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## Do DOM optical parameters improve the prediction of copper availability in vineyard soils?

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## 21 **Abstract**

22 Accumulation of copper (Cu) in soils due to the application of fungicides may be toxic for  
23 organisms and hence affect winegrowing sustainability. Soil parameters such as pH and  
24 dissolved organic matter (DOM) are known to affect the availability of Cu. In this study, we  
25 investigated the contribution of chromophoric and fluorescent DOM properties to the prediction  
26 of Cu availability in 18 organic vineyard soils in the Bordeaux winegrowing area (France). The  
27 DOM parameters, assessed through absorbance and fluorescence analyses, and proxies for Cu  
28 availability (total soluble Cu and free ionic  $\text{Cu}^{2+}$ ) were measured in 0.01 M KCl extracts. Total  
29 soluble Cu ( $\text{Cu}_{\text{KCl}}$ ) varied 23-fold while free ionic  $\text{Cu}^{2+}$  varied by a factor of 4,600 among the  
30 soils. DOC concentrations were similar among the soils, but the samples differed in the quality  
31 of DOM as assessed by optical spectroscopy. Multilinear regression models with and without  
32 DOM quality parameters were investigated to predict Cu availability. The best model for  $\text{Cu}_{\text{KCl}}$   
33 successfully explained 83 % of variance and included pH,  $\text{Cu}_{\text{T}}$  and two DOM fluorescence  
34 quality indices, the FI fluorescence index, which distinguishes between microbial and higher  
35 plant origins, and the HIX humification index. For the prediction of  $\text{Cu}^{2+}$ , pH alone explained  
36 88 % of variance and adding DOM parameters did not improve modelling. The two Cu  
37 availability proxies were related to pH. This study confirms the prominent role of pH in Cu  
38 availability and underlines the importance of DOM quality to better predict Cu solubility.

39

## 40 **Keywords**

41 Copper, organic matter, fluorescence, absorbance, vineyard soils, availability, modelling.

## 42        **1. Introduction**

43        Copper-based fungicides like Bordeaux mixture have been repeatedly applied in vineyards  
44        since the end of the 19<sup>th</sup> century to control downy mildew of grapevine, sometimes causing Cu  
45        to accumulate in the soils (Brun et al. 2001; Ballabio et al. 2018). In European soils, the median  
46        of total Cu concentrations is 13 mg kg<sup>-1</sup>. In vineyards soils, this value reaches 49 and 91 mg kg<sup>-1</sup>  
47        <sup>1</sup> in Europe and France, respectively (Ballabio et al. 2018). In old vineyards, Cu concentrations  
48        in the topsoil can reach several hundred mg Cu kg<sup>-1</sup>, as widely reported in several European  
49        countries including France, Italy, Spain, Greece, Portugal, Czech Republic (Fernández-Calviño  
50        et al. 2009; Komárek et al. 2010; Fernández-Calviño et al. 2010; Flores-Álvarez et al. 2011;  
51        Kelepertzis et al. 2017). Despite the reduction of the authorized application rate to 4 kg Cu ha<sup>-1</sup>  
52        <sup>1</sup> year<sup>-1</sup> (EFSA et al. 2018), Cu-based fungicides continue to be used, particularly in organic  
53        viticulture for which there is currently no alternative that offers equally effective protection  
54        (Andrivon et al. 2018).

55        Cu is an essential micronutrient for all living organisms. Microorganisms need trace levels of  
56        Cu for their growth because it is a cofactor in enzymatic reactions (Trevors and Cotter 1990). In  
57        plants, copper plays key roles in cell wall metabolism and in photosynthetic electron transport  
58        (Yruela 2009). A Cu deficiency can affect vital functions in plant metabolism. However, excess  
59        copper can cause phytotoxicity through the formation of oxygen radicals which damage cells  
60        and inactivate protein structure, or by disturbing photosynthetic activity due to the  
61        photoinhibition induced by excessive Cu (Yruela 2005). In calcareous soils, excess Cu can also  
62        lead to Fe deficiency in graminaceous species such as durum wheat (Michaud et al. 2007) since  
63        Cu(II) has sufficient affinity for phytosiderophores to divert them from their initial objective of  
64        mobilising Fe(III). All these problems may affect plant growth and reduce agricultural  
65        productivity. Cu phytotoxicity has been reported in durum wheat grown in former vineyard  
66        soils with total Cu concentrations beginning at 114 mg kg<sup>-1</sup> (Michaud et al. 2007). Exposure to

67 Cu at soil concentrations above 16 mg Cu kg<sup>-1</sup> in the soil has also been shown to have a serious  
68 impact on earthworm *Eisenia fetida* reproduction by reducing the production of cocoons  
69 (Helling et al. 2000). Fernández-Calviño et al. (2010) reported that microbial enzymatic activity  
70 (phosphatase) in Portuguese vineyard soils decreased drastically above 250 mg Cu kg<sup>-1</sup>. A  
71 recent meta-analysis of Cu in vineyard soils showed that microbial respiration was reduced by  
72 half above a stock of 200 kg Cu ha<sup>-1</sup> (Karimi et al. 2021). Excess copper may thus cause  
73 ecotoxicity in vineyard soils, although the ecotoxic thresholds vary widely depending on certain  
74 soil parameters like organic matter (OM) content or pH. Total Cu inducing 50 % of biomass  
75 decrease (EC<sub>50</sub>) is a widely used indicator in ecotoxicity studies. It has been shown that EC<sub>50</sub>  
76 of lettuce and tomatoes increases with increasing OM content or decreasing pH (Recatalá et al.  
77 2012; Sacristán et al. 2016). Cu toxicity may therefore be linked not only to total Cu amounts  
78 but also to certain bioavailable forms of Cu in the soils.

79 The bioavailability of metals is defined as the fraction of its environmentally available  
80 compound an organism takes up through physiologically driven processes (ISO 2006).  
81 Environmental availability is defined as the availability of metals in the soil measured using  
82 chemical methods (Harmsen 2007). Sauvé et al. (1996) showed that Cu concentrations in the  
83 tissues of several plants were closely linked to Cu in solution, more specifically to free Cu<sup>2+</sup>  
84 activity. The free ion Cu<sup>2+</sup> is thought to be the bioavailable form, and this hypothesis is  
85 supported by other authors who reported that Cu toxicity for different organisms (algae,  
86 earthworms, zooplankton) in soils and aquatic systems depended strongly on free Cu<sup>2+</sup> activity  
87 (Verweij et al. 1992; Qiu et al. 2013; Tait et al. 2016).

88 The concentration of Cu in soil solutions and Cu<sup>2+</sup> activity are controlled by soil properties such  
89 as total Cu content, pH and soil organic matter (SOM) content (Sauvé et al. 1997; Sauvé et al.  
90 2000). pH is known to affect Cu solubility (Temminghoff et al. 1994), but also copper  
91 speciation in solution (Bravin et al. 2009). Cu biogeochemistry is controlled to a large extent

92 by its binding to organic matter, since SOM contains acidic functional groups (mainly phenolic  
93 and carboxylic groups) which have a particular and strong affinity for Cu (Manceau and  
94 Matynia 2010). Organic matter supplied to soils in different forms (fresh materials, compost,  
95 manure, sludge) has been shown to significantly reduce Cu availability in its soluble form  
96 (Navel and Martins 2014; Ferreira et al. 2018; Gonzaga et al. 2020) and its ionic form (Oustriere  
97 et al. 2016). Dissolved organic matter (DOM) plays a key role in the fate of metals in the soil.  
98 While SOM immobilises Cu through sorption onto solid phases (Sauvé et al. 2000), DOM  
99 increases Cu solubility by forming Cu complexes in soil solution. However, the free Cu fraction  
100 may also decrease due to complexation with DOM. Cu mobilisation from the solid phase into  
101 solution has been reported to be exacerbated with increasing aromatic DOM (Amery et al. 2007;  
102 Amery et al. 2008; Araújo et al. 2019). What is more, prediction of Cu in soil leachates was  
103 improved when the DOM aromaticity was taken into account in geochemical modelling (Amery  
104 et al. 2008) rather than only considering a percentage of reactive DOM (Djae et al. 2017).  
105 Phenolic and O-functional groups of aromatic molecules have high affinity for Cu binding and  
106 are highly stable, which may facilitate its solubilisation by DOM (Fuentes et al. 2013). These  
107 findings clearly underline the importance of DOM characteristics in the control of Cu dynamics  
108 in soil solutions.

109 The capacity of DOM to complex Cu and to promote its mobilisation from the solid phase not  
110 only depends on DOM quantity, but also on DOM quality (Baken et al. 2011; He et al. 2016;  
111 Kikuchi et al. 2017). DOM quality can be described through its optical properties assessed by  
112 Ultraviolet visible (UV-Vis) absorption and excitation-emission matrix (EEM) fluorescence  
113 spectroscopy (Jaffé et al. 2014). In the soil literature, DOM is largely characterised by its  
114 specific UV-absorbance index at 254 nm ( $SUVA_{254}$ ), which is related to its aromaticity  
115 (Weishaar et al. 2003). But the measurements of  $SUVA_{254}$  may be biased due to the presence  
116 of colloids or iron at high concentrations (Weishaar et al. 2003). Other spectroscopic indices

117 are widely used in studies on aquatic environments to characterise DOM (Yang and Hur 2014;  
118 Derrien et al. 2017) and its interactions with Cu (He et al. 2016). Another UV-Vis absorption  
119 index is the spectral slope ratio  $S_R$  which is inversely proportional to molecular size (Helms et  
120 al. 2008). Fluorescence indices include the humification index HIX, which expresses the degree  
121 of DOM condensation (Zsolnay et al. 1999), the biological index BIX, used to assess the recent  
122 production of autochthonous components (Huguet et al. 2009), and the fluorescence index FI  
123 which makes it possible to distinguish microbial versus higher plant origin of DOM (McKnight  
124 et al. 2001). In aquatic media, concentrations of metals (Cu, Zn, Cd) in sediment solutions were  
125 shown to be related to DOM aromaticity (HIX) and to the autochthonous (BIX) or microbial  
126 (FI) origins of molecules and not to DOM molecular size ( $S_R$ ) (He et al. 2016; Zhang et al.  
127 2020).

128 Up to now, only a few studies combined all these tools to describe DOM in contrasted  
129 agricultural soils (Romero et al. 2017; Li et al. 2019; Sun et al. 2020) and their effects on metal  
130 speciation in soil solution (Guo et al. 2015) or sediments (Xu et al. 2019). To our knowledge,  
131 only one paper reported the use of these spectroscopic indices for vineyard soils exposed to  
132 herbicide glyphosate and Cu-based fungicides (Daouk et al. 2015). The main goal of the present  
133 study was thus to examine the contribution of quality of DOM in predicting Cu availability in  
134 organic vineyard soils. We hypothesized that taking the DOM optical indices into account  
135 would improve the prediction of Cu environmental availability, assessed with total soluble Cu  
136 or free  $Cu^{2+}$ .

## 137 **2. Material and methods**

### 138 **2.1 Soil sampling and analyses**

139 Eighteen vineyard topsoils were sampled in the vine inter-rows at a depth of between 0 and 15  
140 cm in March 2019 in the region of Bordeaux (France), located in the AOC (i.e., controlled

141 designation of origin) zones of ‘*Bordeaux supérieur*’, ‘*Blaye Côtes de Bordeaux*’ and ‘*Graves*’.  
142 All the vineyards sampled are organic and the only fungicide treatment they receive is Cu. The  
143 fungicide applied was mainly Bordeaux mixture (mixture of copper sulphate and lime).  
144 The 18 soils were contrasted in terms of organic matter and pH based on the pedological  
145 bedrock and agronomic practices. After sampling, the fresh soils were sieved to 4 mm and kept  
146 at 4 °C before analysis to minimise the effects of sampling on dissolved organic carbon (Jones  
147 and Willett 2006).

148 Soil pH ( $\text{pH}_{\text{water}}$ ), total copper ( $\text{Cu}_{\text{T}}$ ), organic carbon ( $\text{C}_{\text{org}}$ ) and total nitrogen contents were  
149 determined on separate air-dried soils.  $\text{pH}_{\text{water}}$  was determined by potentiometry in distilled  
150 water in a 1:2.5 (w/v) soil-to-solution ratio (NF ISO 10390).  $\text{Cu}_{\text{T}}$  was determined after *aqua*  
151 *regia* microwave-assisted digestion (Anton Paar Multiwave PRO Microwave Reaction  
152 System®). Aliquots (0.5 g) of crushed dried sample were placed in a microwave Teflon flask  
153 with an acid mix  $\text{HNO}_3\text{:HCl}$  (3.75:1.25, v:v) (Aristar® for trace analysis, VWR Chemicals).  
154 Extracts were obtained after centrifugation at 1,300 W at 180 °C for 20 minutes and filtration  
155 on a 2- $\mu\text{m}$  Whatman cellulose acetate filter. The concentration of Cu in the extracts was  
156 measured by ICP-OES (Agilent®). A certified reference material (BCR-143R, 98 % Cu  
157 recovery rate) was used to validate extraction and quantification.

158  $\text{C}_{\text{org}}$  and N contents in soils were assessed by dry combustion (950 °C) with a CN analyser  
159 (Flashea 1112 series®) after carbonates were removed with HCl (NF ISO 10694). An organic  
160 soil (ISE 973) was used as control.

## 161 **2.2 Extraction of soil solutions**

162 Before the soil solution was extracted, the wet soils were all adjusted to 80 % water holding  
163 capacity (WHC) and incubated at 20 °C for one week to avoid a possible microbial flush and  
164 its effects on Cu solubility and speciation (Cornu et al. 2007).

165 Soil solutions were extracted as follows: 15 g of wet soil were shaken with 30 mL of 0.01 M  
166 KCl (purity 99.5 %, Merck®) with 1:2 w/v (soil-to-solution ratio) at 35 rev. mn<sup>-1</sup> for 2 h. The  
167 soil suspensions were centrifuged at 4,600 g for 10 min. The supernatants were collected and  
168 filtered through 0.2 µm cellulose acetate filters. Extractions were performed in triplicate. The  
169 extracted soil solutions were stored at 4 °C in the dark before spectroscopic analysis, which was  
170 performed less than seven days after extraction.

171 The 0.01 M KCl extraction was chosen for two reasons: (i) to be in the range of common ionic  
172 strength for soil pore water and (ii) to avoid DOM coagulation which may occur in the case of  
173 CaCl<sub>2</sub> extraction (Römken and Dolfing 1998). The 1:2 w/v ratio was used because lower  
174 extraction ratios tend to overestimate the concentrations of DOC recovered (Jones and Willett  
175 2006).

## 176 **2.3 Analyses of soil extracts**

### 177 **2.3.1 Total dissolved metals and Cu<sup>2+</sup> concentrations**

178 The pH of the extracts (pH<sub>KCl</sub>) was measured immediately after extraction by potentiometry  
179 using a microelectrode (E16M331, Radiometer Analytical®). The total concentrations of  
180 dissolved Ca, Cu, Fe, Mn and Zn were determined by ICP-OES (Agilent ®) in a 5-mL aliquot  
181 acidified with 2 % HNO<sub>3</sub> (Aristar® for trace analysis purity 69 %, VWR Chemicals®). Free  
182 Cu activity was measured in non-acidified 5-mL aliquots using a cupric ion-selective electrode  
183 (ISE, 9629BNWP, Thermo Scientific Orion®). The ISE was calibrated in Cu solutions buffered  
184 with iminodiacetic acid and potassium phthalate with pCu (= - log<sub>10</sub> aCu<sup>2+</sup>) ranging from 5.5 to  
185 13 as described in (Bravin et al. 2009). The calibration curve (R<sup>2</sup>=0.98, n=67) showed 85 %  
186 slope efficiency compared to the theoretical Nernstian slope (**Fig. S1**). The concentrations of  
187 free Cu<sup>2+</sup> were determined from pCu values and from the Cu<sup>2+</sup> activity coefficient, calculated  
188 from the extended Debye-Hückel equation (Ritsema 1993).

189        **2.3.2 Dissolved organic matter parameters**

190        The concentrations of dissolved organic carbon (DOC) in the soil extracts were determined  
191        using a TOC analyser (TOC-VCPH, Shimadzu®, Japan). The DOC was calculated as the  
192        difference between total carbon (TC) and inorganic carbon (IC) (DOC=TC - IC). The analyser  
193        was calibrated with a solution prepared from sodium carbonate, sodium bicarbonate, potassium  
194        hydrogen phthalate, and the 0.01 M KCl was used as the zero point in the calibration.

195        **Absorbance indices**

196        Soil solution UV-visible absorbance was measured using a Jasco® V-560 spectrophotometer,  
197        scanning from 210 to 700 nm with a 0.5 cm path length quartz cuvette. A 0.01 M KCl solution  
198        was used as a blank. Several indices were determined from absorbance spectra to characterise  
199        the DOM in the extracts. The specific UV absorbance at 254 nm (SUVA<sub>254</sub>, L gC<sup>-1</sup> cm<sup>-1</sup>) was  
200        calculated as follows:

201        
$$\text{SUVA}_{254} = \frac{A_{254} \cdot 1000}{b \cdot \text{DOC}}$$

202        where A<sub>254</sub> is the measured absorbance at 254 nm (dimensionless) (**Fig. S2**), b is the path length  
203        (cm) and DOC the organic carbon concentration (mg L<sup>-1</sup>).

204        The S<sub>R</sub> index was calculated as the spectral slope ratio over 275-295 nm to 350-400 nm (Helms  
205        et al. 2008).

206        **Fluorescence indices**

207        The excitation-emission matrix (EEM) fluorescence spectra were recorded in a 0.5 cm quartz  
208        cuvette, thermostatted at 20 °C in the cell holder, using an Aqualog spectrofluorometer (Horiba  
209        Scientific®). Excitation scans were performed using a double-grating monochromator, a 150-  
210        Watt Xenon lamp with a 5 nm bandpass, and 2-s integration time at wavelengths of 240–800 nm  
211        at 5-nm intervals. Emission spectra were collected with high CCD detector gain at

212 approximately 0.58 nm (1 pixel) intervals at wavelengths of 245-830 nm. A blank EEM was  
213 acquired daily from the 0.01 M KCl solution used for soil extraction and subtracted from EEM  
214 spectra of all the samples to eliminate scattering peaks. EEM spectra were corrected for  
215 instrumental biases. When the maximum intensity of the absorbance spectrum was higher than  
216 0.1, the samples were diluted with 0.01 M KCl to avoid inner filter effects. Fluorescence data  
217 are expressed in Raman units (R.U) after normalization to the area under the Raman peak of an  
218 ultrapure water blank (Milli-Q, Millipore®) at an excitation wavelength of 350 nm. TreatEEM  
219 software (<https://sites.google.com/site/daromasoft/home/treateem>) was used to remove  
220 remaining scatter peaks in EEMs and to calculate the fluorescence indices.

221 Several indices were obtained from fluorescence analysis. As defined by Zsolnay et al. (1999),  
222 the humification index HIX was calculated as the ratio H/L of the area under the curve for two  
223 spectral regions (between emission wavelengths 435 nm and 480 nm for H and between 300  
224 nm and 345 nm for L) from the emission spectrum scanned for excitation at 254 nm (**Fig. S3**).  
225 The higher the HIX, the higher the degree of condensation and aromaticity. The biological index  
226 BIX was determined as the ratio of fluorescence intensity at an emission wavelength of 380 nm  
227 to that at 430 nm, at an excitation wavelength of 310 nm (Huguet et al. 2009). High BIX values  
228 are related to predominantly recent production of DOM linked to biological activity (Huguet et  
229 al. 2009). The fluorescence index FI was calculated as the ratio of the fluorescence intensity at  
230 emission wavelength of 450 nm to that at 500 nm while excitation was set at 370 nm (McKnight  
231 et al. 2001). FI is an indicator of the source of DOM, low values are associated with vascular-  
232 plant-derived compounds, while high values indicate microbial origin or microbially processed  
233 terrestrial DOM (Cory and Mcknight 2005; Broder et al. 2017).

#### 234 **2.4 Statistical analyses**

235 All statistical analyses were performed with R software version 4.0.2 (R core Team 2020). First,  
236 principal component analysis (PCA) was used to visualise correlations between variables and

237 possibly groups of soils. PCA was performed using the ‘FactomineR’ package (Husson et al.  
238 2008). For the PCA, all the variables were log-transformed except  $\text{pH}_{\text{water}}$  and  $\text{pH}_{\text{KCl}}$ . Second,  
239 a correlation matrix was performed to investigate the relationships between the same variables  
240 using the R ‘corrplot’ package (Wei and Simko 2021). The Pearson correlation coefficients  
241 were considered as statistically significant at  $p < 0.01$ .

242 Multi-linear log-log regressions were used to identify and rank the contributions of soil  
243 variables to the prediction of Cu availability proxies ( $\text{Cu}_{\text{KCl}}$  and  $\text{Cu}^{2+}$ ). Multi-linear regressions  
244 were performed using the ‘car’ package (Fox and Weisberg 2019). Each replicate was  
245 considered as one sample. For  $\text{Cu}_{\text{KCl}}$  prediction, soil and solution variables were entered as  
246 predictors ( $\text{Cu}_{\text{T}}$ ,  $\text{pH}_{\text{water}}$ , C:N,  $\text{C}_{\text{org}}$ ,  $\text{SUVA}_{254}$ ,  $\text{S}_{\text{R}}$ , DOC, HIX, BIX, FI). Only solution variables  
247 were used for  $\text{Cu}^{2+}$  prediction ( $\text{Cu}_{\text{KCl}}$ ,  $\text{pH}_{\text{KCl}}$ ,  $\text{SUVA}_{254}$ ,  $\text{S}_{\text{R}}$ , DOC, HIX, BIX, FI). The variables  
248 (except  $\text{pH}_{\text{water}}$  and  $\text{pH}_{\text{KCl}}$ ) were log-transformed before the computation. A total of 2,047 and  
249 255 models with one to maximum four predictors (to avoid overparameterization) were  
250 exhaustively tested for  $\text{Cu}_{\text{KCl}}$  and  $\text{Cu}^{2+}$  predictions, respectively. The best models had the  
251 maximum  $R^2_{\text{adj}}$  (goodness of fit) with a minimum number of predictors (parsimony). The  
252 selected models also had a variation inflation factor (VIF) below 2.3 to avoid multicollinearity.  
253 The root mean square error (RMSE) was calculated to compare the accuracy of the models  
254 including or not DOM parameters.

## 255 **2.5 Modelling $\text{Cu}^{2+}$ activity**

256 Cu speciation in solution was predicted with ORCHESTRA software (Meeussen 2003) using  
257 the DOM ion-binding model NICA-Donnan (Kinniburgh et al. 1996; Koopal et al. 2005). The  
258 objective was to compare simulations of amounts of free  $\text{Cu}^{2+}$  with the corresponding  
259 experimental measurements. The input parameters used for the calculations were  $\text{pH}_{\text{KCl}}$ , total  
260 DOM,  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Mn}^{3+}$  concentrations.  $\text{Cl}^-$  and  $\text{K}^+$  concentrations were set to  
261 0.01 M, i.e., their concentration in the extraction solution. DOM concentrations were calculated

262 assuming that DOM contained 50 % of C. Activity coefficients were calculated using the  
263 Davies equation. Fe activity was calculated by assuming its equilibrium with  
264 Fe(OH)<sub>3</sub> (logK<sub>S</sub>=3.19). Equilibrium reactions were described taking the equilibrium with  
265 atmospheric CO<sub>2</sub> (pCO<sub>2</sub> = 3.9x10<sup>-4</sup> atm) into account.

266 Several hypotheses regarding DOM composition were examined: (i) 100 % humic acids (HA),  
267 (ii) 100 % fulvic acids (FA), or an inert DOM pool and an active DOM pool comprised of (iii)  
268 65 % FA, or (iv) 30 % HA and 30 % FA. In each case, proton and metal binding parameters  
269 were set at their generic value (Milne et al. 2001, 2003). Sixty-five percent is the approximation  
270 generally used to determine the amount of free metal cations in soil solutions (Weng et al. 2002,  
271 Djae et al. 2017). In the second step, free Cu was optimised by adjusting the reactive DOM pool  
272 (noted rDOM) in each sample to match the experimental measurements.

273

### 274 **3. Results and discussion**

#### 275 **3.1 Soil properties**

276 The main soil parameters are listed in **Table 1**.

277 Total concentration of Cu in the soil (Cu<sub>T</sub>) ranged from 28 to 238 mg kg<sup>-1</sup> with a median of 107  
278 mg kg<sup>-1</sup>. In the Aquitaine region of France, large quantities of Cu-based fungicides are used to  
279 prevent downy mildew caused by the wet climate. Accordingly, El Hadri et al. (2012) found  
280 that Cu<sub>T</sub> in soils in the Aquitaine region ranged from 0.5 to 491 mg kg<sup>-1</sup>, with maximum values  
281 found in vineyard soils. All the vineyard soils in our study fell within this range. It is worth  
282 noting that Cu<sub>T</sub> concentrations may be very different in the same location, mainly depending  
283 on the age of the vineyard (Fernández-Calviño et al. 2009). This may explain the wide range of  
284 Cu<sub>T</sub> (i.e., 8-fold) observed in the present study.

285 The  $\text{pH}_{\text{water}}$  of the soils ranged from 6.3 to 8.3 (**Table 1**). The most alkaline soils were associated  
286 with the highest carbonate contents (**Table 1**). The organic carbon content ( $\text{C}_{\text{org}}$ ) ranged from  
287 5 to 31  $\text{g kg}^{-1}$ . Vineyard soils are generally poor in organic carbon. Coll et al. (2011) reported  
288  $\text{C}_{\text{org}}$  values ranging from 10 to 13  $\text{g kg}^{-1}$  for vineyard soils in the Languedoc-Roussillon region  
289 (South of France). Our results are also similar to those obtained by Parat et al. (2002) ( $11 < \text{C}_{\text{org}}$   
290  $< 45 \text{ g kg}^{-1}$ ) in a study conducted in Burgundy viticultural region (east-central France).

291 The C:N ratio varied between 10.3 and 17.4 (**Table 1**). The C:N ratio varied between 10.3 and  
292 17.4 (**Table 1**). This ratio provides information on the rate of SOM mineralisation (high for  
293 values below 12) and also on the carbon storage capacity of the soil (Swift et al. 1979).

294

### 295 **3.2 Elemental composition of the soil extracts and Cu availability**

296 Mean Fe, Mn, Ca and Zn concentrations are listed in **Table S1** in supplementary information.

297 The pH of the soil extracts ( $\text{pH}_{\text{KCl}}$ ) fluctuated between 5.9 and 7.6 (**Table S1**).

298 Mean concentrations of DOC in KCl extracts ranged only from 15 to 28  $\text{mg L}^{-1}$  (median 18)  
299 (**Table S1**). This corresponds to 0.08 and 0.33 % of total  $\text{C}_{\text{org}}$ , illustrating the low solubility of  
300 SOM. Furthermore, Zsolnay (1996) reported that DOC is the most mobile and reactive fraction  
301 of  $\text{C}_{\text{org}}$ .

302 The mean total concentration of Cu in the extracts ( $\text{Cu}_{\text{KCl}}$ ) ranged from 0.11 to 2.64  $\mu\text{M}$ , i.e., a  
303 factor of 23 (**Table S1**), with a median of 0.3  $\mu\text{M}$ . Many studies in the literature used salt  
304 solutions - generally  $\text{CaCl}_2$  - to extract Cu and considered it to be a correct surrogate for soil  
305 pore water Cu (Sauvé et al. 1997; Chaignon et al. 2003). Our  $\text{Cu}_{\text{KCl}}$  values were similar to those  
306 found by Djae et al. (2017) ( $3 \times 10^{-2}$  - 3.7  $\mu\text{M}$  Cu) with 2 mM  $\text{KNO}_3$  /  $\text{Ca}(\text{NO}_3)_2$  extractions.  
307 Similar ranges of total Cu assessed in  $\text{CaCl}_2$  extracts were found in the literature: 0.11 – 2  $\mu\text{M}$   
308 (Sauvé et al. 1996), 0.01 – 5  $\mu\text{M}$  (Sauvé et al. 1995).

309 The mean  $\text{Cu}^{2+}$  concentrations varied between  $2.1 \times 10^{-4}$  and  $0.95 \mu\text{M}$ , i.e., by a factor of 4,600  
310 (**Table S1**). This variability shows that our samples were more contrasted in terms of  $\text{Cu}^{2+}$  than  
311  $\text{Cu}_{\text{KCl}}$ . The highest  $\text{Cu}^{2+}$  values were found in the most acidic soils. The percentage of free Cu,  
312 defined as the ratio of concentrations in  $\text{Cu}^{2+}$  and  $\text{Cu}_{\text{KCl}}$  ( $f\text{Cu}^{2+}$ ), ranged from 0.07 % to 50 %  
313 (**Table S1**). Our results are in agreement with Djae et al. (2017) who found  $\text{Cu}^{2+}$  concentrations  
314 between  $3.10^{-7}$  and  $2 \mu\text{M}$  and  $f\text{Cu}^{2+}$  ranged from 0.01 % to 44 % for Cu-contaminated soils.  
315 But in moderately contaminated vineyard soils, Cornu et al. (2019) found  $\text{Cu}^{2+}$  of between  $6 \times 10^{-5}$  –  $0.3 \mu\text{M}$   
316 and  $f\text{Cu}^{2+}$  of between 0.002 % - 10 %. These results reveal dramatic differences  
317 based on free  $\text{Cu}^{2+}$ , even within a collection of soils with the same source of Cu contamination.

318 Soil pore water Cu has been used as a proxy for Cu phytotoxicity (Michaud et al. 2008),  
319 assuming that few complex forms of Cu present in the solution play a role in Cu uptake by plant  
320 roots. The toxicity threshold found in the literature for various plants ( $3.94\text{-}9.55 \mu\text{M}$ ) are all  
321 higher than the maximum  $\text{Cu}_{\text{KCl}}$  ( $2.64 \mu\text{M}$ ) found in the present study (**Table 2**), suggesting  
322 that our 18 vineyard soils do not *a priori* represent a risk for grapevine and also possibly maize,  
323 grapevine and sunflower production (Ouzounidou 1995, Juang et al. 2012, Kolbas et al. 2014).

324 Beyond total soluble Cu, the bioavailable form of Cu is generally considered to be its ionic  
325 species  $\text{Cu}^{2+}$  (Sauvé et al. 1998), so  $\text{Cu}^{2+}$  concentrations would be a far more reliable tool to  
326 assess Cu toxicity for organisms. In the present study, the  $\text{Cu}^{2+}$  measured in some KCl extracts  
327 was the same as that which caused a 50 % reduction ( $\text{EC}_{50} = 6.3 \times 10^{-2} \mu\text{M}$ ) in root growth in  
328 durum wheat (Bravin et al. 2010) (**Table 2**). This  $\text{EC}_{50}$  value was found at  $2.2 \mu\text{M}$   $\text{Cu}^{2+}$  for  
329 cucumber (Kader et al. 2016). To our knowledge, no such toxicity indicator assessed via ionic  
330  $\text{Cu}^{2+}$  has been established for grapevine. Four soils of our panel (**Table S1**) may be problematic  
331 if converted from grapevine to widely consumed crops like wheat. Qiu et al. (2013) reported  
332 that  $\text{Cu}^{2+}$  concentration was also an appropriate proxy to assess Cu toxicity for earthworms and  
333 found a  $\text{EC}_{50}$  of  $1 \mu\text{M}$   $\text{Cu}^{2+}$  for *Eisenia fetida* mortality (**Table 2**). In our panel of soils, this

334 suggests that one soil represents a potential toxic risk for this earthworm species (**Table 2,**  
335 **Table S1**).

336 Overall, the  $\text{Cu}^{2+}$  and  $\text{Cu}_{\text{KCl}}$  levels in the soils sampled in this study cannot be considered  
337 completely risk-free for various organisms, and may therefore affect the sustainability of the  
338 vineyards. The  $\text{Cu}^{2+}$  concentrations in about one-quarter of the soils were above the toxicity  
339 thresholds reported in the literature and may be toxic for organisms. It is thus important to  
340 examine and understand the factors that control Cu availability and potential toxicity.

341

### 342 **3.3 Diversity of soils in terms of DOM properties**

343 We calculated several spectroscopic indices to assess DOM quality (**Fig. S4**).  $\text{SUVA}_{254}$  values  
344 varied between 5 and 61  $\text{L g C}^{-1} \text{cm}^{-1}$  (**Fig. 1, Table S1**). This range is similar to that reported  
345 in other studies on agricultural soils: 6–65  $\text{L g C}^{-1} \text{cm}^{-1}$  (Amery et al. 2007); 27–46  $\text{L g C}^{-1} \text{cm}^{-1}$   
346 (Amery et al. 2008); 8–58  $\text{L g C}^{-1} \text{cm}^{-1}$  (Supriatin et al. 2015). DOM is considered to be more  
347 aromatic when the  $\text{SUVA}_{254}$  index is higher (Weishaar et al. 2003). It has been reported that  
348  $\text{SUVA}_{254}$  values below 30  $\text{L g C}^{-1} \text{cm}^{-1}$  suggest that DOM is composed mainly of hydrophilic  
349 substances (Yeh et al. 2014), which is the case of about 80 % of our samples.  $S_{\text{R}}$  values varied  
350 three-fold between 0.42 and 1.28 (**Fig. 1, Table S1**), which is the same range as those reported  
351 by Tang et al. (2019) for Chinese agricultural soils (0.36–1.7). The  $S_{\text{R}}$  index is inversely related  
352 to molecular size, high  $S_{\text{R}}$  values indicate low molecular weight DOM (Helms et al. 2008) while  
353 low  $S_{\text{R}}$  values indicate high molecular weight and highly condensed organic molecules (Gao et  
354 al. 2017).

355 Fluorescence EEM spectra (**Fig. S4**) were acquired to describe heterogeneities in DOM in the  
356 samples more precisely by calculating indices such as FI, HIX and BIX. The FI values ranged  
357 from 1.10 to 1.34 (median 1.22) (**Fig. 1, Table S1**). FI values are usually used to distinguish

358 sources of DOM. McKnight et al. (2001) found that low FI values (~1.3) correspond to higher  
359 plant-derived molecules, while high values (~1.9) indicate microbial or microbially-processed  
360 DOM. This suggests that the majority of DOM collected from our samples originated from  
361 vascular plants, and that microbial contributions were limited. The BIX index is used to assess  
362 the relative production of recent DOM and usually ranges from 0.5 to 1. In the present study,  
363 about 75 % of the samples had BIX between 0.6 and 0.7, indicating a relatively low contribution  
364 of fresh autochthonous DOM substances (**Table S1**), while the other 25 % had BIX between  
365 0.7 and 0.9, suggesting fresh DOM production by organisms (Huguet et al. 2009; He et al.  
366 2016). In our samples the HIX values were contrasted, with almost 10-fold variation from 1.31  
367 to 10.33 (median 5.75) (**Fig. 1, Table S1**). Two of the soils (D and L) were characterised by  
368 very low HIX values (**Table S1**) due to the presence of tyrosine-like fluorescence at an  
369 excitation wavelength of 240-260 nm and emission of 300-340 nm (**Fig. S4a**). This tyrosine-  
370 like fluorescence band was absent from all other EEM spectra that were similar to that of soil  
371 B (**Fig. S4b**). HIX stands for degree of humification or condensation of DOM (Zsolnay et al.  
372 1999), but has also been used to characterise DOM aromaticity (Ohno et al. 2007). Our HIX  
373 results are in the same range as those reported by Daouk et al. (2015) for Swiss vineyard soils  
374 (0.1-7.0) and as those reported by Tang et al. (2019) (2 to 10). However, HIX values varying  
375 from 10 to 30 have been reported in several arable soils (Kalbitz et al. 2003). Compared to the  
376 latter range, our soils had low HIX values and can be characterized as weakly-humified.

### 377 **3.4 Relationships between the variables**

378 We performed a principal component analysis (PCA) of all 18 soil extracts to describe the  
379 relationships between the solution variables and to identify homogeneous groups. 64% of total  
380 variance is explained by the first two principal components (**Fig. 2a**). The first principal  
381 component revealed 44.5 % variance of data and was positively associated with  $\text{Cu}_{\text{KCl}}$ ,  $\text{Cu}^{2+}$ ,  
382 Fe concentrations, and  $\text{SUVA}_{254}$ , and was negatively associated with  $\text{pH}_{\text{water}}$ ,  $\text{pH}_{\text{KCl}}$  and HIX.

383 The second principal component explained 19.2 % of variance and was positively associated  
384 with  $C_{org}$ , the C:N ratio, and negatively associated with FI and BIX. Two groups can be  
385 distinguished on the principal plan plot (**Fig. 2b**): group 1 is composed of the eight most alkaline  
386 soils ( $7.8 < pH_{water} < 8.3$ ), while group 2 is composed of the 10 most acidic soils ( $6.3 < pH_{water}$   
387  $< 7.5$ ). The differences between the groups are mainly explained by the  $pH_{water}$  values (PCA  
388 correlation coefficient with Component 1: -0.92; with Component 2: 0.03). On the first axis,  
389 the soils in group 1 share high values of pH and HIX, and low values of  $Cu_{KCl}$ , Fe, and  $Cu^{2+}$ .  
390 The opposite trend was observed in group 2. On the second axis, soils in group 2 have more  
391 contrasted FI and BIX than those in group 1. Indeed, in group 1, FI values ranged from 1.20 to  
392 1.31; and BIX values from 0.67 to 0.75. Comparatively, these indices show a larger range in  
393 group 2, with 1.10 - 1.34 for FI and 0.59 - 0.86 for BIX. Group 1 is composed of alkaline soils  
394 with low Cu concentrations in solution and relatively highly-humified DOM, while group 2 is  
395 composed of more acidic soils with high concentrations of  $Cu_{KCl}$  and  $Cu^{2+}$ , and contrasted  
396 contributions of microbial DOM.

397 Pearson's correlation coefficients were analysed to describe the links between the variables  
398 measured in the soils and in the extracts (**Fig. 3**).  $pH_{water}$  and  $pH_{KCl}$  were strongly and positively  
399 correlated ( $r=0.94$ ,  $p < 0.01$ ). Total dissolved Fe and Cu concentrations were negatively  
400 correlated with pH (**Fig. 3**), with  $r=0.77$ ,  $0.73$  ( $p < 0.01$ ), respectively. **Fig. 4a** reports total Cu  
401 in extracts as a function of total Cu in the soils. We found no immediate relationship between  
402 Cu availability and the level of contamination.  $Cu_{KCl}$  concentrations globally increased with  
403 decreasing soil pH (**Fig. 4b**). This result confirms the fact that metals are more soluble in acidic  
404 soils, as reported by McBride et al. (1997). None of the Fe and Cu concentrations were  
405 significantly correlated with DOC (**Fig. 3**).  $Cu^{2+}$  was strongly linked to  $Cu_{KCl}$  ( $r=0.82$ ,  $p < 0.01$ )  
406 (**Fig. 3, Fig. 4c**), showing that Cu solubilisation affects its concentration of free ions. A slightly  
407 positive relationship was found between  $pH_{KCl}$  and HIX (**Fig. 3**), suggesting that the most

408 humified DOM was present in the most alkaline samples, as confirmed by the results of the  
409 PCA.

410  $\text{Cu}^{2+}$  concentrations and  $f\text{Cu}^{2+}$  were strongly linked to the pH of the extracts, with correlation  
411 coefficients of -0.94 and -0.91 respectively (**Fig. 3, Fig. 4d**), suggesting that  $\text{Cu}^{2+}$  decreased  
412 with increasing pH. The strong relationship between pH and  $\text{Cu}^{2+}$  has already been reported  
413 (McBride and Blasiak 1979, Sauvé et al. 1995, Laurent et al. 2020), confirming that pH is the  
414 main factor controlling Cu speciation in solution. When pH increases, DOM is more negatively  
415 charged due to the deprotonation of its functional groups. This promotes its complexation with  
416 Cu, leading to a drastic decrease in  $\text{Cu}^{2+}$  activity in solution (Bravin et al. 2009; Ren et al. 2015).  
417 Conversely, at low pH, competition for DOM binding sites is high between  $\text{Cu}^{2+}$  and  $\text{H}^+$ , which  
418 promotes increasing  $\text{Cu}^{2+}$  activity. A pH above 6.5 seems to be the safety threshold for the soils  
419 in our collection in terms of Cu toxicity when  $\text{Cu}^{2+}$  is used as a proxy (**Fig. 4d**).

420 No tangible relationship was found between  $C_{\text{org}}$  values and DOC concentrations in the extracts  
421 (**Fig. 3**), meaning that the solubilisation rates of  $C_{\text{org}}$  may differ with the soil conditions or  
422 properties. None of DOM optical indices was significantly linked to DOC concentrations in the  
423 KCl extracts, indicating that DOM quality was not related to the quantity of DOM in solution.  
424 No significant relationship was found between the  $S_R$  and the  $\text{SUVA}_{254}$  indices.  $\text{SUVA}_{254}$  and  
425 HIX indices were negatively linked in this study ( $r=-0.71$ ,  $p < 0.01$ ) (**Fig. 3**). This negative  
426 relationship is all the more surprising as these indices are both related to DOM aromaticity. In  
427 the literature,  $\text{SUVA}_{254}$  and HIX are generally positively correlated (He et al. 2016; Tang et al.  
428 2019). However, such unexpected differences between these two indices have already been  
429 observed (Inamdar et al. 2011; Broder et al. 2017). Considering the differences in pH between  
430 soils, and given that fluorescence is pH-dependent, one may wonder if the variations observed  
431 in these two indices are not affected by the effect of pH on spectroscopic analyses. However,  
432 an impact on fluorescence analyses is unlikely in our range of soil pH, and would, in any case,

433 be less than differences due to the sources and composition of DOM (Hudson et al. 2007). The  
434 presence of protein-like fluorescence was observed in EEM spectra of only two soils (D and L)  
435 and cannot explain the negative correlation between HIX and SUVA<sub>254</sub>. Moreover, it has been  
436 reported that SUVA<sub>254</sub> measurements may be subject to some bias since Fe, colloids, and other  
437 constituents also absorb at 254 nm (Weishaar et al. 2003; Yang and Hur 2014). Since there were  
438 huge differences in Fe concentrations among our samples (i.e., varying by a factor of 12, **Table**  
439 **S1**), the SUVA<sub>254</sub> results in this study should be interpreted with caution. In a collection of  
440 samples with such contrasted concentrations of dissolved Fe as those measured in the present  
441 study, it would therefore not be appropriate to use the SUVA<sub>254</sub> index to assess DOM  
442 aromaticity, and HIX appears to be a more reliable tool for that purpose. No significant  
443 relationship was found between HIX and S<sub>R</sub>, suggesting that, in our soil extracts, DOM  
444 condensation may not be directly related to its molecular size. Moreover, the negative  
445 correlation between HIX and BIX (**Fig. 4b**) ( $r = -0.82$ ,  $p < 0.01$ ) indicates that the most aromatic  
446 DOM is associated with older molecules derived from higher plants (Tang et al. 2019), which  
447 is quite consistent. No significant link was found between HIX and FI. Nevertheless, the  
448 positive relationship between BIX and FI ( $r = 0.63$ ,  $p < 0.01$ ) was not surprising and suggested  
449 that the soils with DOM originating from a microbial source were related to the highest  
450 autochthonous contributions, as reported in other works (Tang et al. 2019; Musadji et al. 2020).

451 Overall, these results indicate that the 18 soils sampled in our study differed in DOM quality  
452 even though they had similar concentrations of DOC. Given the significant affinity of Cu for  
453 DOM, these differences in DOM quality are expected to affect the geochemistry of Cu in  
454 solution.

### 455 **3.5 Prediction of Cu availability using the properties of the soils and the extracts**

456 The risk of Cu environmental toxicity could be better assessed if its availability could be  
457 predicted using the variables measured in the soil and in the soil solution. However, organisms

458 often use mechanisms to resist the stress caused by Cu toxicity. For instance, some plants take  
459 up and sequester Cu in cells where it cannot cause harm (Küpper et al. 2009). Nevertheless,  
460 as Cu environmental availability is linked to its total ( $Cu_{KCl}$ ) and ionic ( $Cu^{2+}$ ) concentrations in  
461 soil solutions, predicting these concentrations could enable more accurate evaluation of  
462 potential Cu toxicity to a certain extent (see **Section 3.2**). **Table 3** shows the best models  
463 calculated to predict  $Cu_{KCl}$  and  $Cu^{2+}$  among the 18 vineyard soils sampled in this study.

464

### 465 **3.5.1. Prediction of total Cu in extracts $Cu_{KCl}$**

466 Among the one-variable models tested to predict  $Cu_{KCl}$ , the model with  $pH_{water}$  (a) showed the  
467 best goodness of fit ( $R^2_{adj} = 0.575$ , **Table 3**). This suggests that pH is the main factor driving  
468 Cu solubilisation in the soils sampled in this study. However, this model showed marked  
469 dispersion of points around the 1:1 line (**Fig. 5a**), more visible in soils in group 1 than in soils  
470 in group 2. The prediction was better when  $Cu_T$  was added to pH (model (b)), the  $R^2_{adj}$  value  
471 raised to 0.71 and the RMSE decreased from 0.28 to 0.23 (**Table 3**). The points were then less  
472 dispersed than with the preceding model, except in soils in group 2 (**Fig. 5b**). The respective  
473 coefficients of pH and  $Cu_T$  are suitable for the presumed mechanisms. Indeed, it has been  
474 reported that metal desorption increases with a decrease in pH, which is why metals tend to be  
475 more soluble in acidic environments (McBride and Blasiak 1979; Degryse et al. 2009).  
476 Moreover, at higher pH, Cu oxyhydroxides or carbonates may form and precipitate thereby  
477 reducing the Cu in solution (McBride 1989). As expected, the model showed that the higher the  
478  $Cu_T$ , the higher the concentration of soluble Cu in solution ( $Cu_{KCl}$ ). Sauv e et al. (1997)  
479 underlined the importance of pH and  $Cu_T$  in Cu solubilisation, but in the reverse hierarchy.  
480 These authors found that in 66 soils with diverse sources of Cu contamination, 90 % of variation  
481 in soluble Cu was explained by total Cu in the soils and the importance of pH was minimal.

482 This could be explained by the wider range of  $Cu_T$  in their study (14–3000 mg kg<sup>-1</sup>) which  
483 reinforced the contribution of  $Cu_T$  in predicting soluble Cu.

484 The concentration of DOC did not appear in the best models, presumably because of its limited  
485 variability in our initial dataset. However,  $Cu_{KCl}$  predictability was dramatically improved when  
486 DOM quality parameters were added as predictors in addition to pH and  $Cu_T$ . This suggests that  
487 in the present study, Cu availability depended more on DOM characteristics than on its  
488 concentration. Among all the DOM indices tested in the regressions, only HIX and FI emerged  
489 significantly, and the best prediction was obtained by equation (c) (**Table 3, Fig. 5c**), which  
490 explained 83.2 % of variance, showing good accuracy of this model for the prediction of  $Cu_{KCl}$ .  
491 Compared to model (b), model (c) produced lower RMSE (0.17) and a slope closer to 1 (0.85)  
492 (**Fig. 5c**). The contributions of  $pH_{water}$ ,  $Cu_T$ , FI and HIX were all significant in the model ( $p <$   
493  $0.001$ ) (**Table 3**). The variance inflation factor (VIF) did not identify correlations between the  
494 predictors ( $VIF < 2.3$ ). HIX and FI were both expressed with negative coefficients in the model,  
495 suggesting that the solubilisation of Cu is enhanced with lowly-condensed plant-derived DOM  
496 molecules. This suggests that, in the vineyard soils studied here, molecules with low aromaticity  
497 have the most affinity for Cu for its solubilisation. This finding contradicts previous studies  
498 suggesting that the most aromatic DOM had higher affinity for Cu and promoted its  
499 solubilisation through complexation (Amery et al. 2007; Amery et al. 2008; Araújo et al. 2019).  
500 In the present study, DOM composed of more hydrophilic molecules may have higher Cu-  
501 binding capacity because of its higher proportion of O-containing functional groups (hydroxyl,  
502 carboxylic acid, sulfonic acid and amine) than aromatic and hydrophobic substances (Baham et  
503 al. 1978; Leenheer 1981). Moreover, the negative coefficient of FI in the model suggests a  
504 higher plant origin for the DOM components that bind Cu. Our results are consistent with those  
505 of He et al. (2016), who found higher concentrations of heavy metals in sediment solutions with  
506 lower aromatic DOM molecules, which the authors linked to amino acids. Moreover, it has

507 previously been reported that the ability of DOM hydrophilic and low aromatic components to  
508 transport Cu is greater than that of hydrophobic molecules (Han and Thompson 1999; 2003).  
509 These authors explained that low aromatic and hydrophilic molecules were more mobile, while  
510 aromatic and complex components tended to be adsorbed on soil particles.

### 511 **3.5.2. Prediction of free ionic Cu<sup>2+</sup>**

512 Among the models with just one predictor, the one with pH<sub>KCl</sub> (model (d)) appeared to be the  
513 most effective and explained 88.3 % of Cu<sup>2+</sup> variance (**Table 3**). The RMSE value was low  
514 (0.4), implying good accuracy of the prediction (**Fig. 5d**). The high R<sup>2</sup><sub>adj</sub> (0.88) of model (d)  
515 highlights the importance of pH in predicting Cu<sup>2+</sup> concentrations.

516 We hypothesised that free Cu<sup>2+</sup> may be linked to the solubility of Cu, as we found a positive  
517 relationship between Cu<sup>2+</sup> and Cu<sub>KCl</sub> (**Fig. 4c**). When adding Cu<sub>KCl</sub> to pH<sub>KCl</sub> as predictors of  
518 Cu<sup>2+</sup> (model (e)), the R<sup>2</sup><sub>adj</sub> increased from 0.88 to 0.91. This slight improvement can be observed  
519 in **Fig. 5e**. The corresponding VIF was the maximum receivable (2.3), certainly due to the  
520 relative effect of pH on Cu solubilisation. The contributions of pH<sub>KCl</sub> and Cu<sub>KCl</sub> were all  
521 significant ( $p < 0.001$ ) in explaining Cu<sup>2+</sup> variability. The coefficients in the model suggest that  
522 Cu<sup>2+</sup> increases with Cu<sub>KCl</sub>, and decreasing pH. Compared to model (d), model (e) was more  
523 accurate with a lower RMSE (0.34 instead of 0.4) (**Table 3**).

524 BIX was the DOM index that appeared in the best three-variables model (model (f), **Table 3**).  
525 Models with more than three variables did not present any statistical improvement (data not  
526 shown). In model (f), adding BIX to pH<sub>KCl</sub> and Cu<sub>KCl</sub> did not enhance the effectiveness of the  
527 prediction compared to model (e) (**Fig. 5f**), with similar R<sup>2</sup><sub>adj</sub> and RMSE (0.92 and 0.32  
528 respectively) (**Table 3**). Even though the contribution of BIX to the model was significant ( $p <$   
529  $0.05$ ), the addition of BIX was not needed to explain the variability of our collection of samples.  
530 Moreover, the max VIF of model (f) (2.44) was higher than the fixed threshold of 2.3,

531 illustrating multicollinearity between the predictors that probably weakens the stability of the  
532 prediction. Taken together, these results suggest that including DOM indices does not improve  
533 the modelling of  $\text{Cu}^{2+}$  and that only  $\text{pH}_{\text{KCl}}$  and  $\text{Cu}_{\text{KCl}}$  were needed.

534 In the literature, pH is widely reported to play an important role explaining free ionic  $\text{Cu}^{2+}$   
535 activities and the log-log relationship between these two variables is usually linear (McBride  
536 and Blasiak 1979; Sauvé et al. 1995; McBride et al. 1997; Sauvé et al. 1997; Bravin et al. 2009).  
537 Sauvé et al. (1997) proposed the following equation to predict  $\text{Cu}^{2+}$  activity in  $\text{CaCl}_2$  extracts  
538 of a collection of wide Cu-contaminated soils:  $\text{pCu} = -1.7 \log_{10}\text{Cu}_T + 1.4 \text{pH} + 3.4$ . In contrast  
539 to our study, the OM parameters (concentrations and quality) were not included in the model  
540 calculations and the authors underlined the importance of the degree of Cu contamination in  
541 predicting  $\text{Cu}^{2+}$ . However, interestingly, the pH coefficient in our study (model (e)) is consistent  
542 with the results of McBride (1989) who reported that slopes for  $\log\text{Cu}^{2+}$  - pH plots are generally  
543 lower than 2. This suggests that pH plays a constant role in  $\text{Cu}^{2+}$  prediction. But the optical  
544 parameters describing the quality of DOM in soil solutions were of no use in explaining the  
545 concentrations of  $\text{Cu}^{2+}$ .

### 546 **3.6. Influence of DOC composition on Cu speciation**

547 We calculated Cu speciation in solution based on thermodynamic models and on several  
548 hypotheses concerning DOM composition. We found that considering DOM as 100 % HA led  
549 to underprediction of free  $\text{Cu}^{2+}$  compared to experimental observations in all samples (**Fig. S5**).  
550 Considering all DOM as FA produced better results, but even using only 65 % of active DOM  
551 as FA (a widespread assumption in similar studies) resulted in marked discrepancies between  
552 model and experimental results. This observation - made here using the NICA-Donnan model  
553 to describe ion binding to DOM - is in agreement with previous estimations made using the  
554 Windermere humic aqueous model (WHAM) on a large set of soil solutions (Djae et al. 2017).

555 For the model results to fit the experimental observations, the percentage of active DOM,  
556 rDOM, was adjusted separately for each sample. The values of rDOM obtained ranged between  
557 0.10 and 1.68, reflecting the wide variability of the active pool within DOM. This is consistent  
558 with the physically meaningful range of 35-215 % estimated by Djae et al. (2017). The  
559 variations in rDOM were compared to the variations in the chemical and optical parameters,  
560 but no multilinear regression model could be established (data not shown).

561 The amount of Cu bound to carboxylic or phenolic groups of DOM can be estimated using  
562 thermodynamic modelling. Results show that binding to phenolic groups becomes predominant  
563 above pH 7.4 (**Fig. S6**). This suggests that these DOM binding groups, deprotonated at alkaline  
564 pH, have a very high affinity for Cu. Thus, in alkaline soils, the nature of DOM binding sites  
565 will be important in controlling Cu speciation.

566

#### 567 **4. Conclusions**

568 The main goal of this work was to assess the contribution of DOM spectroscopic indices to  
569 predicting two Cu availability proxies in 18 organic vineyard soils. Our results showed that in  
570 the KCl extracts, the total concentration of Cu varied 23-fold while that of free ionic  $\text{Cu}^{2+}$  varied  
571 4,600-fold among soils, pointing to wide diversity of soils in terms of Cu availability. Four soils  
572 had concentrations of  $\text{Cu}^{2+}$  higher than the toxicity levels for organisms reported in the  
573 literature. These samples were considered as potentially harmful for vineyard soil organisms.

574 Our results also showed that Cu environmental availability was weakly related to the level of  
575 Cu contamination in the soils. pH was the main factor controlling Cu environmental  
576 availability, as it was closely and positively correlated with the concentrations of total Cu and  
577 free ionic  $\text{Cu}^{2+}$  measured in KCl extracts. Thus, in theory, toxicity problems can occur even in  
578 moderately Cu-contaminated soils when the soils are particularly acidic. It would thus be safer  
579 to maintain the pH above 6.5. To this end, liming is possible but should be applied with caution,

580 so as not to jeopardise plant uptake of other nutrients like Fe, whose availability is low at  
581 alkaline pH.

582 This work highlighted the fact that even though the DOC concentrations were similar among  
583 the soils, noticeable differences were found in DOM quality, as assessed by optical  
584 spectroscopy. Absorbance and fluorescence analyses are thus appropriate to identify these  
585 differences in chromophoric and fluorescent DOM in soils.

586 The prediction of total Cu in KCl extracts was clearly improved when DOM spectral indices  
587 (HIX and FI) were taken into account, in addition to pH and  $Cu_T$ . This study thus emphasizes  
588 that the quality of DOM affects the availability of Cu in the soil more than its quantity. Although  
589 this finding now needs to be tested on a wider dataset (with different sources of contamination),  
590 it suggests that organic management in Cu-contaminated vineyards needs to be redesigned, to  
591 better control quality of dissolved organic matter production as a function of the nature of  
592 organic matter amendments.

593

#### 594 **Declarations**

#### 595 **Ethics approval and consent to participate**

596 Not applicable

#### 597 **Consent for publication**

598 Not applicable

#### 599 **Availability of data and materials**

600 All data generated or analysed during this study are included in this published article and its  
601 supplementary information files.

#### 602 **Competing interests**

603 The authors declare that they have no competing interests.

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#### 609 **Authors' contributions**

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612 Noémie Janot: Formal analysis, Supervision, Writing-review & editing

613 Christophe Nguyen: Formal analysis, Writing-review & editing

614 Mahaut Sourzac: Investigation

615 Edith Parlanti: Conceptualization, Funding acquisition, Supervision, Writing-original draft

616 Laurence Denaix: Conceptualization, Funding acquisition, Supervision, Writing-original draft

617 All authors read and approved the final manuscript.

618

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Figure 1

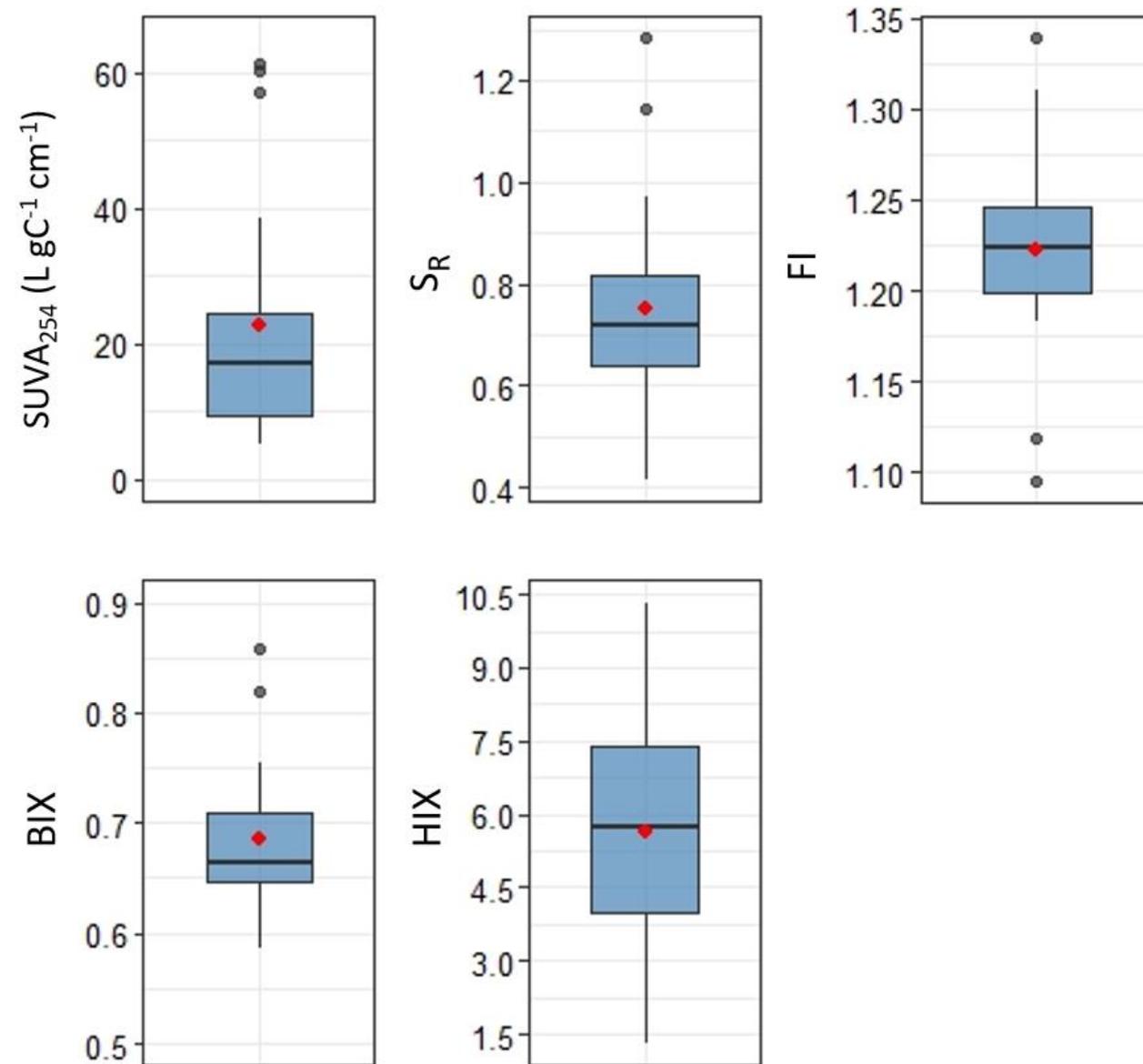


Figure 2

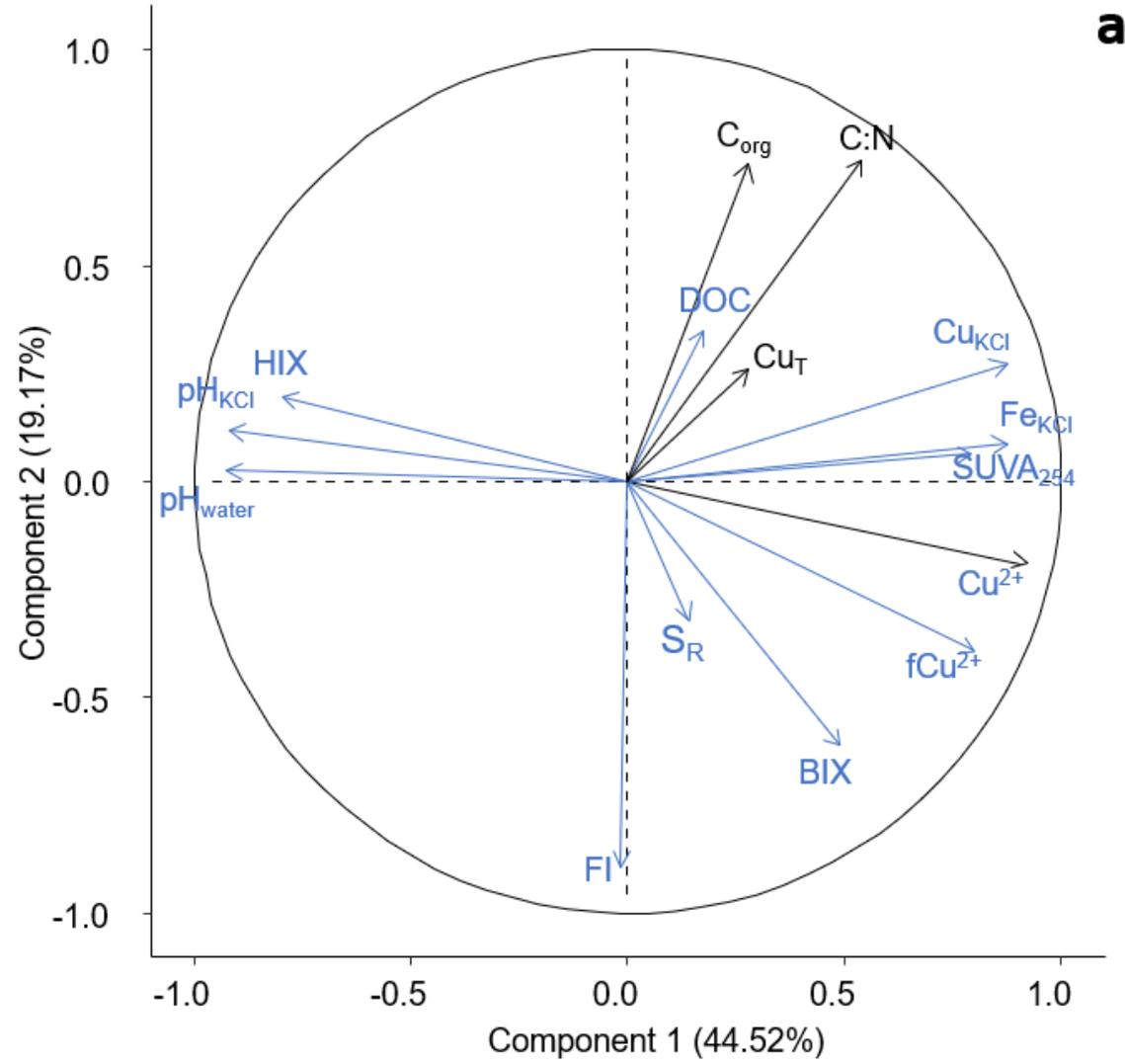


Figure 2

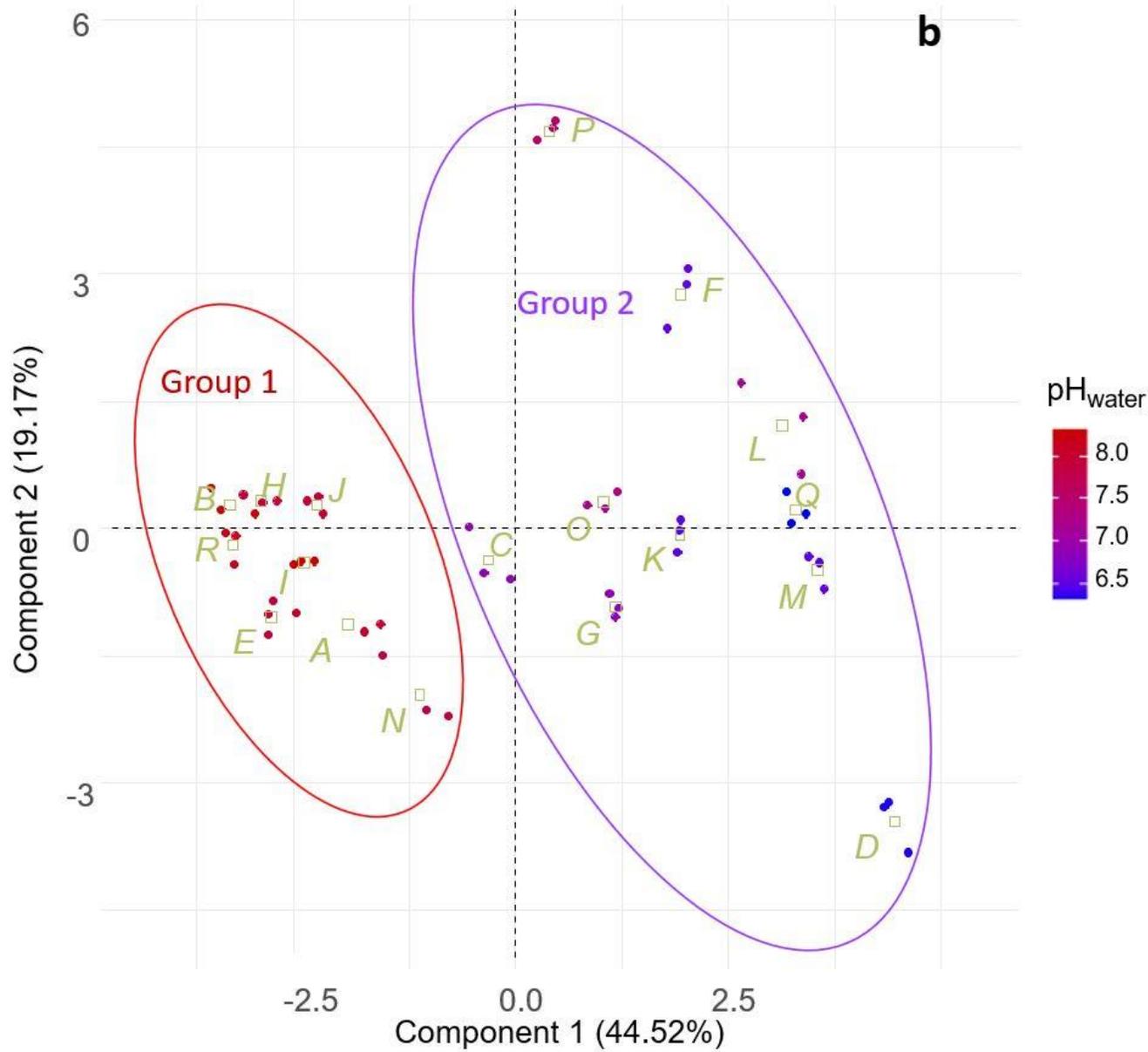




Figure 4

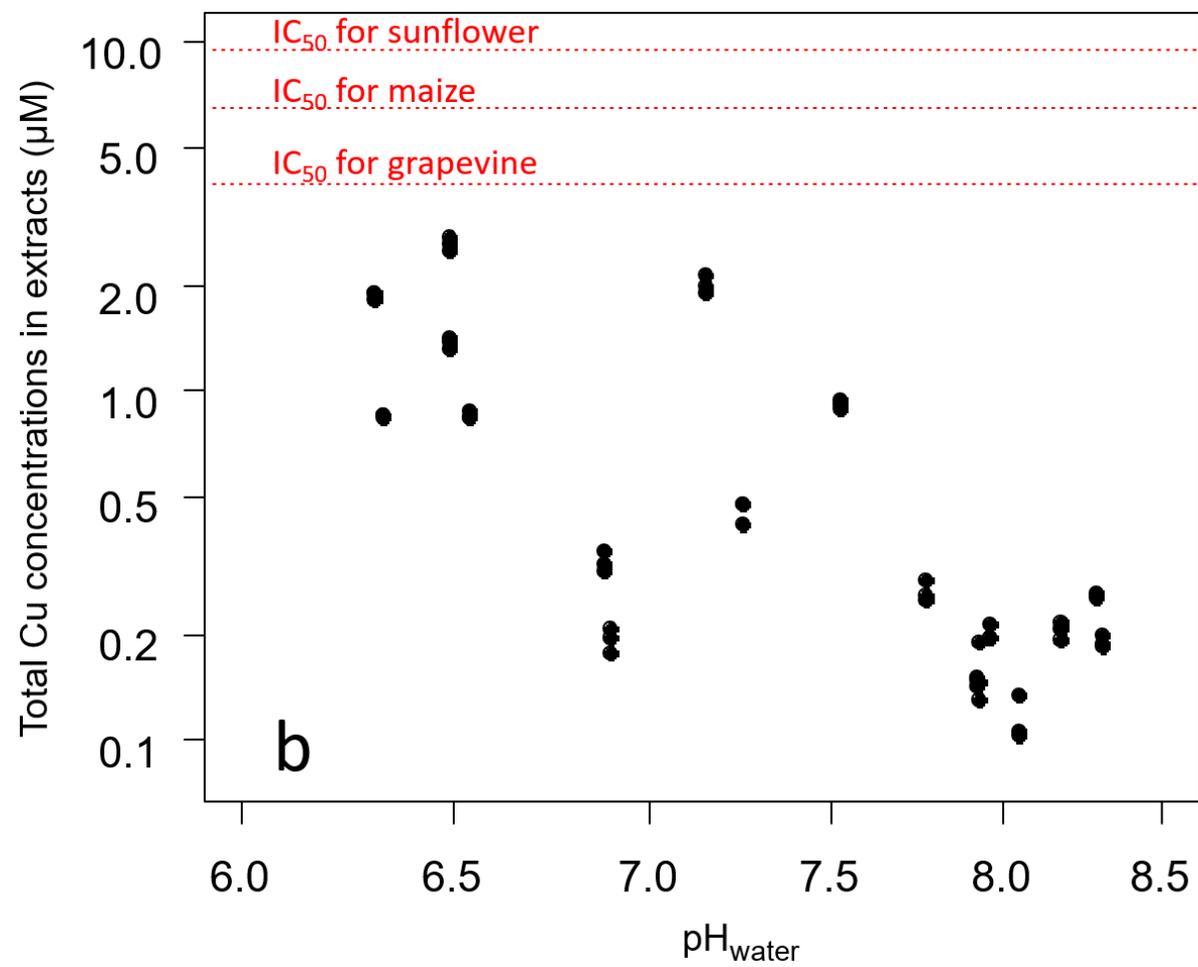
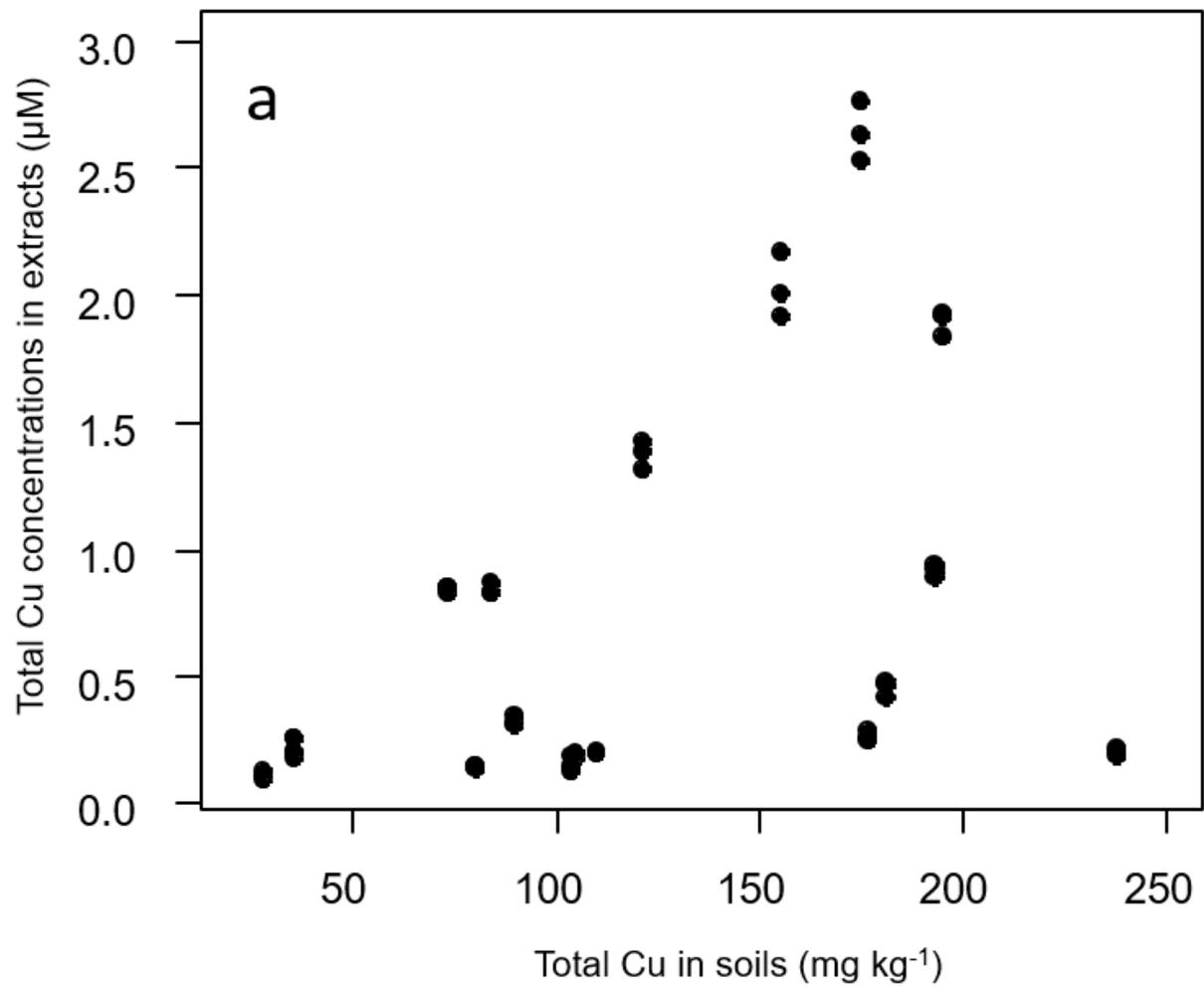
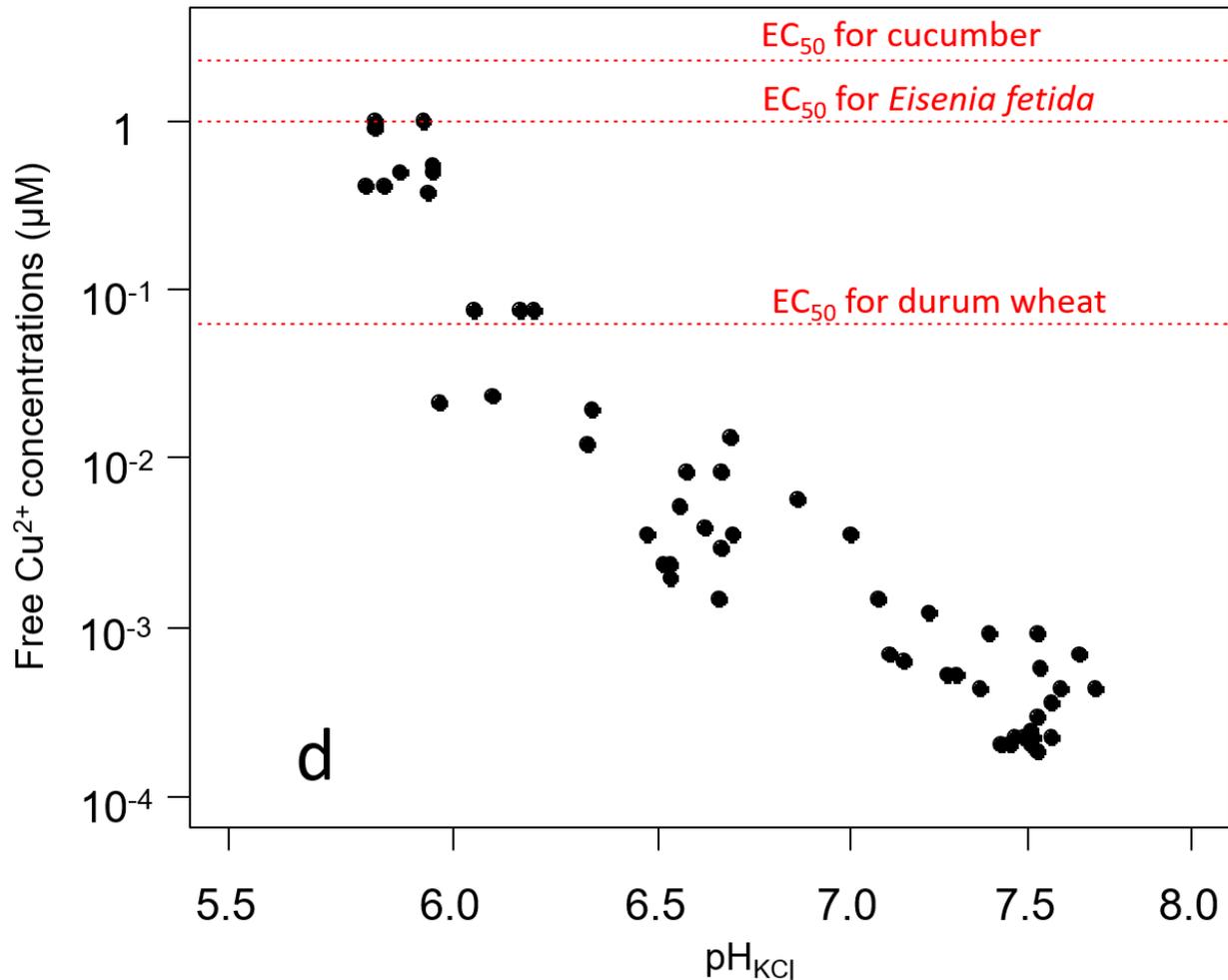
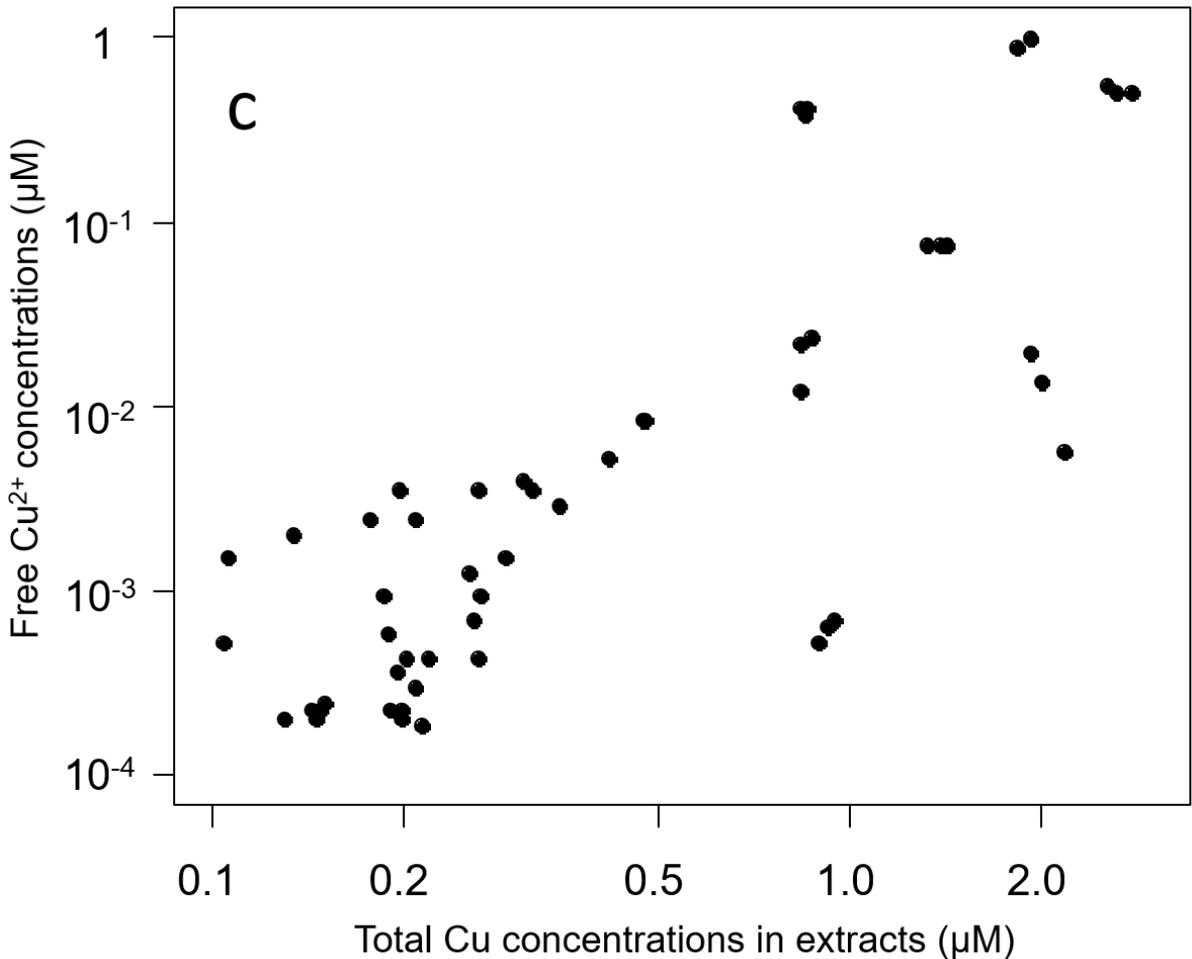
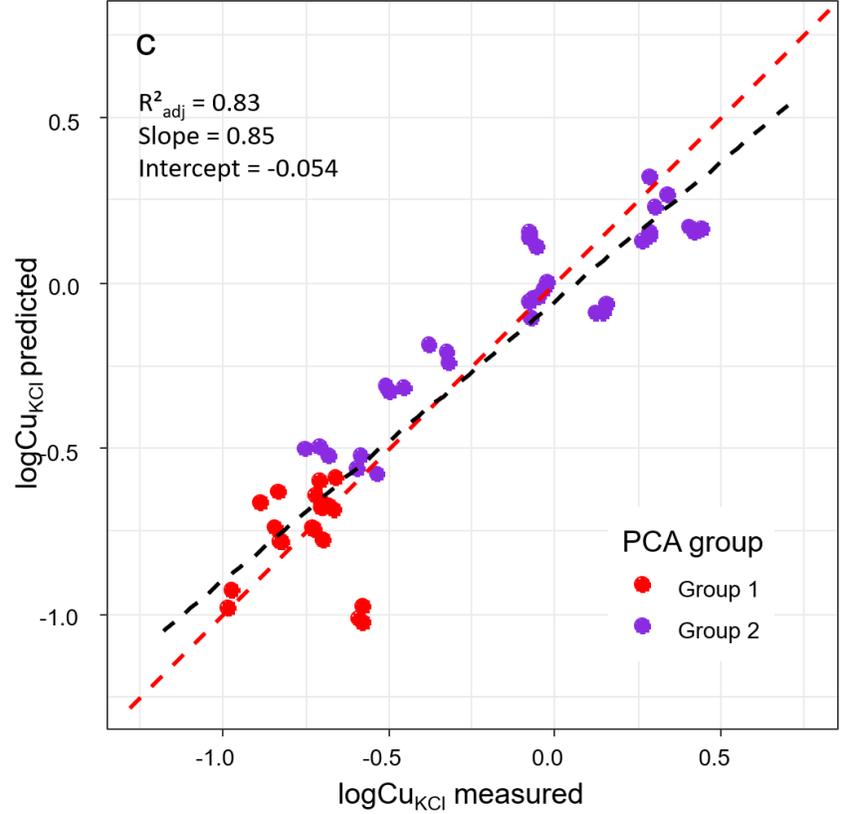
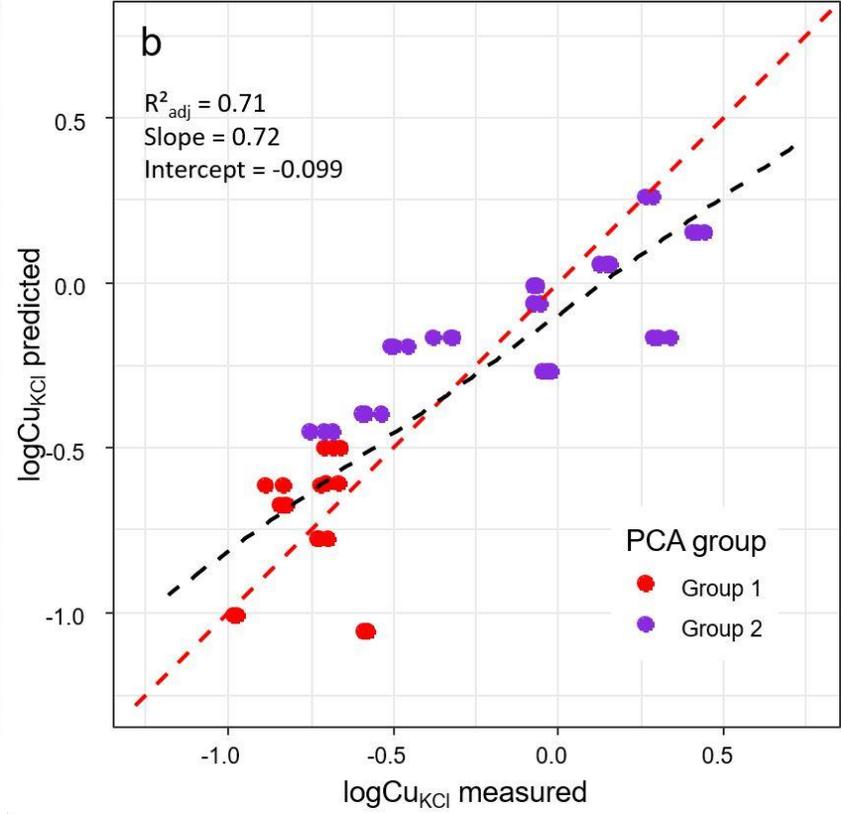
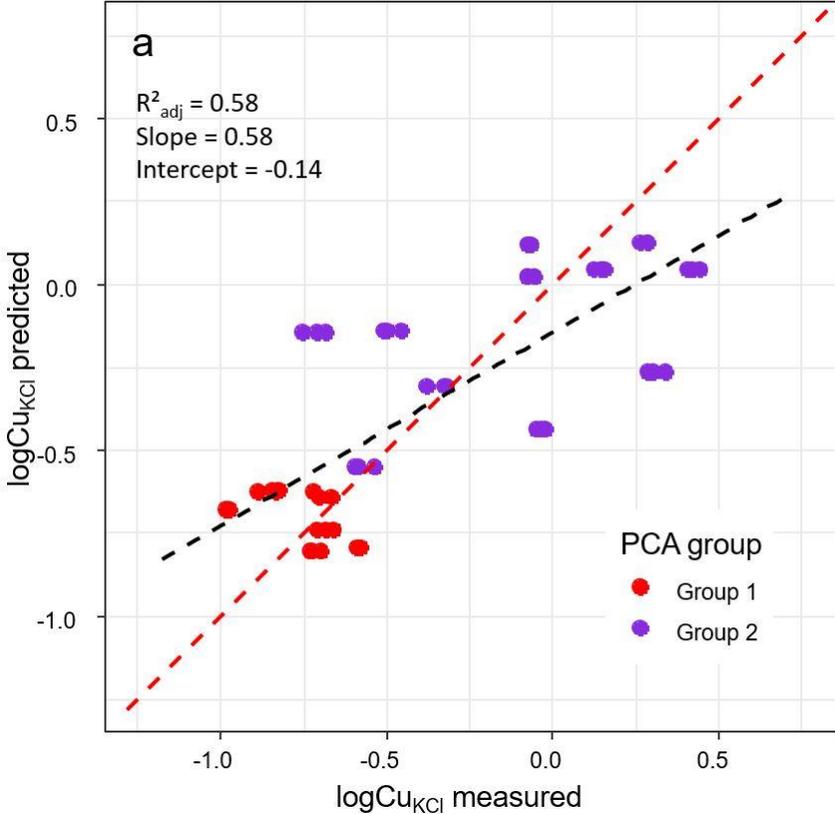


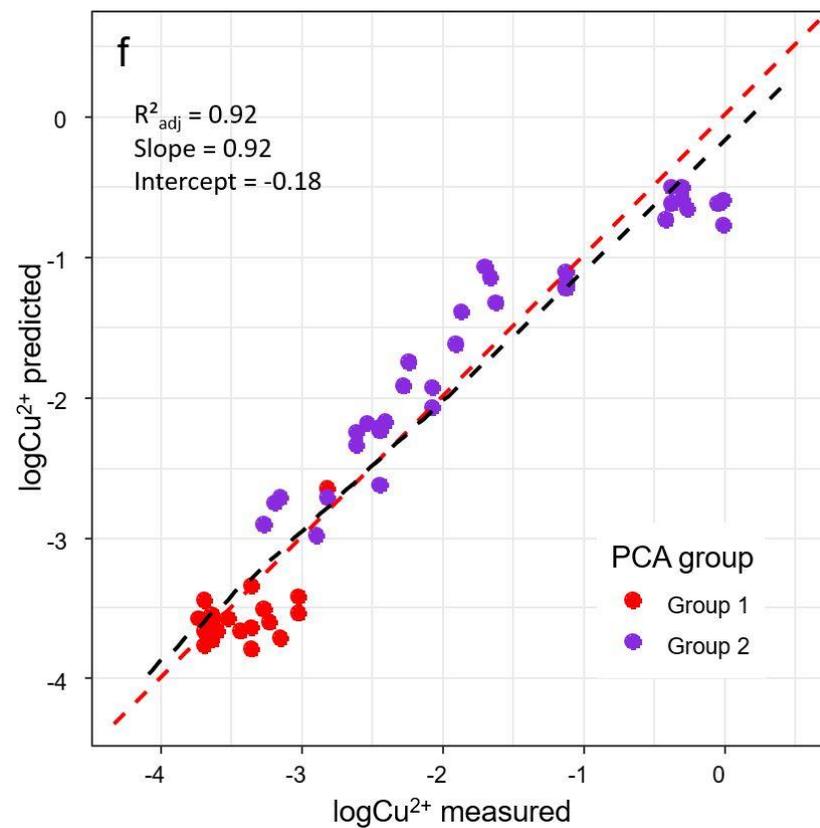
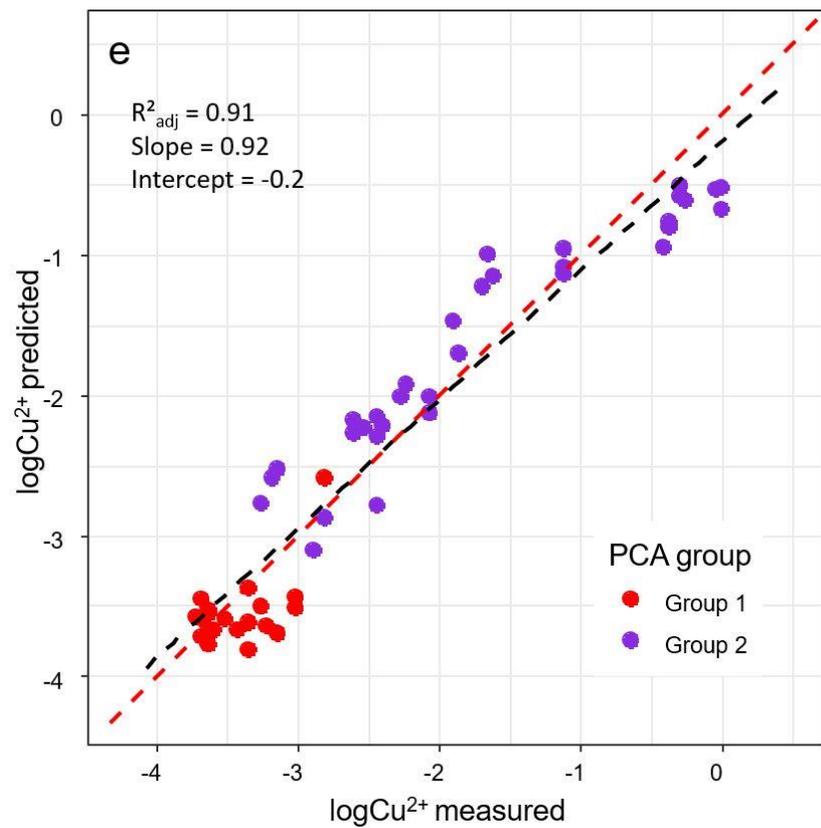
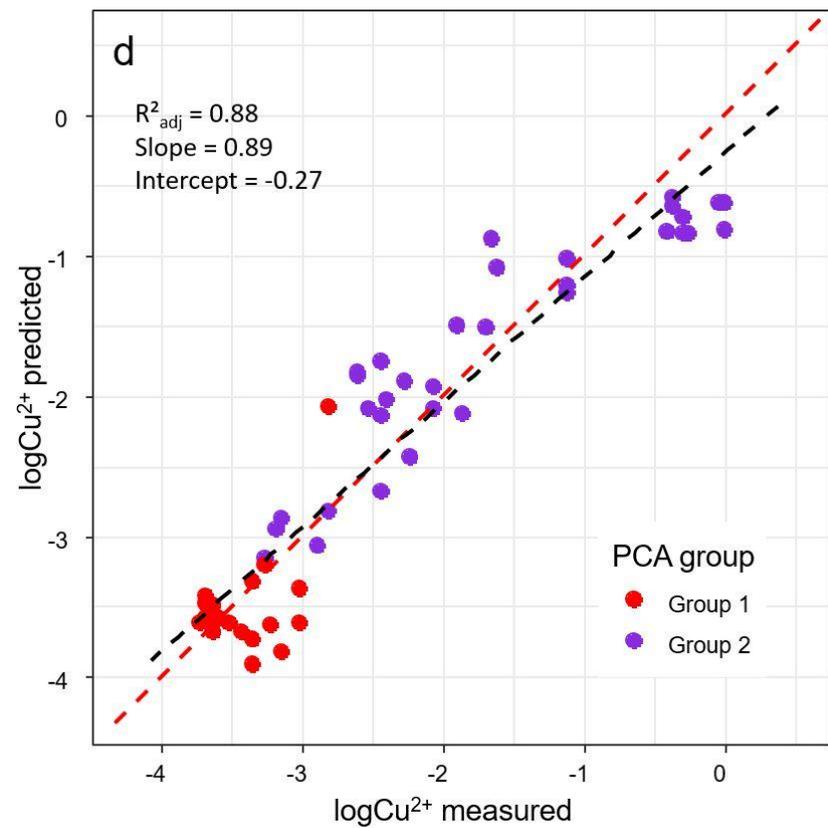
Figure 4



# Figure 5



# Figure 5



**Figure captions**

**Fig. 1:** Distribution of DOM optical parameters (mean of replicates), the red points represent the mean values of all the soils

**Fig. 2:** Principal component analysis (PCA) of soil solution chemistry and Cu availability proxies in the dataset. All the variables except  $\text{pH}_{\text{water}}$  and  $\text{pH}_{\text{KCl}}$  were log-transformed. The correlation plot (a) shows the contribution of each variable to the two first principal components. The soil variables are shown in black, the extract variables in blue. Soil samples (grouped in triplicate) are coloured according to their  $\text{pH}_{\text{water}}$  values (b)

**Fig. 3:** Correlation matrix between soil properties ( $\text{pH}_{\text{water}}$ ,  $C_{\text{org}}$ , C:N,  $\text{Cu}_{\text{T}}$ ), solution parameters: pH of the extracts ( $\text{pH}_{\text{KCl}}$ ), free ionic concentrations ( $\text{Cu}^{2+}$ ), free ionic fractions ( $f\text{Cu}^{2+}$ ), total dissolved metals ( $\text{Cu}_{\text{KCl}}$ ,  $\text{Fe}_{\text{KCl}}$ ) and DOM properties (DOC, HIX, BIX, FI,  $S_{\text{R}}$ ,  $\text{SUVA}_{254}$ ). The colored boxes represent significant correlations ( $p < 0.01$ )

**Fig. 4:** Total Cu concentrations in extracts ( $\text{Cu}_{\text{KCl}}$ ) as a function of total Cu in soils  $\text{Cu}_{\text{T}}$  (a) and pH of the soils ( $\text{pH}_{\text{water}}$ ) (b); free ionic Cu concentrations ( $\text{Cu}^{2+}$ ) as a function of total Cu concentrations in extracts (c) and pH of the extracts ( $\text{pH}_{\text{KCl}}$ ) (d). The log-log plot is shown in (c) and only the y-axis is log-transformed in (b) and (d)

**Fig. 5:** Comparison of the multilinear models predicting  $\text{Cu}_{\text{KCl}}$  (a, b, c) and  $\text{Cu}^{2+}$  concentrations (d, e, f) in the 0.01 M KCl extracts. The dashed red line represents the 1:1 line, the dashed black line represents the regression line

## Tables

**Table 1:** Chemical properties of the 18 vineyard soils analysed in this study. C<sub>org</sub> stands for organic carbon, Cu<sub>T</sub> for total Cu. Carbonate values <1 were not quantifiable

Soil id	AOC Zone	Soil classification (WRB)	Cu <sub>T</sub>	pH <sub>water</sub>	CaCO <sub>3</sub>	C <sub>org</sub>	C:N
			mg kg <sup>-1</sup>		g kg <sup>-1</sup>	g kg <sup>-1</sup>	
<b>A</b>	Blaye Côtes de Bordeaux	Cambisol	28	8.1	< 1	9.9	10.6
<b>B</b>	Blaye Côtes de Bordeaux	Cambisol	35	8.3	27.6	13.7	10.9
<b>C</b>	Blaye Côtes de Bordeaux	Luvic Cambisol	35	6.9	< 1	10.2	11.4
<b>D</b>	Bordeaux supérieur	Luvisol	73	6.3	< 1	10.5	11.5
<b>E</b>	Blaye Côtes de Bordeaux	Cambisol	80	7.9	< 1	8.3	10.7
<b>F</b>	Graves	Podzosol	84	6.5	< 1	16.9	15.3
<b>G</b>	Bordeaux supérieur	Luvisol	89	6.9	< 1	19.2	11.7
<b>H</b>	Bordeaux supérieur	Luvisol	103	7.9	3.2	12.6	10.6
<b>I</b>	Graves	Cambisol	104	8.3	19.5	7.5	11.5
<b>J</b>	Blaye Côtes de Bordeaux	Luvisol	110	8	1.1	11.3	11.6
<b>K</b>	Blaye Côtes de Bordeaux	Podzosol	121	6.5	< 1	11.8	13.0
<b>L</b>	Graves	Leptosol	155	7.1	< 1	14.6	15.5
<b>M</b>	Blaye Côtes de Bordeaux	Luvisol	175	6.5	< 1	9.3	11.9
<b>N</b>	Blaye Côtes de Bordeaux	Luvisol	176	7.8	< 1	5.4	11.7
<b>O</b>	Blaye Côtes de Bordeaux	Luvisol	181	7.3	< 1	15.6	12.6
<b>P</b>	Graves	Leptosol	193	7.5	< 1	30.8	17.4

<b>Q</b>	Blaye Côtes de Bordeaux	Luvisol	195	6.3	< 1	13.3	12.6
<b>R</b>	Blaye Côtes de Bordeaux	Cambisol	238	8.2	30.4	10.6	10.3

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**Table 2:** Values of total Cu in solution and Cu<sup>2+</sup> reported as toxicity thresholds in the literature, compared with the maximum values found in the present study

	Toxicity thresholds	Values (μM)	References
	<b>Max value in this study</b>	<b>2.64</b>	
Total Cu in solution (Cu <sub>KCl</sub> in this study)	IC <sub>50</sub> grapevine	3.94	Juang et al. 2012
	IC <sub>50</sub> maize	6.50	Ouzounidou 1995
	IC <sub>50</sub> sunflower	9.55	Kolbas et al. 2014
	<b>Max value in this study</b>	<b>0.94</b>	
Free ionic Cu <sup>2+</sup>	EC <sub>50</sub> durum wheat	0.06	Bravin et al. 2010
	EC <sub>50</sub> cucumber	2.20	Kader et al. 2016
	EC <sub>50</sub> <i>Eisenia fetida</i>	1.00	Qiu et al. 2013

**Table 3:** Best models for predicting  $\text{Cu}_{\text{KCl}}$  and  $\text{Cu}^{2+}$  in KCl extracts and associated statistics.

$R^2_{\text{adj}}$ : adjusted r-squared, RMSE: root mean square error, max VIF: maximum value of the variance inflation factor, N: number of observations

Statistical significance: \*\*\* for p-value < 0.001, \*\* for p-value < 0.01, \* for p-value < 0.05

	Models	$R^2_{\text{adj}}$	max VIF	RMSE ( $\mu\text{M}$ )	N
$\log_{10}\text{Cu}_{\text{KCl}} =$	$-0.465^{***} \text{pH}_{\text{water}} + 3.066$ (a)	0.575	-	0.28	53
	$-0.434^{***} \text{pH}_{\text{water}} + 0.615^{***} \log_{10}\text{Cu}_{\text{T}} + 1.598$ (b)	0.707	1.02	0.23	53
	$-0.312^{***} \text{pH}_{\text{water}} + 0.443^{***} \log_{10}\text{Cu}_{\text{T}} - 7.362^{***} \log_{10}\text{FI}$	0.832	1.91	0.17	53
	$-0.664^{***} \log_{10}\text{HIX} + 2.148$ (c)				
$\log_{10}\text{Cu}^{2+} =$	$-1.751^{***} \text{pH}_{\text{KCl}} + 9.589$ (d)	0.883	-	0.40	53
	$-1.380^{***} \text{pH}_{\text{KCl}} + 0.731^{***} \log_{10}\text{Cu}_{\text{KCl}} - 7.307$ (e)	0.912	2.30	0.34	53
	$-1.316^{***} \text{pH}_{\text{KCl}} + 0.73^{***} \log_{10}\text{Cu}_{\text{KCl}} + 2.818 \log_{10}\text{BIX} + 7.338$ (f)	0.919	2.44	0.32	53