as well as for pesticides such as glyphosate on the surface  
413 of oxyhydroxides (Kah and Brown, 2006). The main cations involved in the formation  
414 of bridges in alkaline soils are  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . The accumulation of  $\text{Cu}^{2+}$  ions at the  
415 surface of (at least some) soil constituents caused by spiking the soil with  $\text{CuSO}_4$  was  
416 assumed to be sufficiently high in treatments Cu350, Cu850 and Cu1600 to compete  
417 with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and thereby to increase the adsorption of DFOB and Pvd.

418 Figure 3c shows that the amount of metals (Fe+Al) mobilized by DFOB gradually  
419 decreased with an increase in the concentration of Cu in soil. The Cu-induced decrease  
420 in the amount of Fe+Al released was of the same magnitude as that of DFOB measured  
421 in the  $\text{CaCl}_2$  extract (i.e. around 70% loss between Cu100 and Cu1600). In other words,  
422 the 1:1 linear relationship between the amount of DFOB in the  $\text{CaCl}_2$  extract and the  
423 amount of Fe+Al mobilized from the solid phase was conserved regardless of the  
424 concentration of Cu in the soil (suppl. Fig. F5). This suggests that the process of ligand-  
425 controlled dissolution by which DFOB mobilizes Fe and Al in soil is not affected by

426 the level of soil Cu contamination. This may be because DFOB is unable to complex  
427 Cu in soil, as shown in Exp. 1. Unlike DFOB, the amount of metals (Fe+Al+Cu)  
428 mobilized by Pvd gradually increased with an increase in the concentration of Cu in the  
429 soil (Fig. 3d). The amount of metals mobilized by Pvd was 2.6-times higher at Cu1600  
430 than at Cu100, although the amount of Pvd in the CaCl<sub>2</sub> extract was 36% lower at  
431 Cu1600 than at Cu100 (cf. above). This shows that the amount of metal mobilized in  
432 presence of Pvd exceeded the amount of Pvd monitored in the CaCl<sub>2</sub> extract at Cu300,  
433 Cu850 and Cu1600, and suggests that the process by which Pvd mobilized metals in  
434 soil L gradually shifted from ligand-controlled dissolution with an increase in the soil  
435 Cu concentration.

436 Copper is the metal whose mobilization by Pvd was affected most by the increase in  
437 the soil Cu concentration. While Cu accounted for 74% of the metals mobilized by Pvd  
438 at Cu100, it accounted for almost 90% of the metals mobilized by Pvd at Cu1600.  
439 Although there was a one-year delay after the soil was spiked, the adsorption of “fresh”  
440 Cu onto the solid phase was assumed to involve low-energy bonds that were more  
441 inclined to break in presence of Pvd than the high-energy bonds involved in the  
442 retention of “aged” Cu. Therefore, the mechanisms used by Pvd to mobilize “fresh” Cu  
443 and “aged” Cu are almost certainly not the same. The fact that the amount of Cu  
444 mobilized exceeded the amount of Pvd in the CaCl<sub>2</sub> extract at Cu300, Cu850, and  
445 Cu1600, suggests that the mobilization mechanism used for “fresh” Cu enabled the Pvd  
446 involved to be at least partially recycled. According to Akafia et al. (2014), among the  
447 mechanisms involved in ligand-promoted mobilization, only reductive dissolution  
448 allows ligand recycling. Therefore, the Pvd-induced mobilization of “fresh” Cu was  
449 assumed to rely on a reductive dissolution pathway even though no specific  
450 measurements, for instance of the electrode potential of the mineral (Bi et al., 2010),

451 were made in this experiment to confirm it. In reductive dissolution, the ligand acts as  
452 a reductant whose adsorption at the mineral surface induces its dissolution via the  
453 reduction of the metal ion center (Kraemer, 2004). In this scenario, Pvd probably acts  
454 as a reductant able to reduce Cu(II) to Cu(I), but this remains to be demonstrated.

455 Figure 3c shows that, depending on the concentration of Cu in the soil, DFOB did not  
456 mobilize the same relative proportions of Fe and Al. At Cu100, Fe accounted for 9% of  
457 the metals mobilized by DFOB but this fraction gradually increased with an increase in  
458 the soil Cu concentration to reach 52% at Cu1600. As a result, the ability of DFOB to  
459 mobilize Fe increased with an increase in the soil Cu concentration to the detriment of  
460 the mobilization of Al. Similar results were observed for Pvd (Fig. 3d): the amount of  
461 Fe mobilized by Pvd gradually increased with an increase in the soil Cu concentration,  
462 from < 1 nmole at Cu100 to 6 nmoles at Cu1600, while that of Al gradually decreased  
463 from 7 nmoles at Cu100 to 1 nmole at Cu1600. By which process(es) can the  
464 accumulation of Cu in soil promote the mobilization of Fe by these two siderophores  
465 to the detriment of Al? The process is hypothesized to be the same for DFOB and Pvd  
466 and, in accordance with the hypothesis formulated above, a first explanation is that Cu<sup>2+</sup>  
467 ions adsorbed onto the surface of Fe-bearing phases in preference to the Al-bearing  
468 phases in soil L. According to this hypothesis, the accumulation of Cu<sup>2+</sup> ions promoted  
469 the adsorption of siderophores onto the surface of Fe-bearing phases (notably Fe-oxides)  
470 thereby increasing the rate of Fe release. Another possible explanation based on the  
471 study by Dubbin and Bullough (2017) is that the accumulation of Cu<sup>2+</sup> ions at the  
472 surface of Al-bearing phases blocked the reactive sites of Al dissolution thereby  
473 reducing the rate of Al mobilization by DFOB and Pvd.

474

475 3.3 The metal mobilization efficiency of DFOB and Pvd changed drastically over time

476 following their supply

477 Experiment 3 showed the extent to which the ability of DFOB and Pvd to mobilize

478 metals in soil persists over time, by comparing their effect 1, 8 and 22 days after their

479 addition. Soil K was chosen because it is an acidic sandy soil typical of the gravel soils

480 of the Bordeaux area where symptoms of Cu toxicity were observed on grapevines in

481 the 1960s (Delas 1963), and soil L for the same reasons as those given above for Exp.

482 2. Figure 4 first shows that DFOB was no longer detected in the CaCl<sub>2</sub> extract after

483 eight and 22 days in the two soils (Fig. 4a, b). Likewise, no further increase in the

484 amount of Fe and Al in the CaCl<sub>2</sub> extract was observed after eight and 22 days in

485 presence of DFOB, regardless of the soil (Fig. 4c, d; suppl. Table T5). This result

486 suggests that the ability of DFOB to mobilize metals in soil is likely to end within eight

487 days after its release by (e.g. *Streptomyces*) bacteria. In a previous work (Cornu et al.,

488 2017), a decrease in the concentration of DFOB in pore water over time following its

489 addition was already observed, although the decrease was much more gradual.

490 The disappearance of DFOB in the CaCl<sub>2</sub> extract observed between day 1 and day 8

491 may be the result of DFOB adsorption onto soil constituents and/or of DFOB

492 degradation by microorganisms. A study by Zhong et al. (2013) showed that the

493 adsorption of DFOB onto the solid phase was complete within the first 4 h in an Oxisol.

494 However, DFOB is not only adsorbed by electrostatic bonds but also by forming

495 covalent bonds, which may require longer, perhaps more than 24 h in some conditions

496 (e.g. soil, temperature). In addition, several rhizosphere soil bacteria have been found

497 to degrade ferrioxamine-type siderophores such as DFOB, including members of the

498 genus *Azospirillum* (Winkelmann et al., 1999), and to use DFOB as a carbon source

499 (Castignetti and Siddiqui, 1984). However, the rate of microbial degradation of DFOB

500 in soil has not been sufficiently documented to know if all the DFOB present in the

501 CaCl<sub>2</sub> extract could have been microbially degraded in eight days. As reported for  
502 phytosiderophores (Römheld, 1991), the rate of DFOB degradation in soil presumably  
503 depends on a combination of environmental factors, including the size and the  
504 composition of the microbial community.

505 Figure 4 shows that the amount of Pvd in the CaCl<sub>2</sub> extract also decreased over time in  
506 the two soils, but more gradually than DFOB (Fig. 4 a, b). The amount of Pvd in the  
507 CaCl<sub>2</sub> extract was reduced by, on average, 57% after eight days and by 82% after 22  
508 days in soil K, and by 24% after eight days and by 44% after 22 days in soil L, compared  
509 to the amount measured on day 1 (suppl. Table T5). Like for DFOB, the progressive  
510 decrease in the amount of Pvd in the CaCl<sub>2</sub> extract could result from Pvd adsorption  
511 (like DFOB, Pvd is not only adsorbed onto soil constituents by electrostatic bonds)  
512 and/or from Pvd degradation (Parker et al., 2007). <sup>15</sup>N-Pvd was used to help identify  
513 which of the two processes is responsible for the progressive decrease in the  
514 concentration of Pvd in the CaCl<sub>2</sub> extract. Under the Pvd adsorption hypothesis, the  
515 delta <sup>15</sup>N and the concentration of total N in the CaCl<sub>2</sub> extract decrease over time  
516 together with the Pvd concentration, while under the Pvd degradation hypothesis, they  
517 remain constant (provided the Pvd degradation metabolites do not adsorb onto soil  
518 constituents). Figure 5 shows that the concentration of delta <sup>15</sup>N and of total N in the  
519 CaCl<sub>2</sub> extract increased over the incubation period in the two soils, either transitorily in  
520 soil K (Fig. 5a) or continuously in soil L (Fig. 5b). The theoretical concentration of Pvd  
521 in the CaCl<sub>2</sub> extract was calculated from the delta <sup>15</sup>N and the concentration of total N  
522 in the extraction solution assuming Pvd did not degrade over time (for details, see  
523 supplementary data). The good agreement between the calculated and the  
524 experimentally measured concentration of Pvd observed on day 1 (Fig. 5c, d) suggests  
525 that Pvd did not degrade in either soil during the first 24 h following its addition. In soil



526 L, the increase in the calculated concentration of Pvd over time contrasted with the  
527 decrease in the Pvd concentration over time measured experimentally. This discrepancy  
528 may be due to the fact that part of the Pvd adsorbed onto the soil constituents on day 1  
529 degraded over time (presumably by microorganisms) and released nitrogen compounds,  
530 such as ammonium, nitrate and/or amino acids, into the extraction solution that do not  
531 absorb at 380 nm.

532 In soil K, the transitory increase in the delta <sup>15</sup>N and in the concentration of total N in  
533 the CaCl<sub>2</sub> extract between day 1 and day 8 (Fig. 5a), as well as the increase in the  
534 resulting calculated Pvd concentration (Fig. 5c), was also attributed to the degradation  
535 of adsorbed Pvd, whereas their marked decrease between day 8 and day 22 was  
536 attributed to volatilization of <sup>15</sup>N in the CaCl<sub>2</sub> extract by denitrification. Indeed,  
537 anaerobic conditions probably developed in the soil K between day 8 and day 22 when  
538 the addition on day 0 of 1 mL of solution (either Pvd, DFOB or ultra-pure water)  
539 increased the soil water content almost to saturation. As reported previously (Cornu et  
540 al., 2007), anaerobic conditions can develop in a few days after rewetting in water-  
541 saturated soils. In the present study, anaerobic conditions would have developed  
542 selectively in soil K presumably because of its low WHC resulting from its sandy  
543 texture (the soil contained 87% sand), which makes it more sensitive to water saturation  
544 than the heavier textured soil L (that contained only 31% sand).

545 Figure 4 also shows that the amount of Fe+Al+Cu mobilized by Pvd decreased over  
546 time at almost the same magnitude as Pvd, especially in soil K (Fig. 4c). In other words,  
547 the 1:1 linear relationship between the amount of Pvd in the CaCl<sub>2</sub> extract and the  
548 amount of Fe+Al+Cu mobilized from the solid phase was conserved over the 22-day  
549 incubation period in the two soils (suppl. Fig. F6). This suggests that the main process  
550 by which Pvd mobilized Fe, Al and Cu in soil did not change over time and remained

551 ligand-controlled dissolution. In contrast, the relative proportions in which Pvd  
552 mobilized Fe, Al and Cu did change over time in the two soils (Fig. 4c, d). For example,  
553 in soil K, Cu accounted for 21% of the metals mobilized by Pvd on day 1 but this  
554 fraction gradually increased over time to reach 84% on day 22. As a result, the amount  
555 of Cu mobilized by Pvd decreased by less than 20% in this soil between the day 1 and  
556 day 22, while that of Fe and Al decreased by a factor of 18 and 32, respectively. Why  
557 did the capacity of Pvd to mobilize Fe and Al decrease over time in favor of its capacity  
558 to mobilize Cu? One possible hypothesis is that the processes thought to be responsible  
559 for the progressive reduction in the concentration of Pvd in the CaCl<sub>2</sub> extract (see above)  
560 are sensitive to the metal with which Pvd is associated in solution. In other words, the  
561 Pvd-Cu(II) complex is likely relatively less (or more slowly) adsorbed onto soil  
562 constituents and/or degraded by microorganisms than the Pvd-Fe(III) and Pvd-Al(III)  
563 complexes. The difference in the fate of Pvd-Cu(II) and Pvd-Fe(III) over time can be  
564 explained by biotic process(es). Indeed, one can reasonably assume that Pvd-Cu(II) is  
565 more resistant to microbial degradation than Pvd-Fe(III), for which soil microorganisms  
566 (either bacteria or fungi) have developed a specific enzymatic machinery to recover  
567 iron. In contrast, the difference in the fate of Pvd-Cu(II) and Pvd-Al(III) over time is  
568 difficult to explain by biotic process(es) and could instead result from the adsorption of  
569 Pvd-Al(III) onto soil constituents in preference to Pvd-Cu(II), for reasons that may  
570 derive from the “hard and soft (Lewis) acids and bases” (HSAB) theory (Harter and  
571 Naidu, 1995), notably the fact that Al<sup>3+</sup> belongs to the hard Lewis acids while Cu<sup>2+</sup>  
572 belongs to borderline acids.

573 Taken together, the results of Exp. 3 suggest that the speciation of Pvd in the CaCl<sub>2</sub>  
574 extract changed over time in the two soils. Geochemical modeling was performed to  
575 confirm this hypothesis using the characteristics of the CaCl<sub>2</sub> extract listed in section

576 2.6. As shown in suppl. Table T6, there was good agreement between the Pvd speciation  
577 calculated using the relative proportions of metals mobilized in the CaCl<sub>2</sub> extract (cf.  
578 Fig. 4) and the Pvd speciation assessed by geochemical modeling. Thereby, the  
579 geochemical approach confirmed that the fraction of Pvd associated with Cu in the  
580 CaCl<sub>2</sub> extract increased over time in the two soils to the detriment of the fraction of Pvd  
581 associated with Fe and Al. In addition, the geochemical approach suggests that (i) Pvd  
582 was not associated with Ni and almost not associated with Ca, (ii) Al and Fe were almost  
583 only associated with Pvd on day 1 and with fulvic acids on day 22, and (iii) Cu remained  
584 predominantly associated with Pvd in the CaCl<sub>2</sub> extract over time (suppl. Table T6).  
585 Fluorescence measurements were also performed on the CaCl<sub>2</sub> extract to determine the  
586 concentration and the speciation of Pvd (for details on the protocol, see suppl. data).  
587 These measurements confirmed that Pvd fluorescence properties are distinct from those  
588 of the humic-like substances usually present in soil extracts, as previously underlined  
589 by Potysz et al. (2016), and also showed that Pvd fluorescence decreased over time in  
590 the two soils (suppl. Fig. F7 and F8). However, it was impossible to quantify the  
591 concentration of Pvd or to experimentally characterize its speciation in the CaCl<sub>2</sub> extract  
592 because Pvd fluorescence properties depend on its concentration and on the metal with  
593 which it is associated (Braud et al., 2010), and both parameters varied simultaneously  
594 over time in the two soils.

595

#### 596 **4. Conclusions**

597 The three experiments conducted in this study provide new insights into the metal  
598 mobilization ability of siderophores in soil that determines the conditions in which  
599 siderophore-producing bacteria can improve the remediation of Cu-contaminated soils  
600 through phytoextraction. First, this study highlighted the fact that the panel of metals

601 mobilized by Pvd is larger than the amount mobilized by DFOB. Only Pvd mobilized Cu,  
602 thereby supporting the idea of using Pvd-producing bacteria (or more generally bacteria  
603 that produce siderophores with a catecholate moiety) to enhance Cu phytoextraction.  
604 Second, this study showed that DFOB and Pvd mainly mobilized metals through ligand-  
605 controlled dissolution. In the case of DFOB, it even appears to be the only process by  
606 which Fe and Al are mobilized. In ligand-controlled dissolution, the efficiency of  
607 ligands in mobilizing metals depends on the rate at which they adsorb onto the surface  
608 of metal-bearing phases, and on the rate at which the metal-ligand complex (once  
609 formed) detaches from the surface and dissolves in pore water. This second step seems  
610 to be the limiting step in the DFOB- and Pvd-promoted mobilization of metals, as  
611 suggested by the large fraction of the two siderophores bound to the solid phase after  
612 24 h. The addition prior to siderophores of low molecular mass organic acids (LMMOA)  
613 such as oxalate may increase the rate of siderophore-promoted mobilization of metals.  
614 The role of the LMMOA is to adsorb and form labile metal species at the surface of  
615 metal-bearing phases, while that of the siderophore is to remove metal from LMMOA-  
616 metal complexes leaving the uncomplexed LMMOA ligand free to react again.  
617 Investigations are currently underway to help determine if the production/supply of  
618 LMMOA is a driver that should be used for the optimization of Cu phytoextraction.  
619 Third, this study suggests that Pvd can mobilize Cu by reductive dissolution in soil, but  
620 only “fresh” Cu supplied by spiking. This implies a direct link between the process by  
621 which Cu is bound to the soil constituents and the mechanism by which it is mobilized  
622 by (or in presence of) Pvd. It would be interesting to dissect this link because it likely  
623 affects the efficiency and the selectivity by which Pvd mobilizes Cu in Cu-contaminated  
624 soils. In addition, in reductive dissolution, the amount of metals mobilized usually  
625 exceeds the amount of ligands in soil pore water, which is not the case in ligand-

626 controlled dissolution. Consequently, the mechanism used by Pvd to mobilize Cu in  
627 soils could affect the speciation of Cu in soil pore water (notably the ligands with which  
628 it is associated), and, hence, the efficiency with which Pvd increases Cu  
629 phytoavailability and Cu phytoextraction.

630 Finally, this study showed that the metal mobilization efficiency of siderophores in soil  
631 decreased over time following their supply. This decrease is likely due to the adsorption  
632 of siderophore-metal complexes onto soil constituents, which can take hours, days, or  
633 weeks, depending on the nature of the bonds involved, but also on the degradation of  
634 siderophore-metal complexes by soil microorganisms. The relative contribution of  
635 these two processes is not known and requires specific investigation, for instance by  
636 monitoring siderophore degradation metabolites, to better understand the persistence of  
637 siderophores in soils in terms of lifespan and effect.

638

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649

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## Figure captions

**Figure 1.** Concentrations of total Fe (a), total Al (b) and total Cu (c) in the 0.005 M CaCl<sub>2</sub> extract in 14 Cu-contaminated topsoils supplied with DFOB or not (control). Soil extraction was performed 24 h after DFOB was supplied. For each soil, \*, \*\* and \*\*\* indicate that the Al concentration (a), Fe concentration (b) or Cu concentration measured in the presence of DFOB differed significantly from the concentration measured in the control, at a probability level of  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively. Error bars are mean standard deviations; ns: non-significant.

**Figure 2.** Relationship between the amount of DFOB in the 0.005 M CaCl<sub>2</sub> extract and the amount of Fe+Al mobilized from the solid phase (a) and DFOB speciation in 0.005 M CaCl<sub>2</sub> extract (b), in the 14 Cu-contaminated topsoils supplied with DFOB. Soil extraction was performed 24 h after DFOB was supplied. DFOB speciation was calculated from the DFOB concentration and the DFOB-induced increase in Fe and Al contents measured in the 0.005 M CaCl<sub>2</sub> extract in the DFOB treatment, based on the relationships shown in (a).

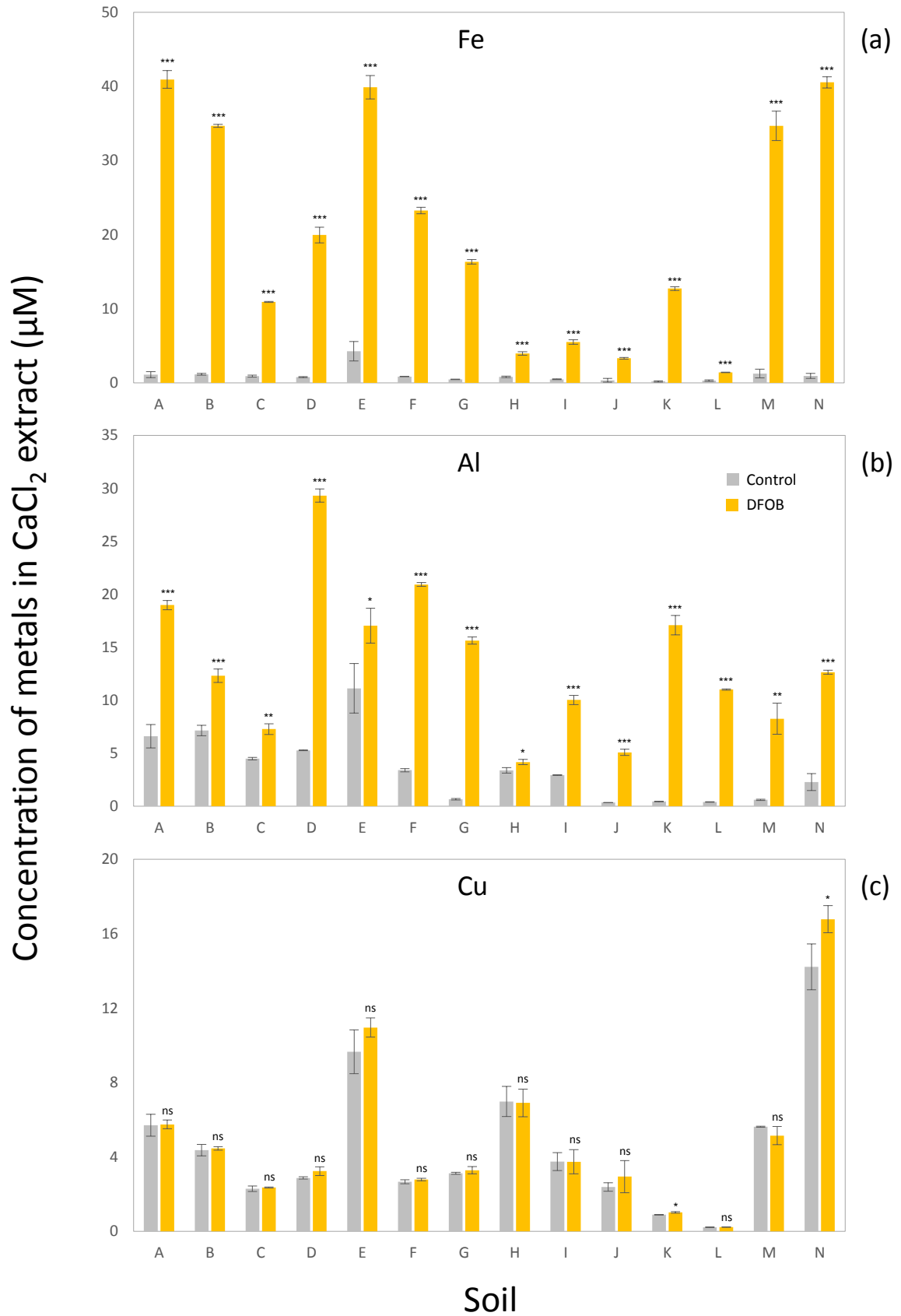
**Figure 3.** Amount of siderophore (DFOB, Pvd) in 0.005 M CaCl<sub>2</sub> extract, and amount of metals (Al, Fe, Cu) mobilized from the solid phase in the vineyard topsoil L contaminated by different concentrations of Cu (100, 350, 850 and 1,600 mg kg<sup>-1</sup>) and supplied with DFOB (a, c) or Pvd (b, d). Soil extraction was performed 24 h after the siderophores were supplied. Error bars are mean standard deviations. Mean values with different letters are significantly different ( $p < 0.05$ ). In (c), the values in the dark blue and grey bar plots correspond to the percentage of DFOB associated with respectively,

Al and Fe, in the CaCl<sub>2</sub> extract. In (d), the values in the light blue bar plots correspond to the percentage of Pvd associated with Cu in the CaCl<sub>2</sub> extract.

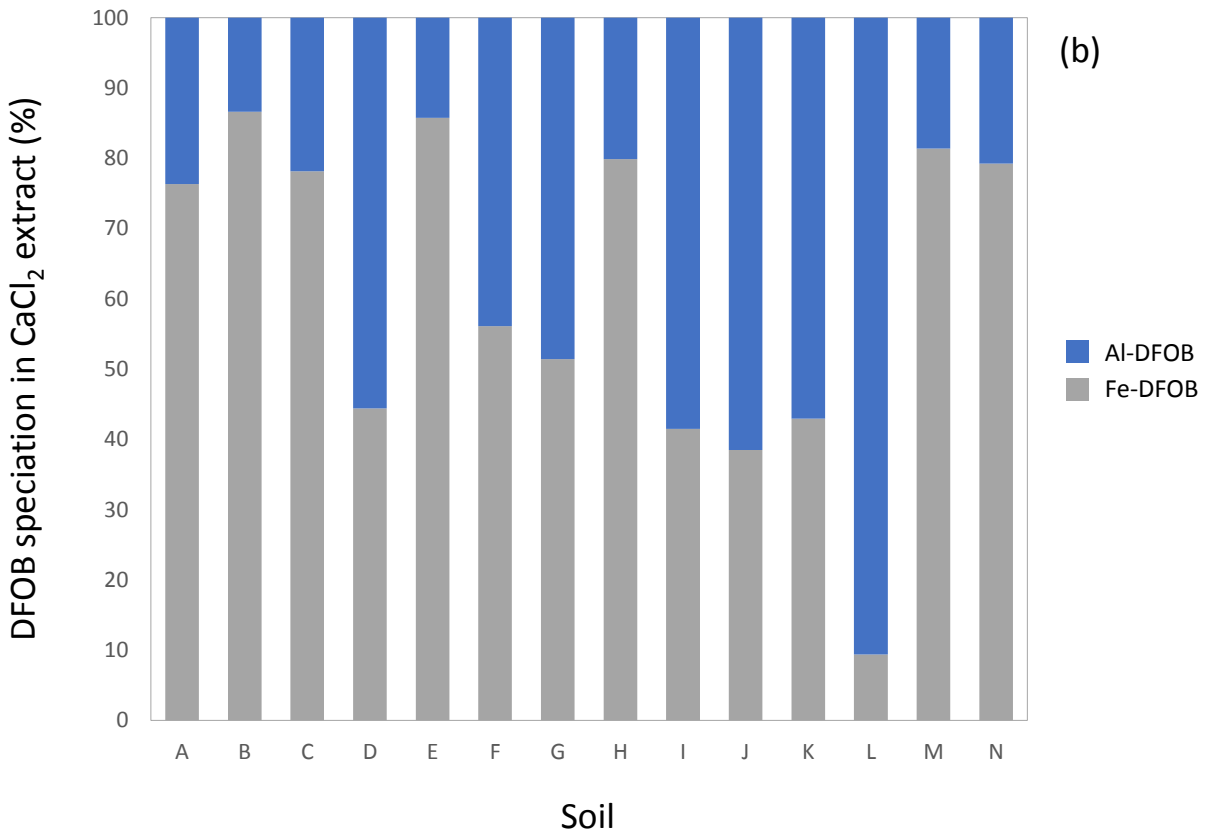
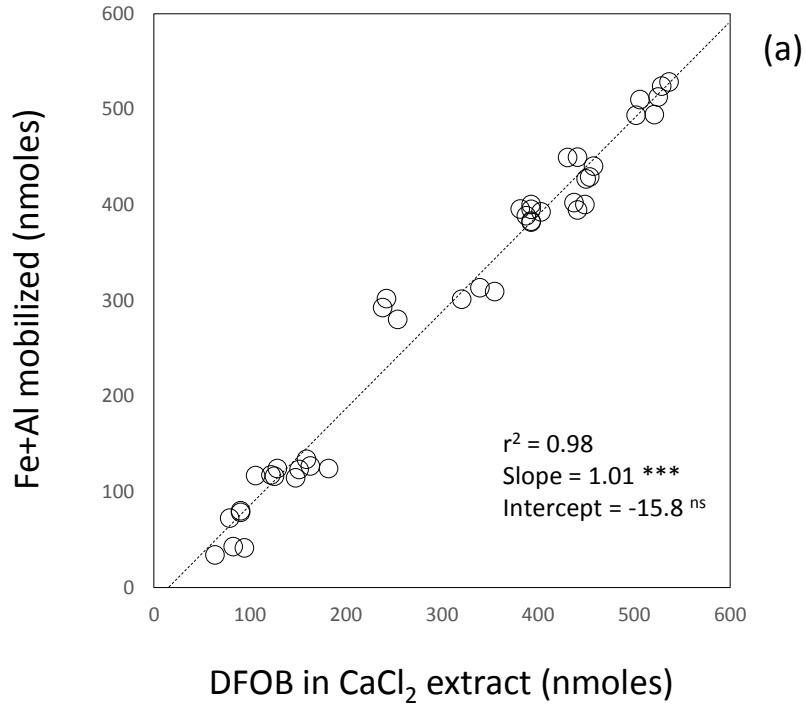
**Figure 4.** Temporal changes in the amount of siderophores (DFOB, Pvd) in the 0.005 M CaCl<sub>2</sub> extract and in the amount of metals (Al, Fe, Cu) mobilized from the solid phase in the vineyard topsoils K (a, c) and L (b, d) supplied with DFOB or Pvd. The incubation time (1, 8 and 22 days) is the time between when the siderophore was supplied and soil extraction with 0.005 M CaCl<sub>2</sub>. Error bars are mean standard deviations. Mean values with different letters are significantly different ( $p < 0.05$ ). In (c) and (d), the values in the light blue bar plots correspond to the percentage of Pvd associated with Cu in the CaCl<sub>2</sub> extract.

**Figure 5.** Temporal changes in the total N concentration and in the delta <sup>15</sup>N in the 0.005 M CaCl<sub>2</sub> extract of the vineyard topsoils K (a) and L (b) supplied with the same concentration of <sup>15</sup>N-Pvd. The incubation time (1, 8 and 22 days) is the time between when <sup>15</sup>N-Pvd was supplied and soil extraction with 0.005 M CaCl<sub>2</sub>. (c) and (d) compare the concentration of Pvd measured in the extraction solution and the theoretical concentration of Pvd calculated from the delta <sup>15</sup>N and the total N concentration in the extraction solution (assuming no degradation of Pvd over time) in soils K and L, respectively.

# Figure 1

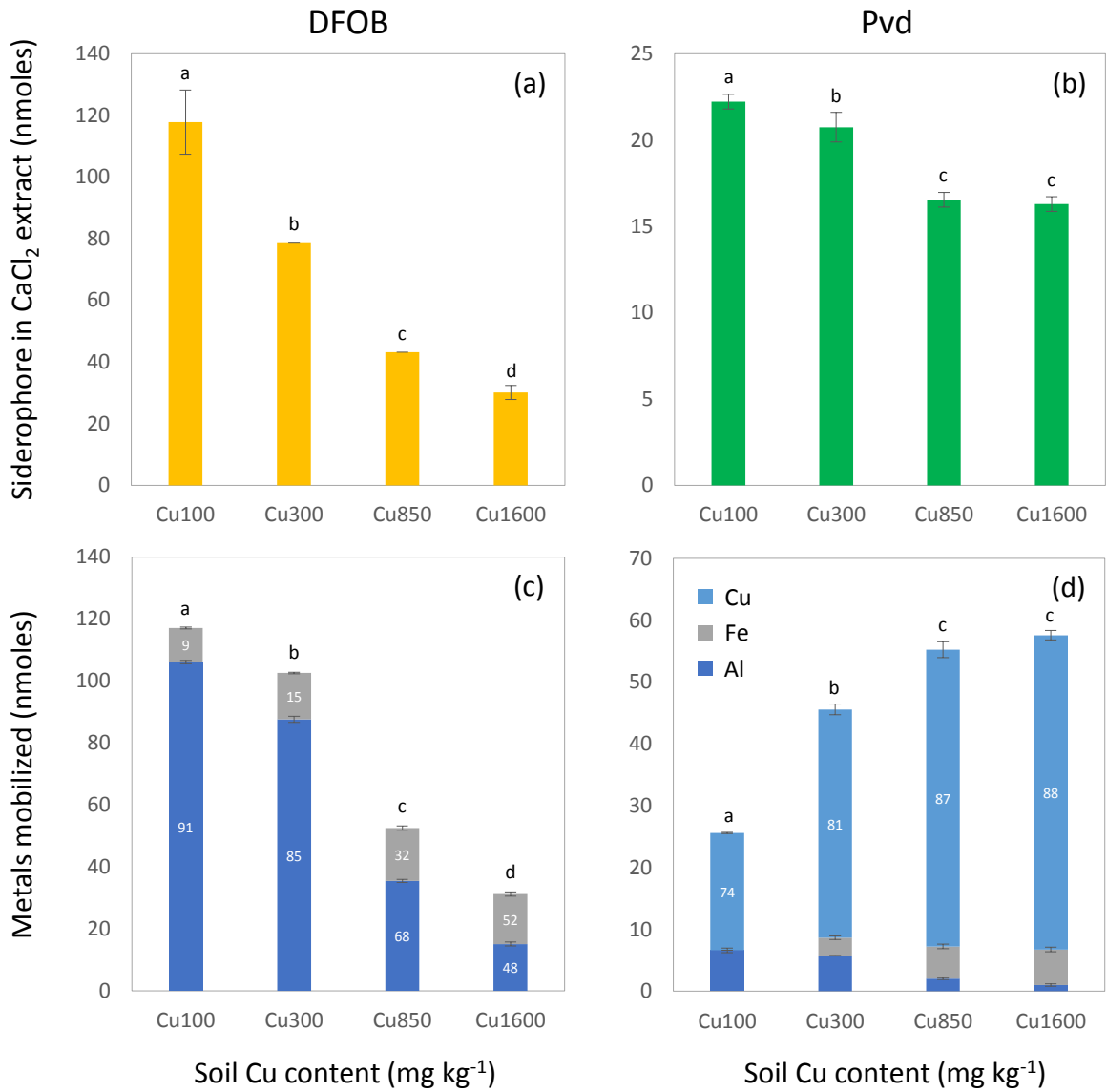


# Figure 2

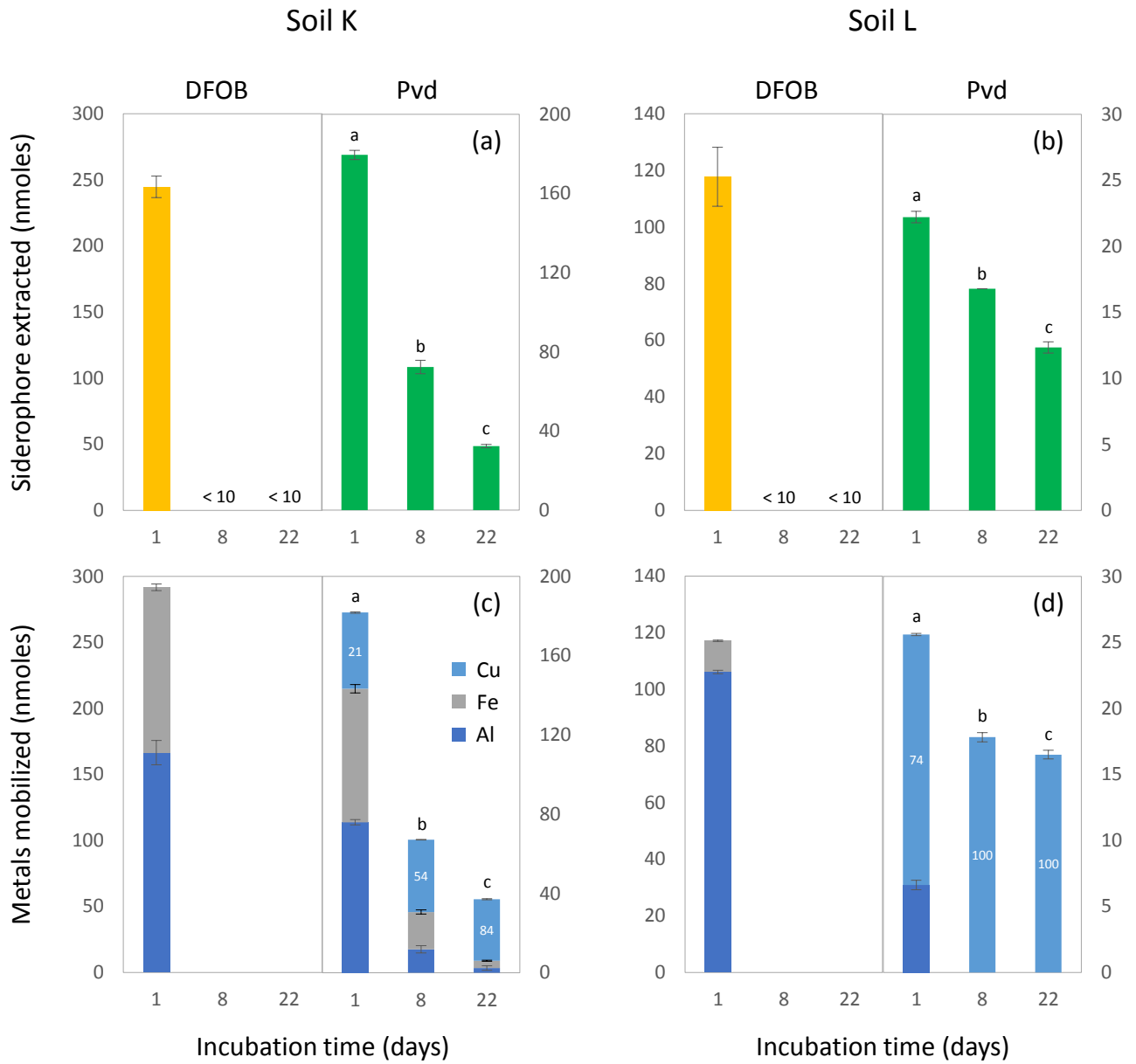




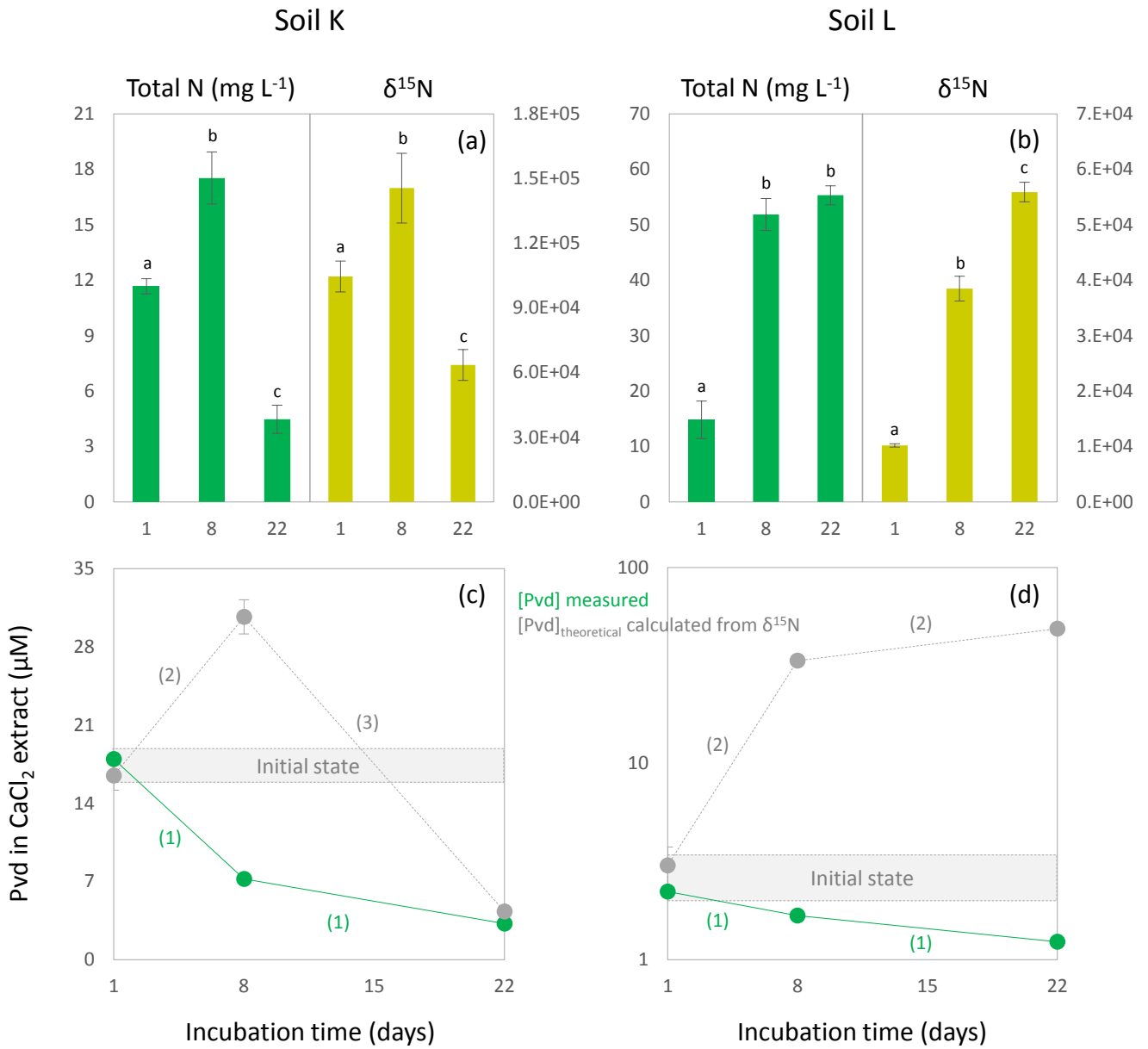
# Figure 3



# Figure 4



# Figure 5



- (1) Degradation and/or adsorption of the Pvd present in the extraction solution on day 1
- (2) Mobilization of degraded Pvd
- (3) Re-adsorption and/or volatilization of degraded Pvd

**Table 1.** Selected physical and chemical properties of the 14 Cu-contaminated topsoils considered in this study. OM: organic matter.

	Sand	Silt	Clay <sup>a</sup>	OM <sup>b</sup>	CEC <sup>c</sup>	CaCO <sub>3</sub> <sup>d</sup>	Al oxide	Fe oxide	Mn oxide <sup>e</sup>	pH <sub>water</sub> <sup>f</sup>	Total Cu <sup>g</sup>	Cu <sub>EDTA</sub> <sup>h</sup>
	g kg <sup>-1</sup>			%	cmol <sub>c+</sub> kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>				mg kg <sup>-1</sup>	
<b>A</b>	875	71	54	0.9	1.8	< 1	0.38	0.66	0.07	5.9	104	96
<b>B</b>	835	119	46	1.4	2.2	< 1	0.54	0.84	0.05	6.5	100	88
<b>C</b>	622	222	156	1.3	5.3	< 1	0.92	1.25	0.05	6.8	103	53
<b>D</b>	825	121	54	1.2	2.6	< 1	0.84	0.96	0.05	6.4	111	76
<b>E</b>	794	88	118	2.2	4.9	< 1	0.78	1.00	0.04	6.5	103	73
<b>F</b>	816	97	87	1.9	5.8	4.4	0.62	1.45	0.08	7.9	113	68
<b>G</b>	857	76	67	1.7	4.9	8.4	0.63	0.94	0.13	8.3	154	132
<b>H</b>	661	109	230	3.1	17.5	105	1.20	1.37	0.25	7.6	159	85
<b>I</b>	872	41	87	1.4	7.6	80	0.63	0.72	0.10	7.9	102	73
<b>J</b>	753	95	152	1.7	9.7	35	0.95	1.07	0.16	8.6	121	77
<b>K</b>	872	84	44	2.0	6.4	9	0.63	1.04	0.14	7.9	174	123
<b>L</b>	309	404	287	1.6	19.1	132	1.56	0.96	0.44	8.5	100	4
<b>M</b>	701	200	99	5.7	8.8	45	1.04	1.57	0.12	6.7	336	223
<b>N</b>	846	110	44	1.5	2.9	< 1	1.06	1.67	0.13	6.4	905	515

<sup>a</sup> ISO 11277<sup>b</sup> NF ISO 10694<sup>c</sup> NF ISO 23470<sup>d</sup> NF ISO 10693<sup>e</sup> Tamm (1922)<sup>f</sup> NF ISO 10390<sup>g</sup> NF X 31-147/NF ISO 22036<sup>h</sup> NF X 31-120/NF ISO 22036