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

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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

Abstract

Siderophores are biogenic metallophores that can play significant roles in the dynamics of a range of metals, including Cu, in the soil. Understanding the impact of siderophores on the mobility and the availability of metals in soil is required to optimize the efficiency of soil remediation processes such as phytoextraction. This study compared the ability of siderophores desferrioxamine B (DFOB) and pyoverdine (Pvd) to mobilize metals in a series of Cu-contaminated soils, and investigated the extent their metal mobilization efficiency changed over time and with the level of Cu contamination of the soil. Siderophores were supplied (or not) to Cu-contaminated soils and metal mobility was assessed through their total concentration in 0.005 M $CaCl_2$ extract. DFOB selectively mobilized Fe and Al while Pvd also mobilized Cu and Ni, Co, V and As but to a lesser degree. The 1:1 relationship between DFOB in the $CaCl_2$ extract and Fe+Al mobilized from the solid phase suggests that DFOB mobilized metals by ligand-controlled dissolution. The accumulation of Cu in soil enhanced the adsorption of DFOB and Pvd at the surface of soil constituents and the mobilization of Fe to the detriment of Al by the two siderophores. The metal mobilization efficiency of DFOB and to a lesser extent of Pvd decreased over 22 days. According to ^{15}N-Pvd analyses, Pvd degradation at least partly contributed to the progressive reduction in the metal mobilization efficiency of Pvd. The processes behind these results and the relevance of these results for manipulating the availability of Cu (and Fe) in soil are discussed.

Keywords

Ligand-promoted mobilization, metals, vineyard soils, phytoextraction, speciation, ^{15}N labeling

1. Introduction

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

Soil Cu contamination in vineyard and orchard soils, although moderate compared to that of Cu-polluted soils located near Cu mines (Zotti et al., 2014), Cu smelting factories (Wang et al., 2014) or at wood preservation sites (Kolbas et al., 2020), has consequences for the functioning and the sustainability of these ecosystems since it has chronic effects on the dynamics of soil populations. Karimi et al. (2021) performed a meta-analysis of Cu ecotoxicity and reported that Cu harms soil microorganisms at concentrations above 200 kg Cu ha^{-1} (Cu of 67 mg kg^{-1} soil), which is currently the level found in many vineyard and orchard topsoils. Excess Cu reduces microbial activity (Soler-Rovira et al., 2013) and biodiversity (Viti et al., 2008) in vineyard topsoils, and reduces microbial biomass and C mineralization rates in apple orchard topsoils (Wang et al., 2009). Ways to reduce the Cu contamination of vineyard and orchard soils are thus needed along with the use of other substances than Cu to protect vines and fruit trees against bacterial and fungal diseases.

Phytoextraction progressively reduces the concentration of metals in soil by accumulating metals in harvestable plant parts (Bert, 2013). Copper phytoextraction is still in the experimental stage as yields do not yet reduce soil Cu contamination sufficiently. The limited phytoavailability of Cu in vineyard or orchard soils (e.g. compared to Ni in serpentine soils) and its preferential accumulation in the roots of most Cu-extracting plants makes phytoextraction of even 1 kg Cu ha^{-1} year^{-1} difficult. One way to increase Cu phytoavailability in the soil without causing Cu leaching to groundwater is to inoculate the rhizosphere of the Cu-accumulating plant with siderophore producing bacteria (SPB). Siderophores are biogenic metallophores released by some plants (*Poaceous* species), soil fungi (e.g. *Aspergillus*) and soil bacteria (e.g. *Streptomyces*, *Pseudomonads*) to guarantee their iron nutrition under iron starvation. Although siderophores are generally considered as biological iron uptake agents, they can form stable complexes (Braud et al., 2010) and play a significant role in the biogeochemical cycling of a range of metals, including Cu (Kraemer et al., 2015). Previous studies (Cornu et al., 2014; 2019) revealed for instance, that the mixed catecholate and hydroxamate siderophore pyoverdine (Pvd) enhanced the mobility (i.e. the solid-solution transfer) of Cu in vineyard soils. However, the *in situ* deployment of bioaugmentation-assisted phytoextraction for Cu requires a better understanding of the processes used by siderophores to mobilize Cu in soil, in order to identify the conditions (type of siderophore, soil characteristics, etc.) needed to optimize their efficiency.

Batch experiments showed that mineral Fe-bearing phases (Fe oxyhydroxides, clays) dissolve almost exclusively via a ligand-controlled dissolution mechanism in presence of siderophores (Cheah et al., 2003; Kraemer, 2004; Akafia et al., 2014). This means that the siderophore-promoted mobilization of Fe primarily relies on Fe complexation by siderophores, in solution and at the mineral surface, which increases both the solubility and the dissolution rate of mineral Fe-bearing phases. However, this theoretical model does not necessarily apply to Cu, because Cu and Fe do not have the same geochemistry, or to soils, because minerals in soils are associated with solid organic matter, and metals and metallophores (other than siderophores) are present in large numbers.

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

2. Material and methods

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

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

2.1 Soils

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

As detailed in Cornu et al. (2019), the vineyard soils A to K have a coarse texture, a rather low organic matter (OM) content, and a low cation exchange capacity (CEC). Their total Cu contents range from 100 to 174 mg kg^{-1} and EDTA-extractable Cu contents (Cu_{EDTA}) range from 53 to 132 mg kg^{-1}. Soil L is characterized by a clay loam texture, high carbonate content and a low EDTA-extractable Cu content. Soil M is characterized by a coarse texture, a high OM content (> 5%), and high total and EDTA-extractable contents of Cu (336 and 223 mg kg^{-1}, respectively). Soil N is characterized by a coarse texture, low OM content and low CEC, and by very high total and EDTA-extractable contents of Cu (905 and 515 mg kg^{-1}, respectively). The soil samples also differ in their pH, ranging from 5.9 in soil A to 8.6 in soil J. Eight out of the 14 samples are carbonate soils (soils F to M), the six others (soils A to E and soil N) are not. For the purposes of Exp. 2, soil L was spiked with Cu (added as $CuSO_4$) one year prior to being used for the present study. The four concentrations of Cu compared in Exp. 2 were 100 (original value), 300, 850 and 1,600 mg Cu kg^{-1} soil DW.

2.2 Desferrioxamine B

DFOB was purchased as mesylate salt ($C_{25}H_{48}N_60_8 \cdot CH_4O_3S$, CAS Number: 138-14-7, M= 656.79 g mol^{-1}) from Merck. DFOB stock solution (1 mM) was prepared 24 h prior to use by dissolving the DFOB mesylate salt in ultra-pure water. The concentration of DFOB in the soil extraction solution (see section 2.5) was assessed by converting all the DFOB present in solution to DFOB-Fe(III), and by measuring the absorbance of the DFOB-Fe(III) complex at 439 nm (Helios Epsilon, Thermo Spectronic), according to the protocol of Cheah et al. (2003). Briefly, a 2 mL-aliquot of extraction solution was acidified to pH < 2 by addition of 4 µL of 70% perchloric acid. Then, 66 µL of 9.4 mM $Fe(ClO_4)_3$ solution was added to obtain an Fe concentration of 302 µM. The molar extinction coefficient (ε) of the DFOB-Fe(III) complex was the same in the extraction solutions as in 0.005 M $CaCl_2$ and was 2650 ± 50 L mol^{-1} cm^{-1}.

2.3 Pyoverdine

Pseudomonas fluorescens (ATCC 13525) was used to produce Pvd using the procedure described in Cornu et al. (2019). Briefly, bacteria were grown for seven days at 25 °C under shaking (200 rpm) in a Dworking & Foster (DF) medium with no iron, in fedbatch mode. After seven days, the culture medium was centrifuged (7,650 *g*) and the Pvd-containing supernatant was filtered and adjusted to pH 6 before being purified in a two-step procedure. First, the Pvd-containing supernatant was put in contact with a hydrophobic polyaromatic resin (Amberlite® XAD-4) for 24 h at 4 °C. After each contact (n= 4), the resin was recovered by filtration and the Pvd was eluted from the resin with 100% methanol and concentrated by evaporation. The Pvd concentrate was then loaded onto a C18 column (Lichroprep® RP-18), eluted with methanol/H_2O 70/30 (v/v) and concentrated by evaporation prior to storage at -20 °C. As detailed in Cornu et al. (2019), three major Pvd isoforms were identified by HILIC-ESI-MS in purified Pvd, with a molecular weight of 1,159.52, 1,160.50 and 1,189.52 Da. The semi-structural formulae of the three isoforms are shown in suppl. Fig. F1. For Exp. 3, ^{15}N-labeled Pvd was produced by growing bacteria in a DF medium in which the nitrogen source was changed to ^{15}N-labeled ammonium sulfate (98 atom % ^{15}N, Merck). Delta ^{15}N analyses (EA-IRMS, see section 2.6) showed that the vast majority of the nitrogen atoms of the ^{15}N-labeled Pvd were ^{15}N. Pvd stock solution (1 mM) was prepared 24 h prior to use by dissolving lyophilized Pvd in ultra-pure water. The concentration of Pvd in the soil extraction solution (see section 2.5) was assessed by measuring the difference in absorbance at 380 nm (Helios Epsilon, Thermo Spectronic) between the Pvd-treated and the control in each soil (for more details, see Cornu et al., 2019). At 380 nm, Pvd absorbance is not sensitive to changes in pH (Moll et al., 2008) or to changes in the metals with which it is associated (Braud et al., 2010).

2.4 Experimental design

Disks of dry soil (5 g, 3 mm thick, 32 mm in diameter) were placed in plastic lids, rewetted to 80% WHC and equilibrated for two weeks at 20 °C before being used for the assay. This delay avoids working during the microbial flush that usually follows soil rewetting and may strongly influence the dynamics of metals in soils (Cornu et al., 2007). In Exp. 1, six disks were prepared for each soil (n= 14). Three soil disks were supplied with desferrioxamine B (DFOB) and the other three with ultra-pure water (control), and equilibrated for one day at 20 °C before extraction. In Exp. 2, nine disks were prepared for each soil Cu concentration (n= 4). Three soil disks were supplied with DFOB, three others with pyoverdine (Pvd) and the last three with ultra-pure water (control), and equilibrated for one day at 20 °C before extraction. In Exp. 3, 27 disks were prepared for each soil (n= 2). Nine soil disks were supplied with DFOB, nine others with Pvd and the last nine with ultra-pure water (control), and equilibrated for one, eight, or 22 days at 20 °C before extraction. In all three experiments, DFOB and Pvd were supplied at a concentration of 200 µmol kg^{-1} soil DW by adding 1 mL from a 1 mM stock solution to the surface of soil disks.

2.5 Soil extraction and analyses of the extraction solution

Soil extraction was performed as follows: 5 g of wet soil were shaken with 9 mL of 0.005 M $CaCl_2$. All the suspensions were shaken for 2 h at 20 °C, centrifuged at 5,000 g for 10 min and the supernatant was filtered through 0.2 mm cellulose acetate filters (Cornu et al., 2014; 2019). After measurement of the pH and the concentration of DFOB and/or Pvd (see below), 10 mL of the extraction solution were acidified with 2% HNO_3 (v/v) and stored at 4 °C until further analysis. In Exp. 3, a 2 mL subsample of the extraction solution was stored non-acidified for A^{254}, DOC, total N and delta ^{15}N analyses. The pH and the concentrations of DFOB and Pvd were measured straight after soil extraction. pH was measured using a combined microelectrode (E16M331, Radiometer Analytical); the concentrations of DFOB and Pvd were measured using the protocols described in section 2.2 and 2.3, respectively. Total concentrations of Al, Fe, Mn, Cu, As, Ni, Co, Cr and V were determined by ICP-MS (7700x, Agilent Technologies) at the central analytical facility of the University of the Basque Country (UPV/EHU, Bilbao, Spain) from a subsample acidified with 2% HNO_3. The absorbance of dissolved organic matter at 254 nm (A^{254}) was measured by UV–VIS spectrometry (Cary 1 Bio, Varian, quartz cells) with a path length of 1 cm. The total concentration of N and the ratio of N isotopes (expressed as delta ^{15}N) were determined at the *PLATeau d'Isotopie de Normandie* (PLATIN', Caen, France). Tin capsules filled with 15 mg of Chromosorb® (inert powder) were used to encapsulate 100 µL of extraction solution. The capsules were placed in an oven at 40 °C for 12 h to dry the sample. The capsules were sealed with tweezers before the sample was analyzed by an elemental analyzer (EA3000, EuroVector) linked to a continuous flow isotope mass spectrometer (IRMS, Isoprime, GV Instruments).

2.6 Geochemical modeling of the extraction solution

Metal speciation in the extraction solution in the presence of Pvd and dissolved organic matter (DOM) was predicted using ORCHESTRA software, version 2021 (Meeussen 2003). Input data for the model were pH, Pvd, DOM, anions (NO_3^-, SO_4^{2-}, PO_4^{3-}, Cl^-) and total dissolved Ca, Fe, Al, Cu, and Ni. The concentrations of NO_3^-, SO_4^{2-} and PO_4^{3-} (fixed at 0.88, 0.1 and 0.02 mM, respectively) were assessed based on the literature, and the concentration of Cl^- was adjusted to equilibrate the charge balance between anions and cations. Thermodynamic constants for Pvd complexation to H^+, Fe^{3+}, Al^{3+}, Cu^{2+}, Ni^{2+} and Ca^{2+} were taken from the literature (Albrecht-Gary et al., 1994; Cornu et al., 2014; Ferret et al., 2015) or estimated (Suppl. Table T1 lists the stability constants used for Pvd-Al and Pvd-Ca) and were added to the default MinteqV4 database. For the calculation of speciation, DOM was described as fulvic acid, and metal binding to DOM was described using the NICA-Donnan model using the corresponding generic parameters (Kinniburgh et al., 1999; Milne et al., 2003; Koopal et al., 2005). The concentration of fulvic acid in the extraction solution was assessed from A^{254} values using the equation described in Amery et al. (2008), and considering a specific UV-absorbance at 254 nm of standard fulvic acid ($SUVA_{FA}$) equal to 55 L g^{-1} cm^{-1} (Benedetti et al., 2002). Activity coefficients were calculated using the Davies equation. Equilibrium reactions are described taking the equilibrium with atmospheric CO_2 into account.

2.7 Statistical analyses

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

3. Results and Discussion

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

Experiment 1 revealed that adding DFOB increased the mobility of Fe and Al in a wide range of soils and over a wide range of soil pH ($5.9 < pH_{water} < 8.6$). The concentrations of total Fe (Fig. 1a) and total Al (Fig. 1b) in the $CaCl_2$ extract were always higher in soils supplied with DFOB than in control soils. The DFOB-promoted mobilization of Fe and Al was observed in both carbonate (F-M) and non-carbonate (A-E, N) soils. This is a crucial point since siderophores are produced only when Fe availability is low (Visca et al., 2007), which is usually the case in soil at alkaline pH. Experiment 1 also showed that Fe and Al were the only two metals (among the eight monitored + As) whose mobility in soil was affected by the addition of DFOB. This finding was evidenced by two-way ANOVA (suppl. Table T2), which showed that the effect of DFOB was highly significant ($p < 0.001$) for the concentrations of Fe and Al whereas it was not ($p > 0.05$) for the concentrations of Mn, Cu, Ni, As, Co, Cr and V, in the $CaCl_2$ extract. Notably, the addition of DFOB did not increase Cu mobility in soil. The concentration of total Cu (Fig. 1c) in the $CaCl_2$ extract was on average the same in DFOB-treated and in control soils, in 12 of the 14 Cu-contaminated soils tested in the present study. In this respect, DFOB contrasts with Pvd, which has been shown to bind and mobilize Cu in series of Cu-contaminated soils (Tansupo et al., 2008; Cornu et al., 2019) (see sections 3.2 and 3.3 for more details on this comparison). The targeted effect of DFOB on Fe and Al is in agreement with the results of previous studies that investigated the effect of DFOB on the mobility of metals in soil (Neubauer et al., 2000; Zhong et al., 2013; Cornu et al., 2017). The high affinity of DFOB for Fe(III) ($\log \beta = 31.9$) and Al(III) ($\log \beta = 25.5$) may explain why DFOB failed to affect the mobility of divalent metals like Cu(II) in Fe-rich and Al-rich matrices such as soil. However, it does not explain why DFOB failed to increase the mobility of Mn(III) for which DFOB exhibits a higher affinity ($\log \beta = 29.9$) than for Al(III). One possible explanation is that Mn(III) was less available for complexation than Fe(III) and Al(III) in these soils due to the low concentration of Mn-bearing phases compared to Fe- and Al-bearing phases

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

Figure 2a shows the relationship between the amount of DFOB in the $CaCl_2$ extract and the amount of Fe+Al released from the solid phase in DFOB-treated soils. The slope equal to one (1.01) and the non-significant intercept of the regression line show that the two variables follow a 1:1 relationship. This 1:1 relationship suggests that Fe and Al were predominantly mobilized by ligand-controlled dissolution (also called complexolysis) and as 1:1 M-DFOB complexes in DFOB-treated soils. It recalls the 1:1: relationship observed for Pvd in almost the same series of soil (Cornu et al., 2019) and confirms that the siderophore-promoted mobilization of metals in soil primarily relies on their complexation by the siderophore (either in pore water or at the surface of metal-bearing phases). For Fe, this result is in line with the results of batch experiments showing that Fe oxyhydroxydes dissolve exclusively via a ligand-controlled mechanism in presence of siderophores (Kraemer, 2004). The 1:1 stoichiometry between the amount of DFOB in the $CaCl_2$ extract and the amount of Fe+Al released from the solid phase was observed even in soils A, B and N, in which the addition of DFOB slightly lowered the pH of the $CaCl_2$ extract (suppl. Fig. F2). This suggests that, in these soils, the amount of Fe+Al released by proton-controlled dissolution (also called acidolysis), if any, was negligible compared to the amount released by ligand-controlled dissolution in presence of DFOB.

In ligand-controlled dissolution, the pool of metallophores irreversibly sorbed onto the solid phase represents a loss of metallophores from the solution that reduces their effect on metal mobilization (Kraemer, 2004). Hence, the extent to which siderophores increase the mobility of metals in soil closely depends on their partitioning between the solid and the liquid phase. The amount of DFOB measured in the $CaCl_2$ extract was used to calculate the solid-liquid partitioning coefficient (K_d) of DFOB by difference with the amount of DFOB supplied. The K_d value of DFOB ranged from 1.8 to 24 L kg^{-1} among soils, which means that between 47% to 92% of the DFOB supplied was bound to the solid phase after 24 h. The strong affinity of DFOB for soil constituents underlines the fact that siderophores can strongly adsorb to soil colloids and particles (Ahmed and Holmstrom, 2014; Harrington et al., 2015; Rai et al., 2020). As detailed in Rai et al. (2020), both DFOB and DFOB-metal complexes may (i) specifically adsorb to metal oxyhydroxide surfaces by forming covalent bonds between hydroxamic acid moieties and surface cations, (ii) adsorb to negatively charged surfaces by electrostatic bonds since they are cationic species at $pH < 8$, and (iii) be associated with solid organic matter by hydrophobic interactions. As expected, the relationship between the K_d of DFOB and the amount of metals mobilized by DFOB was close and negative (suppl. Fig. F3). The linear regression coefficients obtained between the K_d of DFOB and the physico-chemical characteristics of soils are shown in suppl. Table T3. The fact that the K_d of DFOB was closely and positively correlated with the clay content ($r = 0.77$, $p = 0.001$) and to the CEC ($r = 0.79$, $p = 0.001$) suggests that DFOB was mainly bound to the fine soil fraction and through ion-exchange mechanisms. Therefore, DFOB is hypothesized to most affect the mobility of Fe and Al in soils with a very coarse texture like soils A and N. The negative relationship between the clay content and the amount of metals mobilized by DFOB ($r = -0.73$, $p = 0.003$) supports this hypothesis and showed that adding DFOB (at 200 µmol kg^{-1}) to a soil containing 5% clay (soil A) led to the mobilization of 13 times more metals (Fe+Al) than in a soil containing 23% clay (soil H).

DFOB speciation in solution was calculated for each soil from the difference in Al and Fe concentrations measured in the $CaCl_2$ extract in the DFOB treatment and in the control. Figure 2b shows that DFOB was mainly associated with Fe in soils A, B, C, E, H, M, N and with Al in soil L, while in the six other soils, DFOB was associated with the two metals in similar proportions. This suggests rather selective DFOB-promoted dissolution of Fe-bearing phases in soils A, B, C, E, H, M, N and of Al-bearing phases in soil L. One possible hypothesis is that DFOB associated with either Fe or Al in the $CaCl_2$ extract, depending on which was more accessible for complexation. Under this hypothesis, Al was more accessible for complexation than Fe in soil L, which may be consistent with the high concentration of Al oxide in this soil (Table 1). However, the absence of a clear relationship between the ratio of Fe oxide to Al oxide in soil and the ratio of DFOB-Fe to DFOB-Al in the $CaCl_2$ extract (suppl. Fig. F4) suggests that other factors than the mineral composition of the soil determine the extent to which a metal-bearing phase is prone to DFOB-promoted dissolution. As reviewed in Kraemer et al. (2015), the rate of ligand-controlled dissolution is influenced by environmental factors that may change the speciation of the ligand (in this case DFOB) in pore water and at the surface of metal-bearing phases, including pH, metal ion surface coverage (see section 3.2 below), and surfactant adsorption, as well as the presence of other organic molecules such as humic substances or low molecular weight organic acids (LMWOA).

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

Experiment 2 showed how the addition of DFOB and Pvd affected the mobility of metals in soil L, whose Cu concentration was artificially increased by spiking. Soil L was chosen because it is a calcareous soil in which symptoms of Cu-induced Fe deficiency (interveinal chlorosis) were observed in durum wheat (Michaud et al., 2007). It is therefore a soil in which the link between the level of Cu contamination and Fe dynamics needed to be examined in more detail.

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

Figure 3 shows that the amount of DFOB and to a lesser extent that of Pvd in the $CaCl_2$ extract gradually decreased with an increase in the concentration of Cu in the soil. The amount of DFOB in the $CaCl_2$ extract decreased from 118 nmoles at Cu100 to 30 nmoles at Cu1600 (Fig. 3a), which means that the K_d of DFOB increased from 15 to 64 L kg^{-1} when the concentration of Cu in soil increased from 100 to 1600 mg kg^{-1}. Likewise, the K_d of Pvd increased from 88 to 121 L kg^{-1} when the concentration of Cu in soil increased from 100 to 1600 mg kg^{-1}. By which process(es) can the accumulation of Cu in soil promote the adsorption of DFOB and Pvd at the surface of soil constituents? The most likely hypothesis is that the process is the same for the two siderophores and that Cu^{2+} ions serve as cation bridges linking negative sites on soil constituents and an anionic or polar functional group on siderophores. The hypothesis is based on the fact that at pH above 8, which is the case of soil L (Table 1), DFOB and Pvd (as well as their metal-complexes) are neutral and negatively-charged species, respectively, and most metal-bearing phases (including metal oxyhydroxides) have a negative surface charge. In addition, the high solubility of both DFOB and Pvd in water as well as their large polar surface area (206 Å and 636 Å, respectively) suggest that they both contain polar functional groups. Bridging with multivalent cations has been proposed as a possible adsorption mechanism for siderophores to humic substances (Harrington et al., 2015; Boiteau et al., 2020) as well as for pesticides such as glyphosate on the surface of oxyhydroxides (Kah and Brown, 2006). The main cations involved in the formation of bridges in alkaline soils are Ca^{2+} and Mg^{2+}. The accumulation of Cu^{2+} ions at the surface of (at least some) soil constituents caused by spiking the soil with $CuSO_4$ was assumed to be sufficiently high in treatments Cu350, Cu850 and Cu1600 to compete with Ca^{2+} and Mg^{2+} and thereby to increase the adsorption of DFOB and Pvd.

Figure 3c shows that the amount of metals (Fe+Al) mobilized by DFOB gradually decreased with an increase in the concentration of Cu in soil. The Cu-induced decrease in the amount of Fe+Al released was of the same magnitude as that of DFOB measured in the $CaCl_2$ extract (i.e. around 70% loss between Cu100 and Cu1600). In other words, the 1:1 linear relationship between the amount of DFOB in the $CaCl_2$ extract and the amount of Fe+Al mobilized from the solid phase was conserved regardless of the concentration of Cu in the soil (suppl. Fig. F5). This suggests that the process of ligand-controlled dissolution by which DFOB mobilizes Fe and Al in soil is not affected by the level of soil Cu contamination. This may be because DFOB is unable to complex Cu in soil, as shown in Exp. 1. Unlike DFOB, the amount of metals (Fe+Al+Cu) mobilized by Pvd gradually increased with an increase in the concentration of Cu in the soil (Fig. 3d). The amount of metals mobilized by Pvd was 2.6-times higher at Cu1600 than at Cu100, although the amount of Pvd in the $CaCl_2$ extract was 36% lower at Cu1600 than at Cu100 (cf. above). This shows that the amount of metal mobilized in presence of Pvd exceeded the amount of Pvd monitored in the $CaCl_2$ extract at Cu300, Cu850 and Cu1600, and suggests that the process by which Pvd mobilized metals in soil L gradually shifted from ligand-controlled dissolution with an increase in the soil Cu concentration.

Copper is the metal whose mobilization by Pvd was affected most by the increase in the soil Cu concentration. While Cu accounted for 74% of the metals mobilized by Pvd at Cu100, it accounted for almost 90% of the metals mobilized by Pvd at Cu1600. Although there was a one-year delay after the soil was spiked, the adsorption of “fresh” Cu onto the solid phase was assumed to involve low-energy bonds that were more inclined to break in presence of Pvd than the high-energy bonds involved in the retention of “aged” Cu. Therefore, the mechanisms used by Pvd to mobilize “fresh” Cu and “aged” Cu are almost certainly not the same. The fact that the amount of Cu mobilized exceeded the amount of Pvd in the $CaCl_2$ extract at Cu300, Cu850, and Cu1600, suggests that the mobilization mechanism used for “fresh” Cu enabled the Pvd involved to be at least partially recycled. According to Akafia et al. (2014), among the mechanisms involved in ligand-promoted mobilization, only reductive dissolution allows ligand recycling. Therefore, the Pvd-induced mobilization of “fresh” Cu was assumed to rely on a reductive dissolution pathway even though no specific measurements, for instance of the electrode potential of the mineral (Bi et al., 2010), were made in this experiment to confirm it. In reductive dissolution, the ligand acts as a reductant whose adsorption at the mineral surface induces its dissolution via the reduction of the metal ion center (Kraemer, 2004). In this scenario, Pvd probably acts as a reductant able to reduce Cu(II) to Cu(I), but this remains to be demonstrated.

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

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

following their supply

Experiment 3 showed the extent to which the ability of DFOB and Pvd to mobilize metals in soil persists over time, by comparing their effect 1, 8 and 22 days after their addition. Soil K was chosen because it is an acidic sandy soil typical of the gravel soils of the Bordeaux area where symptoms of Cu toxicity were observed on grapevines in the 1960s (Delas 1963), and soil L for the same reasons as those given above for Exp. 2. Figure 4 first shows that DFOB was no longer detected in the $CaCl_2$ extract after eight and 22 days in the two soils (Fig. 4a, b). Likewise, no further increase in the amount of Fe and Al in the $CaCl_2$ extract was observed after eight and 22 days in presence of DFOB, regardless of the soil (Fig. 4c, d; suppl. Table T5). This result suggests that the ability of DFOB to mobilize metals in soil is likely to end within eight days after its release by (e.g. *Streptomyces*) bacteria. In a previous work (Cornu et al., 2017), a decrease in the concentration of DFOB in pore water over time following its addition was already observed, although the decrease was much more gradual.

The disappearance of DFOB in the $CaCl_2$ extract observed between day 1 and day 8 may be the result of DFOB adsorption onto soil constituents and/or of DFOB degradation by microorganisms. A study by Zhong et al. (2013) showed that the adsorption of DFOB onto the solid phase was complete within the first 4 h in an Oxisol. However, DFOB is not only adsorbed by electrostatic bonds but also by forming covalent bonds, which may require longer, perhaps more than 24 h in some conditions (e.g. soil, temperature). In addition, several rhizosphere soil bacteria have been found to degrade ferrioxamine-type siderophores such as DFOB, including members of the genus *Azospirillum* (Winkelmann et al., 1999), and to use DFOB as a carbon source (Castignetti and Siddiqui, 1984). However, the rate of microbial degradation of DFOB in soil has not been sufficiently documented to know if all the DFOB present in the

$CaCl_2$ extract could have been microbially degraded in eight days. As reported for phytosiderophores (Römheld, 1991), the rate of DFOB degradation in soil presumably depends on a combination of environmental factors, including the size and the composition of the microbial community.

Figure 4 shows that the amount of Pvd in the $CaCl_2$ extract also decreased over time in the two soils, but more gradually than DFOB (Fig. 4 a, b). The amount of Pvd in the $CaCl_2$ extract was reduced by, on average, 57% after eight days and by 82% after 22 days in soil K, and by 24% after eight days and by 44% after 22 days in soil L, compared to the amount measured on day 1 (suppl. Table T5). Like for DFOB, the progressive decrease in the amount of Pvd in the $CaCl_2$ extract could result from Pvd adsorption (like DFOB, Pvd is not only adsorbed onto soil constituents by electrostatic bonds) and/or from Pvd degradation (Parker et al., 2007). ^{15}N-Pvd was used to help identify which of the two processes is responsible for the progressive decrease in the concentration of Pvd in the $CaCl_2$ extract. Under the Pvd adsorption hypothesis, the delta ^{15}N and the concentration of total N in the $CaCl_2$ extract decrease over time together with the Pvd concentration, while under the Pvd degradation hypothesis, they remain constant (provided the Pvd degradation metabolites do not adsorb onto soil constituents). Figure 5 shows that the concentration of delta ^{15}N and of total N in the $CaCl_2$ extract increased over the incubation period in the two soils, either transitorily in soil K (Fig. 5a) or continuously in soil L (Fig. 5b). The theoretical concentration of Pvd in the $CaCl_2$ extract was calculated from the delta ^{15}N and the concentration of total N in the extraction solution assuming Pvd did not degrade over time (for details, see supplementary data). The good agreement between the calculated and the experimentally measured concentration of Pvd observed on day 1 (Fig. 5c, d) suggests that Pvd did not degrade in either soil during the first 24 h following its addition. In soil

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

In soil K, the transitory increase in the delta ^{15}N and in the concentration of total N in the $CaCl_2$ extract between day 1 and day 8 (Fig. 5a), as well as the increase in the resulting calculated Pvd concentration (Fig. 5c), was also attributed to the degradation of adsorbed Pvd, whereas their marked decrease between day 8 and day 22 was attributed to volatilization of ^{15}N in the $CaCl_2$ extract by denitrification. Indeed, anaerobic conditions probably developed in the soil K between day 8 and day 22 when the addition on day 0 of 1 mL of solution (either Pvd, DFOB or ultra-pure water) increased the soil water content almost to saturation. As reported previously (Cornu et al., 2007), anaerobic conditions can develop in a few days after rewetting in water-saturated soils. In the present study, anaerobic conditions would have developed selectively in soil K presumably because of its low WHC resulting from its sandy texture (the soil contained 87% sand), which makes it more sensitive to water saturation than the heavier textured soil L (that contained only 31% sand).

Figure 4 also shows that the amount of Fe+Al+Cu mobilized by Pvd decreased over time at almost the same magnitude as Pvd, especially in soil K (Fig. 4c). In other words, the 1:1 linear relationship between the amount of Pvd in the $CaCl_2$ extract and the amount of Fe+Al+Cu mobilized from the solid phase was conserved over the 22-day incubation period in the two soils (suppl. Fig. F6). This suggests that the main process by which Pvd mobilized Fe, Al and Cu in soil did not change over time and remained ligand-controlled dissolution. In contrast, the relative proportions in which Pvd mobilized Fe, Al and Cu did change over time in the two soils (Fig. 4c, d). For example, in soil K, Cu accounted for 21% of the metals mobilized by Pvd on day 1 but this fraction gradually increased over time to reach 84% on day 22. As a result, the amount of Cu mobilized by Pvd decreased by less than 20% in this soil between the day 1 and day 22, while that of Fe and Al decreased by a factor of 18 and 32, respectively. Why did the capacity of Pvd to mobilize Fe and Al decrease over time in favor of its capacity to mobilize Cu? One possible hypothesis is that the processes thought to be responsible for the progressive reduction in the concentration of Pvd in the $CaCl_2$ extract (see above) are sensitive to the metal with which Pvd is associated in solution. In other words, the Pvd-Cu(II) complex is likely relatively less (or more slowly) adsorbed onto soil constituents and/or degraded by microorganisms than the Pvd-Fe(III) and Pvd-Al(III) complexes. The difference in the fate of Pvd-Cu(II) and Pvd-Fe(III) over time can be explained by biotic process(es). Indeed, one can reasonably assume that Pvd-Cu(II) is more resistant to microbial degradation than Pvd-Fe(III), for which soil microorganisms (either bacteria or fungi) have developed a specific enzymatic machinery to recover iron. In contrast, the difference in the fate of Pvd-Cu(II) and Pvd-Al(III) over time is difficult to explain by biotic process(es) and could instead result from the adsorption of Pvd-Al(III) onto soil constituents in preference to Pvd-Cu(II), for reasons that may derive from the “hard and soft (Lewis) acids and bases” (HSAB) theory (Harter and Naidu, 1995), notably the fact that Al^{3+} belongs to the hard Lewis acids while Cu^{2+} belongs to borderline acids.

Taken together, the results of Exp. 3 suggest that the speciation of Pvd in the $CaCl_2$ extract changed over time in the two soils. Geochemical modeling was performed to confirm this hypothesis using the characteristics of the $CaCl_2$ extract listed in section

2.6. As shown in suppl. Table T6, there was good agreement between the Pvd speciation calculated using the relative proportions of metals mobilized in the $CaCl_2$ extract (cf. Fig. 4) and the Pvd speciation assessed by geochemical modeling. Thereby, the geochemical approach confirmed that the fraction of Pvd associated with Cu in the $CaCl_2$ extract increased over time in the two soils to the detriment of the fraction of Pvd associated with Fe and Al. In addition, the geochemical approach suggests that (i) Pvd was not associated with Ni and almost not associated with Ca, (ii) Al and Fe were almost only associated with Pvd on day 1 and with fulvic acids on day 22, and (iii) Cu remained predominantly associated with Pvd in the $CaCl_2$ extract over time (suppl. Table T6). Fluorescence measurements were also performed on the $CaCl_2$ extract to determine the concentration and the speciation of Pvd (for details on the protocol, see suppl. data). These measurements confirmed that Pvd fluorescence properties are distinct from those of the humic-like substances usually present in soil extracts, as previously underlined by Potysz et al. (2016), and also showed that Pvd fluorescence decreased over time in the two soils (suppl. Fig. F7 and F8). However, it was impossible to quantify the concentration of Pvd or to experimentally characterize its speciation in the $CaCl_2$ extract because Pvd fluorescence properties depend on its concentration and on the metal with which it is associated (Braud et al., 2010), and both parameters varied simultaneously over time in the two soils.

4. Conclusions

The three experiments conducted in this study provide new insights into the metal mobilization ability of siderophores in soil that determines the conditions in which siderophore-producing bacteria can improve the remediation of Cu-contaminated soils through phytoextraction. First, this study highlighted the fact that the panel of metals mobilized by Pvd is larger than the panel mobilized by DFOB. Only Pvd mobilized Cu, thereby supporting the idea of using Pvd-producing bacteria (or more generally bacteria that produce siderophores with a catecholate moiety) to enhance Cu phytoextraction.

Second, this study showed that DFOB and Pvd mainly mobilized metals through ligand-controlled dissolution. In the case of DFOB, it even appears to be the only process by which Fe and Al are mobilized. In ligand-controlled dissolution, the efficiency of ligands in mobilizing metals depends on the rate at which they adsorb onto the surface of metal-bearing phases, and on the rate at which the metal-ligand complex (once formed) detaches from the surface and dissolves in pore water. This second step seems to be the limiting step in the DFOB- and Pvd-promoted mobilization of metals, as suggested by the large fraction of the two siderophores bound to the solid phase after 24 h. The addition prior to siderophores of low molecular mass organic acids (LMMOA) such as oxalate may increase the rate of siderophore-promoted mobilization of metals. The role of the LMMOA is to adsorb and form labile metal species at the surface of metal-bearing phases, while that of the siderophore is to remove metal from LMMOA-metal complexes leaving the uncomplexed LMMOA ligand free to react again. Investigations are currently underway to help determine if the production/supply of LMMOA is a driver that should be used for the optimization of Cu phytoextraction.

Third, this study suggests that Pvd can mobilize Cu by reductive dissolution in soil, but only “fresh” Cu supplied by spiking. This implies a direct link between the process by which Cu is bound to the soil constituents and the mechanism by which it is mobilized by (or in presence of) Pvd. It would be interesting to dissect this link because it likely affects the efficiency and the selectivity by which Pvd mobilizes Cu in Cu-contaminated soils. In addition, in reductive dissolution, the amount of metals mobilized usually exceeds the amount of ligands in soil pore water, which is not the case in ligand-controlled dissolution. Consequently, the mechanism used by Pvd to mobilize Cu in soils could affect the speciation of Cu in soil pore water (notably the ligands with which it is associated), and, hence, the efficiency with which Pvd increases Cu phytoavailability and Cu phytoextraction.

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

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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 $CaCl_2$ 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 $CaCl_2$ extract and the amount of Fe+Al mobilized from the solid phase (a) and DFOB speciation in 0.005 M $CaCl_2$ 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 $CaCl_2$ extract in the DFOB treatment, based on the relationships shown in (a).

Figure 3. Amount of siderophore (DFOB, Pvd) in 0.005 M $CaCl_2$ 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^{-1}) 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 $CaCl_2$ extract. In (d), the values in the light blue bar plots correspond to the percentage of Pvd associated with Cu in the $CaCl_2$ extract.

Figure 4. Temporal changes in the amount of siderophores (DFOB, Pvd) in the 0.005 M $CaCl_2$ 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 $CaCl_2$. 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 $CaCl_2$ extract.

Figure 5. Temporal changes in the total N concentration and in the delta ^{15}N in the 0.005 M $CaCl_2$ extract of the vineyard topsoils K (a) and L (b) supplied with the same concentration of ^{15}N-Pvd. The incubation time (1, 8 and 22 days) is the time between when ^{15}N-Pvd was supplied and soil extraction with 0.005 M $CaCl_2$. (c) and (d) compare the concentration of Pvd measured in the extraction solution and the theoretical concentration of Pvd calculated from the delta ^{15}N and the total N concentration in the extraction solution (assuming no degradation of Pvd over time) in soils K and L, respectively.

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Figure 1

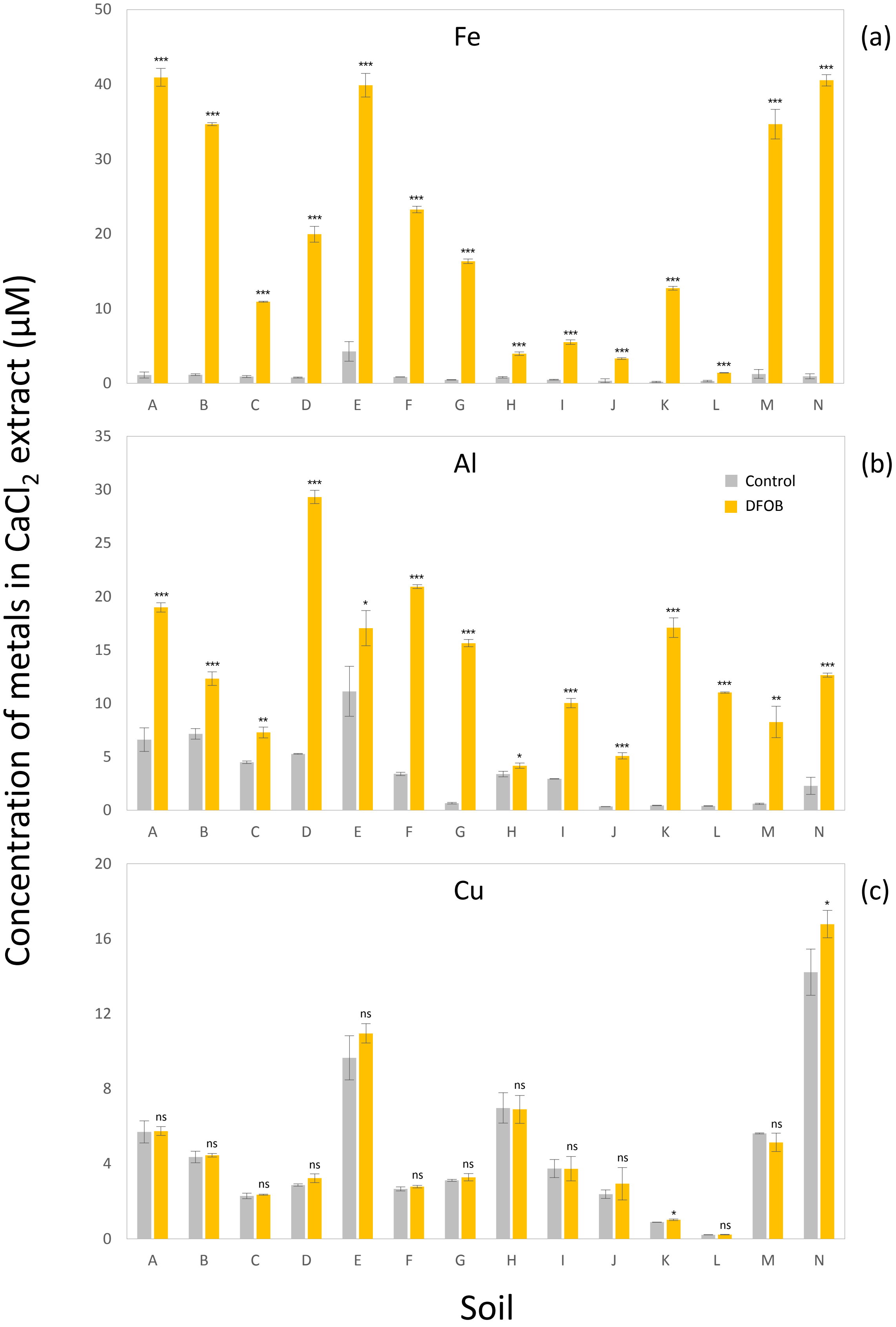
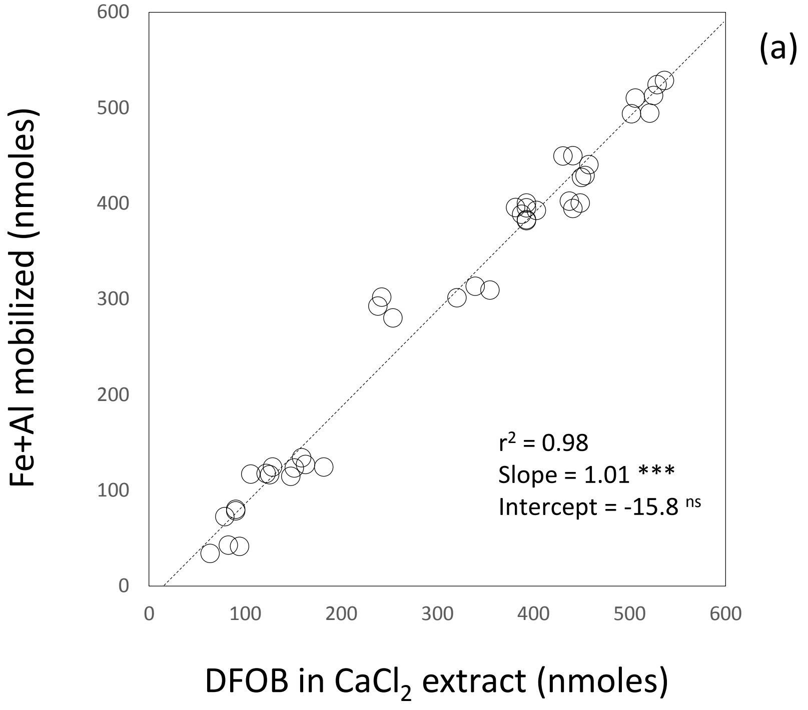
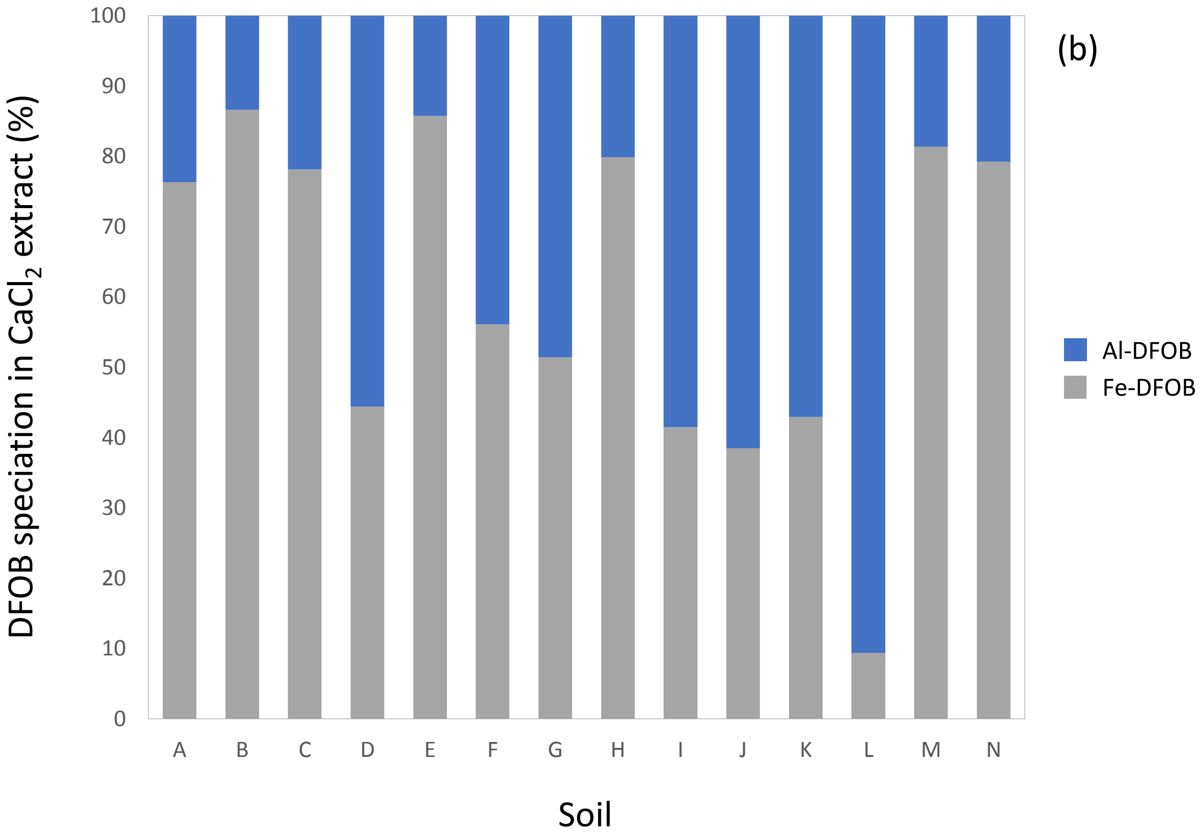


Figure 2



(a)
Fe+Al mobilized (nmoles)
600
500
400
300
200
100
0
0
100
200
300
400
500
600
DFOB in CaCl$_2$ extract (nmoles)
r^2 = 0.98
Slope = 1.01 ***
Intercept = -15.8 ^{ns}



(b)
DFOB speciation in CaCl$_2$ extract (%)
100
90
80
70
60
50
40
30
20
10
0
A
B
C
D
E
F
G
H
I
J
K
L
M
N
Soil
Al-DFOB
Fe-DFOB

Figure 3

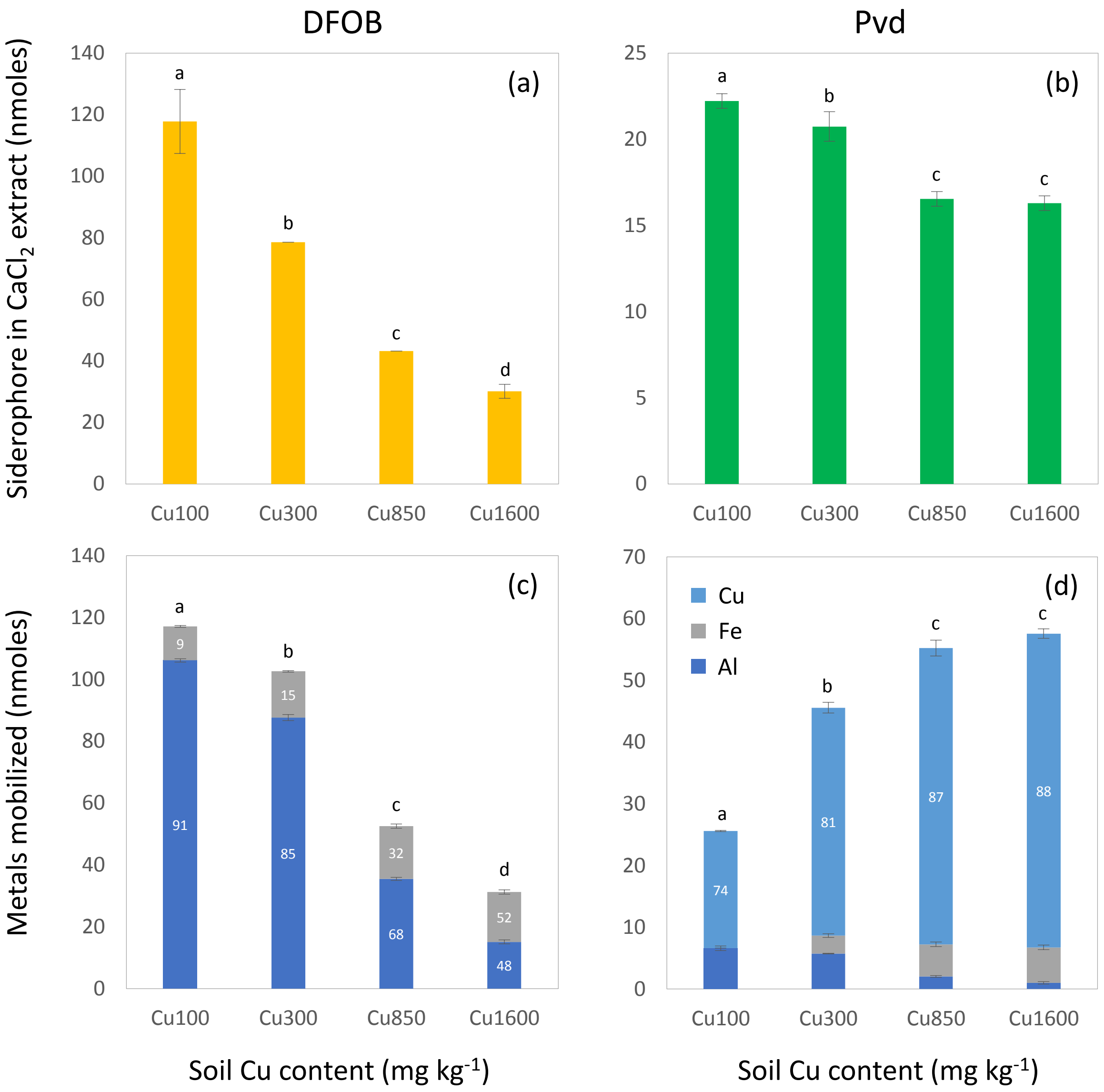

DFOB
Pvd
Siderophore in CaCl$_2$ extract (nmoles)
(a)
140
120
100
80
60
40
20
0
a
b
c
d
Cu100
Cu300
Cu850
Cu1600
(b)
25
20
15
10
5
0
a
b
c
c
Cu100
Cu300
Cu850
Cu1600
Metals mobilized (nmoles)
(c)
140
120
100
80
60
40
20
0
a
9
91
b
15
85
c
32
68
d
52
48
Cu100
Cu300
Cu850
Cu1600
Soil Cu content (mg kg^{-1})
(d)
70
60
50
40
30
20
10
0
Cu
Fe
Al
a
74
b
81
c
87
c
88
Cu100
Cu300
Cu850
Cu1600
Soil Cu content (mg kg^{-1})

Figure 4

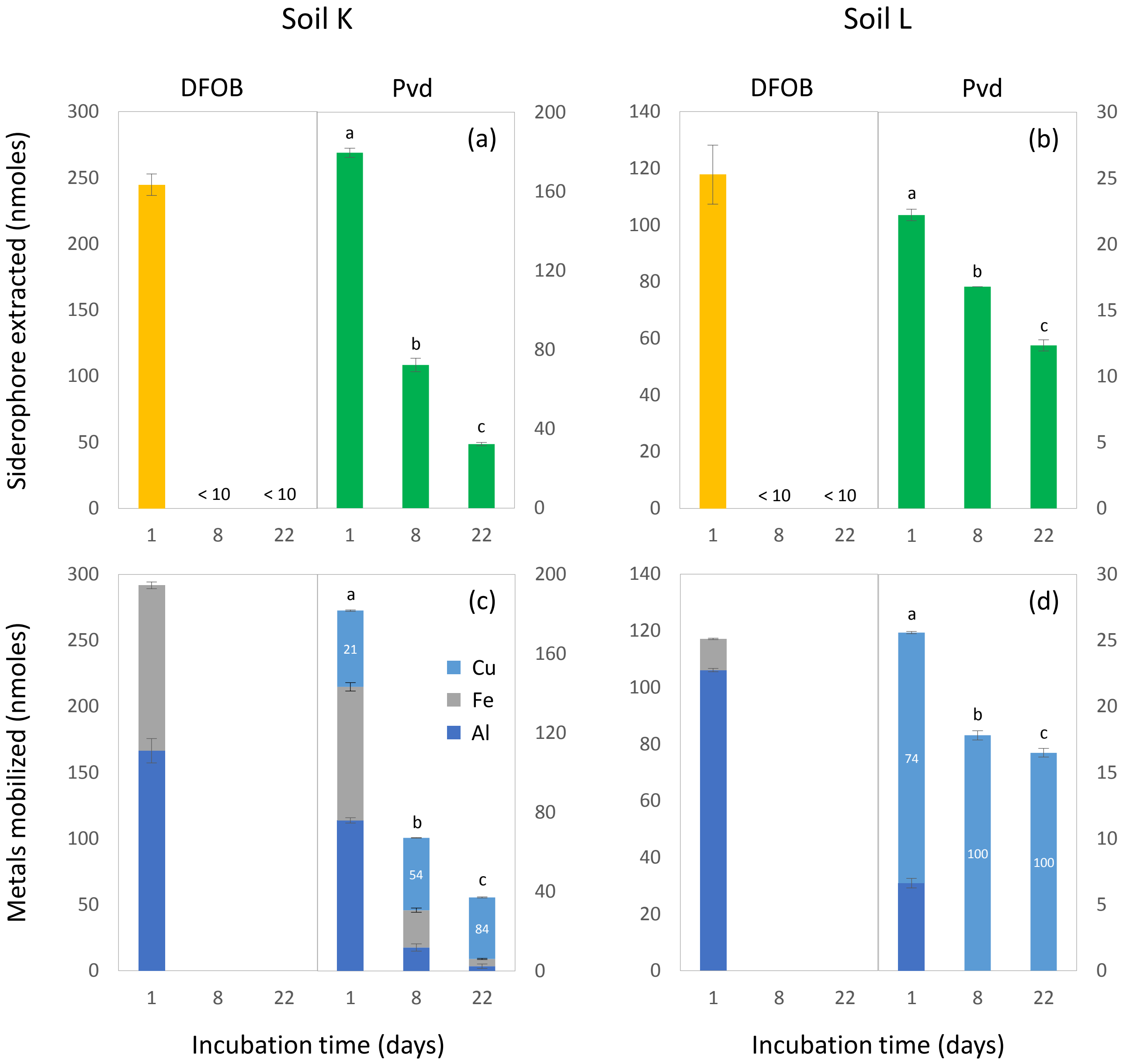
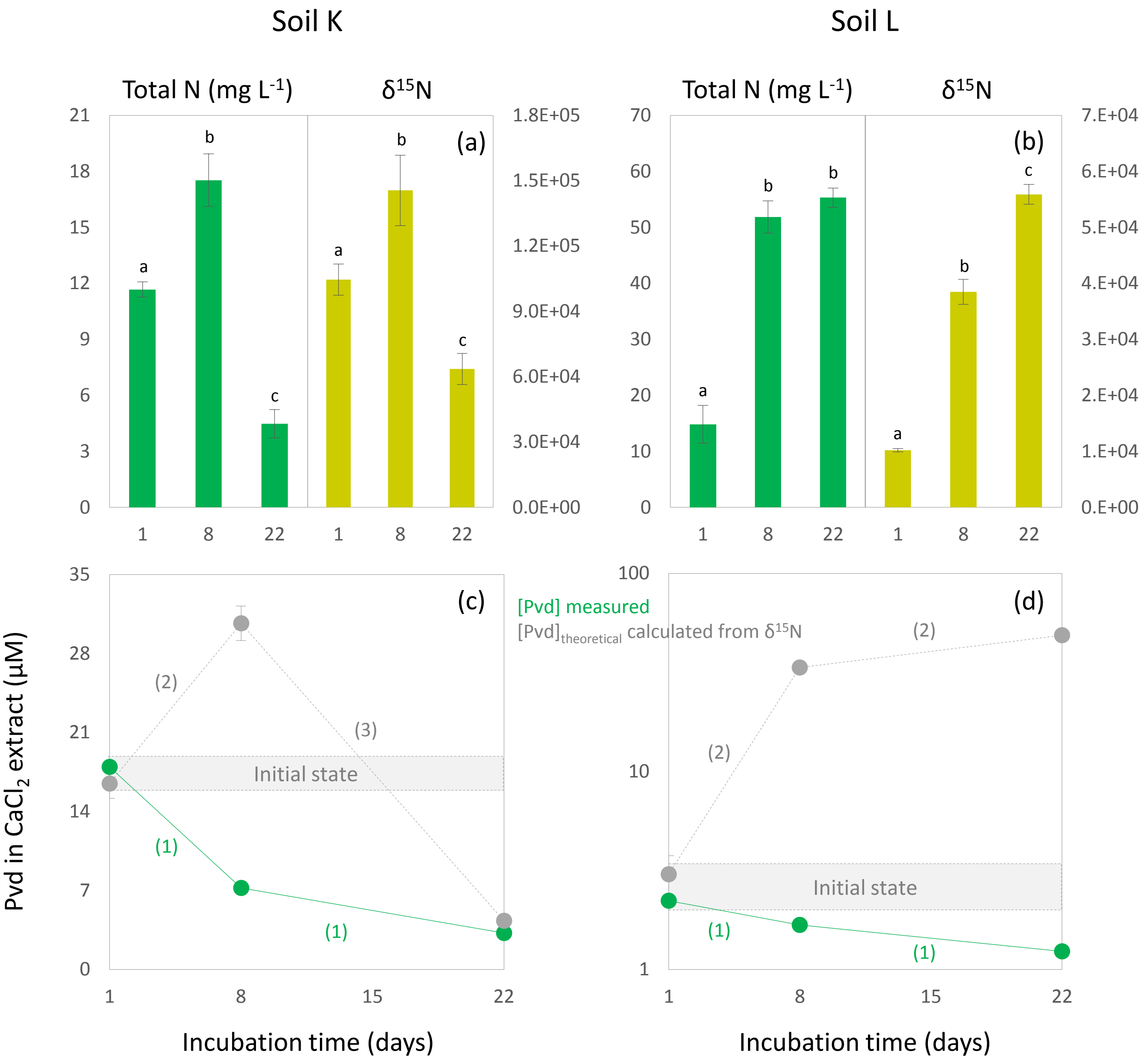

Soil K
Soil L
DFOB
Pvd
DFOB
Pvd
Siderophore extracted (nmoles)
Metals mobilized (nmoles)
300
250
200
150
100
50
0
200
160
120
80
40
0
140
120
100
80
60
40
20
0
30
25
20
15
10
5
0
(a)
(b)
(c)
(d)
a
b
c
< 10
< 10
< 10
< 10
1
8
22
Cu
Fe
Al
21
54
84
74
100
100
Incubation time (days)
Incubation time (days)

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

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

	Sand	Silt	Clay [a]	OM [b]	CEC [c]	$CaCO_3$ [d]	Al oxide	Fe oxide	Mn oxide [e]	pH $_{water}$ [f]	Total Cu [g]	Cu_{EDTA} [h]
	$g\ kg^{-1}$			%	$cmol_{c+}\ kg^{-1}$	$g\ kg^{-1}$	$g\ kg^{-1}$				$mg\ kg^{-1}$	
A	875	71	54	0.9	1.8	< 1	0.38	0.66	0.07	5.9	104	96
B	835	119	46	1.4	2.2	< 1	0.54	0.84	0.05	6.5	100	88
C	622	222	156	1.3	5.3	< 1	0.92	1.25	0.05	6.8	103	53
D	825	121	54	1.2	2.6	< 1	0.84	0.96	0.05	6.4	111	76
E	794	88	118	2.2	4.9	< 1	0.78	1.00	0.04	6.5	103	73
F	816	97	87	1.9	5.8	4.4	0.62	1.45	0.08	7.9	113	68
G	857	76	67	1.7	4.9	8.4	0.63	0.94	0.13	8.3	154	132
H	661	109	230	3.1	17.5	105	1.20	1.37	0.25	7.6	159	85
I	872	41	87	1.4	7.6	80	0.63	0.72	0.10	7.9	102	73
J	753	95	152	1.7	9.7	35	0.95	1.07	0.16	8.6	121	77
K	872	84	44	2.0	6.4	9	0.63	1.04	0.14	7.9	174	123
L	309	404	287	1.6	19.1	132	1.56	0.96	0.44	8.5	100	4
M	701	200	99	5.7	8.8	45	1.04	1.57	0.12	6.7	336	223
N	846	110	44	1.5	2.9	< 1	1.06	1.67	0.13	6.4	905	515

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