

1 **Title: Increased soil pH and dissolved organic matter after a decade of organic fertilizer application**  
2 **mitigates copper and zinc availability despite contamination**

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21 **Abstract**

22 Long-term organic fertilizer (OF) application on agricultural soils is known to induce soil Cu and Zn  
23 contamination, along with pH and organic matter changes, which in turn alter the soil Cu and Zn  
24 availability. Our study was aimed at assessing Cu and Zn availability in long-term OF-amended soils by  
25 distinguishing the importance of increased contamination levels versus pH and organic matter changes in  
26 soil. Seventy-four soil samples were collected over time from fields corresponding to three soil types  
27 upon which no, mineral, or organic fertilization had been applied over a decade, and thus exhibited a  
28 gradient of Cu and Zn contamination, pH, and organic matter concentration. Soil Cu and Zn  
29 contamination (i.e. total and DTPA-extractable Cu and Zn concentration), soil solution chemistry (i.e. pH  
30 and dissolved organic matter concentration and aromaticity) and Cu and Zn availability (i.e. total  
31 concentration and free ionic activity in solution and DGT-available concentration in soil) levels were  
32 measured. The Windermere humic aqueous model (WHAM) was used to estimate  $Zn^{2+}$  activity and  
33 dissolved organic matter (DOM) binding properties in soil solution. Regardless of the soil type, organic  
34 fertilization increased Cu and Zn contamination in soil, in addition to the pH and the DOM concentration,  
35 aromaticity and binding properties in soil solution. The pH increase prompted a decrease in the total Zn  
36 concentration and  $Zn^{2+}$  activity in soil solution. The concomitant pH increase and DOM concentration,  
37 aromaticity and binding properties boosted the total Cu concentration but decreased the  $Cu^{2+}$  activity in  
38 soil solution. DGT-available Cu and Zn varied very little between the three fertilization modalities. Our  
39 results suggest that pH and DOM changes were able to regulate Cu and Zn availability in long-term OF  
40 amended soils by exerting a protective effect that offset the concomitant increase in soil Cu and Zn  
41 contamination.

42 **Keywords:** Ecotoxicity; Livestock residues; Sorption; Speciation; Trace elements

43

## 44 **1. Introduction**

45 The recycling of organic fertilizers (OF) in crop fields is known to improve the soil physical-chemical  
46 properties in the long-term, contribute to fertilization by bringing essential nutrients to crops, while  
47 offering economic and environmental benefits in terms of waste management. Despite these  
48 advantages, OF contain contaminants that lead to diffuse contamination of agricultural soils that have  
49 been repeatedly amended with OF over a long period (Wassenaar et al., 2014). Trace elements  
50 quantitatively appear to be the main type of contaminants affecting agricultural soils following OF  
51 amendment (Senesi et al., 1999). The fate of copper (Cu) and zinc (Zn) seems particularly worrisome as  
52 Cu and Zn are quantitatively the main trace elements involved in this soil contamination. Indeed,  
53 livestock feed is supplemented with Cu and Zn for their growth factor and antibiotic properties, so  
54 animal manure may contain high amounts of Cu and Zn (Jondreville et al., 2003). Furthermore, the  
55 estimated time necessary to increase the soil concentration from the natural background level to  
56 regulatory limits is shorter for Cu and Zn than for other trace elements (Belon et al., 2012; Luo et al.,  
57 2009; Nicholson et al., 2003).

58 The environmental risk related to trace element input in agricultural soils via long-term OF application  
59 was recently assessed by a life-cycle assessment (LCA) approach and the findings showed that Cu and Zn  
60 were the trace elements that most contributed to aquatic and terrestrial ecotoxicity (Leclerc and  
61 Laurent, 2017; Sydow et al., 2018). The country-specific ecotoxicological impacts calculated by LCA were  
62 closely correlated with the total mass of trace elements introduced in soils by OF application in the  
63 concerned country but not with the trace element availability. This suggests that the environmental risk  
64 would mainly be related to the Cu and Zn contamination level rather than to changes in Cu and Zn  
65 availability in amended soils. However, this conclusion contradicts the analytical research findings which  
66 highlighted substantial changes in Cu and Zn availability in soils following long-term OF amendment.

67 Depending on the analytical approach adopted, OF application was shown to alternatively decrease or  
68 increase Cu and Zn availability in amended soils compared to the corresponding non-amended soils  
69 contaminated to the same extent with soluble Cu and Zn salts. Smolders et al. (2012) found that Cu was  
70 on average 6-fold less available in OF-amended soils than in the corresponding non-amended soils  
71 freshly spiked with  $\text{Cu}^{2+}$ . By contrast, Plaza et al. (2006, 2005) found that the Cu and Zn binding  
72 properties of humic substances were lower in OF-amended soils than in the corresponding non-amended  
73 soils, suggesting that Cu and Zn availability would be higher in OF-amended soils than in non-amended  
74 soils independently of the soil contamination level. Note that in all of these studies: (i) pH was set at the  
75 same level, and (ii) the difference in the organic matter metal binding properties did not account for the  
76 higher organic matter concentration usually found in OF-amended soils compared to non-amended soils.  
77 Hence, temporal variations in pH and organic matter content and properties usually observed in soil  
78 following long-term OF amendment were not accounted for.

79 Long-term OF application usually alters pH and organic matter levels in soils (Hargreaves et al., 2008;  
80 Smolders et al., 2012), i.e. two soil properties that are widely known to substantially influence Cu and Zn  
81 availability. The increase in dissolved organic matter (DOM) concentration and aromaticity usually  
82 observed in OF-amended soils likely boosts the total Cu concentration in soil solution (Amery et al. 2008;  
83 Araujo et al. 2019), while decreasing the  $\text{Cu}^{2+}$  activity, with the concomitant effect of increasing the pH,  
84 as usually observed in OF-amended soils (Bravin et al., 2012; Minnich and McBride, 1987). Zinc  
85 availability has been investigated to a much lesser extent than Cu in OF-amended agricultural soils.  
86 However, contrary to Cu, Zn is known to have much lower affinity for DOM (Bonten et al., 2008) such  
87 that the total Zn concentration and  $\text{Zn}^{2+}$  activity in soil solution are mainly driven by soil pH rather than  
88 by the DOM concentration (Bonten et al., 2008). Accordingly, the pH increase usually observed in OF-  
89 amended soils likely decreases the total Zn concentration and  $\text{Zn}^{2+}$  activity in soil solution.

90 Note therefore that long-term OF application on agricultural soils not only increases the Cu and Zn  
91 contamination level but also changes the pH and DOM concentration and their Cu and Zn binding  
92 properties. So far this issue has, however, only been studied by correlating Cu and Zn availability solely  
93 with the increased soil contamination level (e.g. Guan et al., 2018) or by assessing changes in Cu and Zn  
94 availability at a given soil contamination level (e.g. Smolders et al., 2012). Consequently, absolute  
95 changes in Cu and Zn availability due to long-term OF application on agricultural soils are still very  
96 difficult to infer in a comprehensive and mechanistic way.

97 Our study therefore aimed at comprehensively assessing Cu and Zn availability in soils that had been  
98 amended with OF over a decade, by unravelling the respective importance in soil of the increasing level  
99 of contamination versus the changes in pH and the concentration and binding properties of organic  
100 matter. Hence, 74 soil samples were collected from three field trials with different types of soil upon  
101 which no fertilizer, mineral fertilizers or OF had been applied over a decade. The soil samples exhibited a  
102 Cu and Zn contamination, pH and organic matter concentration gradient under these conditions. The soil  
103 Cu and Zn contamination level was assessed by measuring the total and DTPA-extractable Cu and Zn  
104 concentrations. The soil solution chemistry was characterized by measuring the pH and DOM  
105 concentration and aromaticity. Cu and Zn availability was comprehensively assessed through three  
106 proxies by deploying the diffusive gradient in thin film technique (DGT) on incubated soil samples and  
107 measuring the total Cu and Zn concentration and  $\text{Cu}^{2+}$  activity in soil solution. WHAM was used to  
108 estimate  $\text{Zn}^{2+}$  activity and DOM binding properties in soil solution.

109

## 110 **2. Material and Methods**

### 111 2.1. Soil and field trial characteristics

112 The three field trials studied were located on the island of Réunion in the Indian Ocean (55°30'E,  
113 21°05'S). They were conducted on soils belonging to three reference soil groups (hereafter called soil  
114 type): andosol, andic cambisol, and arenosol (WRB, 2014). The characteristics of each field trial and soil  
115 type were previously outlined by Nobile et al. (2018). Considering the mineralogy of the three soil types  
116 (Nobile et al. 2018), their ability to retain trace elements was expected to increase from the arenosol to  
117 the andosol. The main characteristics of the three soil types as a function of fertilization types are given  
118 in Table 1

119 The andosol and arenosol were under permanent grassland cover from 2004 to 2012. Plots were  
120 arranged in three randomized blocks and were either not fertilized or amended with mineral fertilizer  
121 ( $10 \text{ g N m}^{-2} \text{ y}^{-1}$ ), dairy slurry ( $20 \text{ to } 55 \text{ dm}^3 \text{ m}^{-2} \text{ y}^{-1}$ ), or dairy manure compost ( $2.5 \text{ to } 5 \text{ kg m}^{-2} \text{ y}^{-1}$ ) (Table S1;  
122 see Appendix A, Supplementary material). The cambisol was cultivated with two market garden crop  
123 cycles per year from 2004 to 2011. Plots were arranged in three randomized blocks and amended with  
124 mineral fertilizer ( $40 \text{ g N m}^{-2} \text{ y}^{-1}$ ), pig slurry compost ( $5 \text{ kg m}^{-2} \text{ y}^{-1}$ ), or poultry litter compost ( $7 \text{ kg m}^{-2} \text{ y}^{-1}$ )  
125 (Table S1). Soils were sampled once per year from the top soil layer, roughly corresponding to the  
126 fertilizer incorporation depth, i.e. 0-15 cm for the andosol and arenosol because there was no tillage and  
127 0-30 cm for the ploughed cambisol. The studied organic fertilizers in the three field trials were livestock  
128 effluents but the results obtained could also be extrapolated to other organic fertilizers (e.g. green waste  
129 compost, municipal organic waste, sewage sludge).

130 Amongst the yearly sampled soils, seventy-four soil samples (andosol,  $n = 34$ ; cambisol,  $n = 17$ ; arenosol,  
131  $n = 23$ ) were selected over time under all fertilization modalities in each field trial to ensure that the  
132 collected soil samples would exhibit a Cu and Zn contamination, pH and organic matter concentration  
133 gradient that could influence the Cu and Zn availability (Table 1 and Fig. S1). For each field trial, we  
134 selected one or more samples for each fertilization modality in each sampling year considered, to ensure

135 that the gradients observed could not be attributed to the comparison of samples from different  
136 sampling year. All analytical determinations and modelling calculations were performed without  
137 replication on composite samples prepared with an equal amount of soil from the three blocks.

138 The time-cumulated rate of Cu and Zn inputs by mineral and OF were calculated ( $F_{M-rate}$ , mg Cu or Zn kg<sup>-1</sup>  
139 soil) as follows:

$$F_{M-rate} = \frac{n \cdot r \cdot M}{dp \cdot \rho} \quad (1)$$

141 where  $n$  is the number of years since the beginning of fertilization,  $r$  is the yearly rate of OF or mineral  
142 fertilizer applied (kg dry matter m<sup>-2</sup> y<sup>-1</sup>),  $M$  is the Cu or Zn concentration in the OF or mineral fertilizer  
143 (mg Cu or Zn kg<sup>-1</sup> dry matter),  $dp$  is the soil depth (m), and  $\rho$  is the bulk soil density (kg m<sup>-3</sup>).

## 144 2.2. Comprehensive framework of Cu and Zn contamination and availability in soils

145 The Cu and Zn input in soil due to anthropogenic activities and notably fertilizer applications, i.e. soil Cu  
146 and Zn contamination, was assessed by measuring the concentration of total and DTPA-extractable Cu  
147 and Zn in soil. As Réunion soils are known to exhibit a high natural pedo-geochemical background for Cu  
148 and Zn related to the composition of the soil parent material (Doelsch et al., 2006) and the availability of  
149 this soilborne Cu and Zn is considered to be low (Doelsch et al., 2008), we assumed that the soil DTPA-  
150 extractable Cu and Zn concentration measurement would be more sensitive to cumulative inputs of Cu  
151 and Zn derived from organic fertilization than to the total soil Cu and Zn concentration.

152 The Cu and Zn availability, as defined by ISO (ISO 17402, 2008), was assessed with three proxies. DGT  
153 (DGT Research Ltd) was used to assess the re-supply of trace elements in soil solution from the solid  
154 phase (Zhang et al., 2004). The total Cu and Zn concentration in soil solution was measured to assess the  
155 size of the pool in which it is considered that soil organisms readily take up Cu and Zn. Cu<sup>2+</sup> and Zn<sup>2+</sup>

156 activity in soil solution was measured to assess the size of the pool of the main (if not the sole) species of  
157 Cu and Zn that was considered to be taken up by soil organisms. Moreover, the total Cu and Zn  
158 concentration and  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  activity in soil solutions was used to explain DGT measurement  
159 variations.

### 160 2.3. DTPA extractions from soils

161 Soil extraction with diethylenetriaminepentaacetic acid (DTPA) was performed according to Lindsay and  
162 Norvell, (1978). Briefly, soil extractions were conducted with a solution composed of 5 mM DTPA, 10 mM  
163  $\text{CaCl}_2$  and 100 mM triethanolamine buffered at pH 7.3, with a soil:solution ratio of 1:2. The soil-solution  
164 mixture was stirred for 2 h and then centrifuged at 1000 *g*, and the supernatant was filtered with ashless  
165 medium speed filter paper with an 8  $\mu\text{m}$  pore size. Solutions were analyzed with a flame atomic  
166 absorption spectrophotometer. The DTPA extraction procedure was validated by using an uncertified  
167 internal reference soil from Réunion when the extracted Cu and Zn recovery was within the 90-110 %  
168 range and the coefficient variation on the three replicated internal reference was within 10%.

### 169 2.4. DGT soil analysis

170 Each soil sample was analyzed with the DGT technique using devices equipped with a Chelex binding  
171 layer. Soils were incubated at 100% of their water holding capacity at 25°C for 24 h and the devices were  
172 then deployed on the soil surface for 24 h. DGT devices were finally removed from the soil samples and  
173 the Chelex layer was eluted in 1.2 ml of 1 M  $\text{HNO}_3$  for 24 h. Cu and Zn concentrations in eluates were  
174 determined by inductively coupled plasma-mass spectrometry (ICP-MS, ICAP Q, Thermo Scientific). DGT-  
175 available Cu and Zn was expressed as the Cu and Zn concentration at the interface between the soil and  
176 the DGT surface ( $C_{DGT}$ ,  $\text{mol l}^{-1}$ ) as follows:

$$177 \quad C_{DGT} = \frac{m \cdot \Delta y}{M \cdot D \cdot A \cdot t} \quad (2)$$



178 where  $m$  is the mass of Cu or Zn accumulated at the resin surface (g),  $\Delta g$  is the thickness of the diffusive  
179 layer plus filter paper ( $9.2 \cdot 10^{-3}$  dm),  $M$  is the molar mass of Cu or Zn ( $\text{g mol}^{-1}$ ),  $D$  is the diffusion  
180 coefficient of Cu or Zn ( $\text{dm}^2 \text{s}^{-1}$ ),  $A$  is the active surface area of the DGT device ( $3.14 \cdot 10^{-2}$   $\text{dm}^2$ ) and  $t$  is the  
181 soil-DGT contact time (s). Eight DGT controls were eluted without any soil exposure to assess the mean  
182 Cu and Zn background concentration in the DGT resins.

### 183 2.5. Soil solution extraction and analysis

184 The solution of each soil sample was extracted with 2.5 mM  $\text{Ca}(\text{NO}_3)_2$  and 3.5 mM  $\text{KNO}_3$  and analyzed as  
185 described by Djae et al. (2017). Each soil solution was subdivided and subsamples were stabilized with  
186 either  $\text{NaN}_3$  to avoid a shift in metal speciation while preventing DOM degradation for measurement of  
187  $\text{Cu}^{2+}$  activity and the specific ultraviolet absorbance (SUVA) at 254 nm ( $A_{254}$ ) or  $\text{HNO}_3$  for measurement of  
188 the dissolved organic carbon (DOC) and total major cation concentration. Total concentrations of major  
189 and trace cations (Ca, Mg, K, Na, Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn) were determined by ICP-MS. A  
190 solid-solution partitioning coefficient ( $K_D$  in  $\text{l kg}^{-1}$ ) for Cu and Zn ( $K_{\text{DCu}}$  and  $K_{\text{DZn}}$ ) was then calculated  
191 according to Sauvé et al. (2000):

$$192 \quad K_D = \frac{M_{\text{DTPA}}}{M_{\text{solution}}} \quad (3)$$

193 where  $M_{\text{DTPA}}$  is the DTPA-extractable Cu or Zn concentration from soil ( $\text{mg kg}^{-1}$ ) and  $M_{\text{solution}}$  is the total Cu  
194 or Zn concentration in the soil solution ( $\text{mg l}^{-1}$ ).

195 The DOC concentration was determined using a TOC analyzer (Schimadzu, TOC-L). The SUVA at 254 nm  
196 was measured by UV-visible spectrometry (Spectronic Unicam, Helios Beta). The SUVA of DOM ( $\text{l g}^{-1} \text{cm}^{-1}$ )  
197 was calculated as follows to estimate the DOM aromaticity (Weishaar et al., 2003):

$$198 \quad \text{SUVA} = \frac{A_{254}}{b \cdot \text{DOC}} \quad (4)$$

199 where  $A_{254}$  is dimensionless,  $b$  is the path length (1 cm) and  $DOC$  is the DOC concentration ( $\text{g l}^{-1}$ ).  
200 The pH was measured in parallel to  $\text{pCu}^{2+}$  (i.e.  $-\log_{10}\{\text{Cu}^{2+}\}$ ) using a combined electrode (Radiometer  
201 analytical PHC2051-8). Free Cu activity was measured using a  $\text{Cu}^{2+}$  specific electrode (Thermo Scientific  
202 Orion, 9629BNWP).

## 203 2.6. Modeling $\text{Cu}^{2+}$ and $\text{Zn}^{2+}$ activity

204 Free Cu and Zn activity in each soil solution was predicted using the humic ion-binding model VII  
205 included in WHAM (Tipping et al., 2011), as already described by Djae et al. (2017), to estimate the  $\text{Zn}^{2+}$   
206 activity and compare the experimental  $\text{Cu}^{2+}$  with theoretical calculations to assess the impact of the  
207 variability in DOM binding properties on the determination of Cu and Zn speciation in soil solution. Free  
208 Cu and Zn activities were predicted by three approaches, and for each of these the deviation between  
209 predicted and measured  $\text{Cu}^{2+}$  values was determined by calculating the regression slope and coefficient  
210 as well as the root mean square residual (RMSR). Free Cu and Zn activity were first predicted as  
211 commonly described in the literature with the WHAM default settings (Tipping et al., 2011). Accordingly,  
212 the DOM site density (hereafter referred to as  $\%r\text{-DOM}$ ) was fixed by considering that 65% of DOM was  
213 able to bind metal cations with the same binding constants as the colloidal fulvic acid defined in WHAM  
214 (e.g.  $\log K_{\text{Cu}}$  and  $\log K_{\text{Zn}}$ , of 2.16 and 1.68, respectively), with the remaining 35% of DOM being inert. Free  
215 Cu and Zn activities were also predicted by optimizing the  $\%r\text{-DOM}$  by relating it to the SUVA values of  
216 DOM, as previously suggested by Amery et al. (2008). Free Cu activity was finally predicted by adjusting  
217  $\%r\text{-DOM}$  and  $\log K_{\text{Cu}}$  to within physically meaningful value ranges, i.e. respectively 35-215% and 1.84-  
218 2.46, as previously suggested by Djae et al. (2017). Free Zn activity was then calculated on the basis of  
219  $\text{Cu}^{2+}$  prediction using the same adjusted  $\%r\text{-DOM}$ , while  $\log K_{\text{Zn}}$  remained at the default value. The  
220 uncertainty in predicted  $\text{pCu}^{2+}$  and  $\text{pZn}^{2+}$  (i.e.  $\pm 2$  standard deviation) resulting from analytical  
221 uncertainties regarding the pH ( $\pm 0.2$ ), total Cu concentration ( $\pm 0.4\%$ ), total Zn concentration ( $\pm 1.1\%$ )

222 and DOM concentration ( $\pm 12$  and 40% for the first and second modelling approaches, respectively) was  
223 calculated using the Monte Carlo procedure (1999 simulations/sample) included in WHAM.

## 224 2.7. Data processing and analysis

225 The whole dataset is accessible via Dataverse (Laurent et al., 2019). All statistical analyses were  
226 performed with R software (R Core Team 2016). Differences were considered statistically significant if  
227  $p < 0.05$ . The effects of the fertilization types (none, mineral, or organic) on the soil variables (i.e. pH,  
228 organic carbon, total Cu and Zn concentrations, and DTPA-extractable Cu and Zn) were assessed for each  
229 soil by non-parametric tests, i.e. the Kruskal-Wallis rank test and the Dunn post-hoc multiple test  
230 comparison, as the residual normality and homoscedasticity conditions were not satisfied. The effects of  
231 soil (andosol, cambisol or arenosol) or fertilization types on soil DGT and solution values were assessed  
232 by performing either a one-way ANOVA and Tukey HSD post-hoc multiple comparison test when residual  
233 normality and homoscedasticity conditions were satisfied, or non-parametric rank tests (the same as  
234 above) when the conditions were not satisfied.

235 Principal components analysis (PCA) was used to visually illustrate the respective effects of soil and  
236 fertilization types on DTPA-extractable Cu and Zn concentration, the solution chemistry and Cu and Zn  
237 availability in soils without accounting for pH and organic carbon content which were strongly correlated  
238 to soil solution pH and DOC concentration. PCA was performed with FactoMineR, factoextra and  
239 missMDA packages. Multi-linear regressions were then used to mathematically characterize and  
240 hierarchize the respective contributions of pH, DOC, SUVA, DTPA-extractable Cu and Zn concentration in  
241 soils, and  $K_D$  on the three proxies of Cu and Zn availability (i.e. DGT measurement in soil as well as the  
242 total concentration and free ionic activity in soil solution). Multi-linear regressions were performed using  
243 two R packages (olsrr and MASS), thereby combining two approaches based on stepwise regressions and  
244 the Akaike information criteria.

245

### 246 **3. Results and Discussion**

#### 247 3.1 Organic fertilization over a decade induces copper and zinc contamination in soil

248 While soil Cu and Zn inputs due to organic fertilization were much higher than those due to mineral  
249 fertilization or without any fertilization (Fig. S2), total Cu and Zn concentrations in soils receiving organic  
250 fertilization did not clearly increase compared to non-fertilized soils and soil receiving mineral  
251 fertilization (Table 1). This could first be explained by the fact that the initial total Cu and Zn contents in  
252 the three soils were high (Table 1) and even about 5-fold higher than the cumulative Cu and Zn input due  
253 to organic fertilization over a decade in the arenosol for Cu and in the cambisol for Cu and Zn. Réunion  
254 soils are indeed known to exhibit a high natural pedo-geochemical background for Cu and Zn (Døelsch et  
255 al., 2006). This could also be explained by the high Cu and Zn loss from the upper soil layer due to  
256 erosion and leaching, particularly in the andosol and arenosol in which OF were not mechanically  
257 incorporated. The magnitude of these processes was not determined, but the yearly rainfall is known to  
258 be high under the tropical climatic conditions that prevail in Réunion, ranging from 1 000 to 2 000 mm on  
259 average (Météo France, 2017), which could partly flush away any OF not incorporated in the soil. The  
260 cumulative Cu and Zn input due to organic fertilization that actually remained in the soil was thus not  
261 sufficient to induce a clear increase in the total soil Cu and Zn concentration.

262 As soilborne Cu and Zn availability is considered to be low in Réunion soils (Doelsch et al., 2008), we  
263 assumed that the DTPA-extractable Cu and Zn concentration, which represented less than 10 % of the  
264 total Cu and Zn, would be more sensitive to cumulative inputs of OF-derived Cu and Zn in soil than the  
265 total Cu and Zn concentration. In agreement, OF significantly increased the DTPA-extractable Cu and Zn  
266 concentration in soils compared to the non-fertilized treatment or to mineral fertilization (Fig. 1). This

267 result is consistent with the expected moderate but chronic Cu and Zn contamination of soils under OF  
268 and this pattern could be better highlighted through DTPA extraction than by measuring the total  
269 concentration.

270 3.2 Soil and fertilization types substantially but differently influence the solution chemistry and copper  
271 and zinc availability in soils

272 Soil and fertilization types independently structured the distribution of the dataset for the soil solution  
273 chemistry and Cu and Zn availability proxies in the PCA (Fig. 2). The first component (PC1, 40% of the  
274 explained variance) was mainly determined via the pH,  $pZn_{SS}$ ,  $pZn^{2+}$  and SUVA findings, the second  
275 component (PC2, 21% of the explained variance) was mainly determined by DOC and Zn-DTPA, while  
276  $pCu_{SS}$ ,  $pCu^{2+}$  and  $pZn_{SS}$  contributed to both PC1 and PC2 (Fig. 2a). However,  $pCu_{DGT}$ , Cu-DTPA and even  
277 more so  $pZn_{DGT}$  contributed very little to PC1 and PC2, in accordance with their small variation ranges.  
278 The soil types were clearly dissociated in two clusters along the PC2 (Fig. 2b). This clustering according to  
279 soil type was related to the DOC concentration ranges, which significantly increased from 9-30 mg l<sup>-1</sup> in  
280 the arenosol to 23-107 mg l<sup>-1</sup> in the andosol (Fig. S3b) and similar Zn-DTPA variation ranges in the  
281 andosol and cambisol compared to the arenosol (Fig. 1). Conversely, the mineral and organic fertilization  
282 types were dissociated in two independent clusters along the PC1 with the no fertilization modality in  
283 between (Fig. 2c). Accordingly, these results were consistent with the fact that the soil and fertilization  
284 types both had a substantial but different influence on the solution chemistry and Cu and Zn availability  
285 proxies in the soils.

286 By performing PCA separately on each soil type, the effects of the fertilization types on the solution  
287 chemistry and on the Cu and Zn availability proxies in soils were even more clearcut (Fig. 3). Both PC1  
288 and PC2 explained 75%, 84% and 69% of the variance for the andosol, cambisol and arenosol,  
289 respectively (Fig. 3a, c, and e). Regardless of the soil type, PC1 was mainly determined via the pH, DOC,

290 SUVA, Cu-DTPA, Zn-DTPA,  $pCu_{SS}$ ,  $pZn_{SS}$ , and  $pZn^{2+}$  findings, while  $pCu_{DGT}$ ,  $pZn_{DGT}$  and  $pCu^{2+}$  contributed to  
291 PC1 or PC2, or to both depending on the soil type. For each soil, the fertilization types were clearly  
292 dissociated in two (Fig. 3d) or three (Fig. 3b and f) clusters along the PC1 axis. These results highlight the  
293 substantial and similar relative influence of the fertilization type on the solution chemistry and Cu and Zn  
294 availability proxies in all of the soils, with a clear distinction between mineral and organic fertilization.

295 3.3 The increase in pH and DOM induced by organic fertilization mitigates the copper and zinc availability  
296 in soil

297 Organic fertilization significantly increased the soil solution pH for the three soil types compared to  
298 mineral fertilization (Table 1; Figs. S4a, S5a and S6a). The soil solution pH increased by up to 0.7 u (unit)  
299 with OF compared to no fertilization in the andosol and arenosol, and also compared to mineral  
300 fertilization in the cambisol. By contrast, the soil solution pH decreased by up to 0.4 u with mineral  
301 fertilization compared to no fertilization in the andosol and arenosol. This increase in pH after long-term  
302 OF application has already been reported (Haynes and Mokolobate, 2001; Vanden Nest et al., 2016) and  
303 was attributed to a range of processes, notably including the decarboxylation of organic anions during  
304 organic C mineralization that releases  $OH^-$  and the protonation of organic molecules that consume  $H^+$   
305 (Mkhabela and Warman, 2005; Shi et al., 2019; Yan et al., 1996). A similar decrease in pH with long-term  
306  $NH_4NO_3$  application has also been previously reported (Stroia et al., 2011) and attributed to the  
307 production of protons associated with  $NH_4^+$  nitrification that was not completely balanced by  $OH^-$   
308 production associated with the incomplete  $NO_3^-$  consumption by plants. Accordingly, a decade of organic  
309 fertilization strongly alkalinized the soil, while mineral N fertilization tended to acidify the soil.

310 Organic fertilization increased the soil organic C concentration (Table 1) as well as (by almost two-fold)  
311 the DOC concentration and SUVA in the soil solution for the three soil types compared to mineral or no  
312 fertilization (Figs. S4b&c, S5b&c, and S6b&c). The increase in DOC concentration following OF application

313 was consistent with previous findings (Cambier et al., 2014; Seo et al., 2018; Wright et al., 2008) and was  
314 attributed to the input of organic matter whose solubilization was further favored by the increase in soil  
315 pH (Bolan et al., 2011). Araújo et al. (2019) similarly found an increase in SUVA in the solution of a soil  
316 that had received dairy manure amendments for 3 years, which suggests an increase in DOM  
317 aromaticity. We thus conclude that a decade of organic fertilization increased the soil DOM  
318 concentration and aromaticity.

319 The total Cu concentration in soil solution significantly increased in the three soil types with organic  
320 fertilization compared to no fertilization and/or mineral fertilization, but the total Zn concentration in  
321 soil solution significantly decreased with organic fertilization (Figs. S4d&g, S5d&g and S6d&g). These two  
322 distinct effects could be explained by the fact that the main chemical parameters driving the total Cu and  
323 Zn concentration in soil solution differed for Cu and Zn (Table 2). The increase in total Cu concentration  
324 in soil solution was mainly driven by the increased DOC concentration and SUVA. Conversely, the  
325 decrease in total Zn concentration in soil solution was mainly driven by the increase in the soil solution  
326 pH. DTPA-extractable Cu and Zn in soil was only a secondary variable that could explain the variation in  
327 the total Cu and Zn concentration in soil solution, except for Zn in the cambisol. In this latter soil, DTPA-  
328 extractable Zn was the sole explanatory variable of the total Zn in solution, which had surprisingly  
329 decreased as the DTPA-extractable Zn increased. This negative correlation between the DTPA-  
330 extractable Zn and the total Zn in solution could, however, be explained by the indirect effect of pH. The  
331 concomitant increase in the Zn contamination and pH levels in soil under organic fertilization increased  
332 Zn sorption to the soil solid phase and consequently increased the DTPA-extractable Zn, while decreasing  
333 the total Zn concentration in soil solution.

334 The effects of organic fertilization observed on the total Cu and Zn concentration in soil solution were  
335 generally consistent with previously reported results. While some authors have reported that the Cu and

336 Zn concentration in soil solution increased with organic fertilization, they found that the total Cu  
337 concentration in soil solution was mainly driven by the increase in DOC concentration, while the total Zn  
338 concentration was mainly driven by the soil solution pH (Cambier et al., 2014; De Conti et al., 2016;  
339 Pérez-Esteban et al., 2012). Araújo et al. (2019) also found a positive correlation between the total Cu  
340 concentration in soil solution and SUVA in a soil after dairy manure application for 3 years. The well-  
341 known high affinity of Cu for DOM could explain why the Cu release in the soil solution with organic  
342 fertilization was mainly driven by complexation with DOM, which depends on the DOM concentration  
343 and aromaticity, despite the concomitant increase in soil solution pH that increased the binding capacity  
344 of the soil solid phase for Cu. Conversely, the lower affinity of Zn for DOM as compared to Cu could  
345 explain why Zn removal from the soil solution with organic fertilization continued to be driven by  
346 deprotonation of the soil solid phase as the soil solution pH increased. This explanation is in line with our  
347 results (Fig. S7) and those found in the literature (Bonten et al., 2008), showing that Cu was almost  
348 completely complexed with DOM in soil solution, while Zn occurred mainly as  $Zn^{2+}$ .

349  $Cu^{2+}$  and  $Zn^{2+}$  activity similarly decreased in the solutions of the three soil types with organic fertilization  
350 compared to no fertilization and mineral fertilization (Figs. S4e&h, S5e&h and S6e&h). The decrease in  
351  $Cu^{2+}$  and  $Zn^{2+}$  activity in soil solution was mainly driven by the decrease in soil solution pH, either directly  
352 for  $Cu^{2+}$  or indirectly for  $Zn^{2+}$ . Indeed,  $Zn^{2+}$  activity was mainly driven by total Zn in soil solution, which  
353 itself was mainly driven by the soil solution pH (Tables 2 and 3). A similar decrease in  $Cu^{2+}$  and  $Zn^{2+}$   
354 activity with increasing pH has already been reported (Bravin et al., 2012; Ma et al., 2006; Vulkan et al.,  
355 2000), including in the organic fertilization context (De Conti et al., 2016). The deprotonation of DOM  
356 and of the soil solid phase with increasing soil solution pH under organic fertilization could likely explain  
357 the decrease in  $Cu^{2+}$  and  $Zn^{2+}$  activity, respectively.



358 DGT-available Cu and Zn did not significantly change when comparing the different fertilization types in  
359 the andosol and arenosol (Figs. S4f&i and S6f&i). In the cambisol, however, DGT-available Cu increased  
360 with organic fertilization compared to mineral fertilization, while DGT-available Zn decreased (Fig. S5f&i).  
361 No physical-chemical parameter could significantly explain the DGT-available Cu and Zn, except in the  
362 cambisol, where the increase in DGT-available Cu concentration was driven by the increase in total Cu  
363 concentration in soil solution and the decrease in SUVA (Table S2).

364 The measurement of DGT-available metals in soil includes: (i) equilibrium processes that govern the  
365 solid-solution partitioning of available metals ( $K_D$ ), and (ii) kinetic processes that govern metal lability  
366 (Degryse et al., 2009). DGT-available Cu and Zn was reported to increase in soils receiving long-term OF  
367 applications (Grüter et al., 2017; Guan et al., 2018). In both studies, the substantial increase in the total  
368 Cu and Zn concentration in soils likely increased the corresponding  $K_D$  and consequently drove the  
369 increase in DGT-available Cu and Zn. In our study,  $K_D$  was not significantly correlated with DGT-available  
370 Cu and Zn (Table S2). Accordingly, it is likely that DGT-available Cu and Zn were driven by kinetic  
371 processes rather than equilibrium processes.

372 Although we did not perform any kinetic measurements, changes in the total Cu and Zn concentration  
373 and activity in soil solution were indicators of Cu and Zn lability in the soil solid phase and in the soil  
374 solution, respectively. The total Cu concentration increased while  $\text{Cu}^{2+}$  activity decreased in the solution  
375 of the three soil types with organic fertilization, thus indicating that Cu lability was more or less the same  
376 whatever the fertilization type. In addition, the negative correlation between the SUVA and DGT-  
377 available Cu in the cambisol, while DGT-available Cu was positively correlated with the total Cu  
378 concentration in soil solution, suggests that Cu bound to aromatic DOM, such as phenolic compounds,  
379 was weakly labile, in agreement with previous findings obtained under laboratory conditions (Amery et  
380 al., 2010; Kalis et al., 2006; Welikala et al., 2018). The total Zn concentration and  $\text{Zn}^{2+}$  activity decreased

381 in the solution of the three soil types with organic fertilization, implying that Zn lability decreased with  
382 organic fertilization. However, DTPA-extractable Zn substantially increased in the three soil types with  
383 organic fertilization (Figs. 1d, e&f), which may have counteracted the decrease in Zn lability, resulting in  
384 a similar DGT-available Zn concentration regardless of the fertilization type.

385 3.4 The increase in DOM binding properties induced by organic fertilization drives copper but not zinc  
386 speciation in soil solution

387 Whatever the soil type, measured  $\text{Cu}^{2+}$  activity in soil solution was partially predicted by multi-linear  
388 regression ( $\text{adjR}^2 = 0.5\text{-}0.64$ ), while  $\text{Zn}^{2+}$  activity estimated with WHAM was accurately predicted by  
389 multi-linear regression ( $\text{adjR}^2 = 0.97\text{-}0.99$ ; Table 3). In addition, measured  $\text{Cu}^{2+}$  activity was also partially  
390 predicted with WHAM using default parameters, irrespective of the soil type ( $\text{adjR}^2 = 0.54$  and  
391  $\text{RMSR} = 0.74$ ; Fig. 4a). Indeed, WHAM prediction tended to respectively over- and under-estimate  
392 measurements above and below 10  $\text{pCu}^{2+}$  units. Djae et al. (2017) and Li et al. (2017) similarly showed, in  
393 a broad range of soils, that using the WHAM default settings did not enable them to adequately predict  
394  $\text{Cu}^{2+}$  activity in soil solution. Moreover, Djae et al. (2017) showed, in a sensitivity analysis, that DOM  
395 binding properties (i.e.  $\%r\text{-DOM}$  and  $\log K_{\text{Cu}}$ ) were the most sensitive parameters in soil solution to  
396 optimize WHAM to achieve adequate  $\text{Cu}^{2+}$  activity predictions. Accordingly, accounting for the variability  
397 in DOM binding properties in addition to other soil solution parameters could have improved the  
398 prediction of  $\text{Cu}^{2+}$  activity in the soil solutions we studied. However,  $\text{Zn}^{2+}$  activity is likely much less  
399 sensitive to DOM binding properties due to the much lower affinity of  $\text{Zn}^{2+}$  for DOM compared to  $\text{Cu}^{2+}$   
400 (see section 3.3), thus explaining why it seems unnecessary to account for the variability in DOM binding  
401 properties to adequately predict  $\text{Zn}^{2+}$  activity in soil solutions.

402 Whatever the soil type, WHAM parameterization by optimizing the  $\%r\text{-DOM}$  of each soil solution with  
403 their respective SUVA only slightly improved the prediction of measured  $\text{Cu}^{2+}$  activity ( $\text{adjR}^2 = 0.55$  and

404 RMSR = 0.56; Fig. 4b) compared to the prediction obtained with WHAM using default parameters.  
405 Contrary to our results, the WHAM parameterization carried out by Amery et al. (2008), whereby %*r*-  
406 DOM was optimized with SUVA, improved the prediction of Cu binding to DOM and consequently of Cu  
407 speciation in soil solution. Note that Amery et al. (2008) studied soil solutions that were barely impacted  
408 by anthropogenic inputs as the solutions were collected at 45-cm depth in soil that was either not  
409 fertilized or fertilized with a single application of pig manure at a moderate rate (i.e. 20 m<sup>3</sup> ha<sup>-1</sup>).  
410 Contrary to Amery et al. (2008), our solutions were extracted from soils that received high  
411 anthropogenic inputs through OF applications at relatively high rates (see section 2.1) over a decade.  
412 Baken et al. (2011) showed that the Cu binding properties of DOM were no longer driven by its  
413 aromaticity in freshwaters impacted by high anthropogenic inputs related to the discharge of human  
414 effluents and livestock waste. In freshwater systems, such anthropogenic inputs led to the presence of  
415 organic molecules such as aminopolycarboxylates exhibiting a high affinity for Cu but low aromaticity  
416 compared to humic substances that commonly occur in natural DOM. Based on the findings of Baken et  
417 al. (2011), we considered that the application of OF over a decade may have altered the DOM binding  
418 properties towards Cu by introducing some organic molecules with a high affinity for Cu but with low  
419 aromaticity, which could explain why the DOM binding properties towards Cu were not driven by DOM  
420 aromaticity. However, the nature of these added organic molecules and their binding properties are  
421 unknown and would require further investigation. Consequently, our results suggest that, although  
422 organic fertilization increased DOM aromaticity (see section 3.3), DOM aromaticity was not a good proxy  
423 of DOM binding properties towards metals and consequently of Cu speciation in the solution of soils that  
424 had received OF over a decade.

425 Whatever the soil type, WHAM parameterization by manually optimizing the %*r*-DOM and then the  
426 logK<sub>Cu</sub> in each soil solution within physically meaningful ranges substantially improved the prediction of  
427 measured Cu<sup>2+</sup> activity compared to predictions conducted with default parameterization or SUVA

428 optimization of WHAM ( $\text{adj}R^2 = 0.94$  and  $\text{RMSR} = 0.21$ ; Fig. 4c). The optimized %*r*-DOM and  $\log K_{\text{Cu}}$  values  
429 came within the variation range we set for these two parameters, as previously observed for the WHAM  
430 prediction of  $\text{Cu}^{2+}$  activity in 72 soil samples exhibiting a very broad range of physical-chemical properties  
431 (Djae et al. 2017; Li et al. 2017). These results suggest that organic fertilization induced substantial  
432 changes in DOM binding properties, which must be accounted for to adequately predict Cu speciation in  
433 soil solution using geochemical models.

434 Whatever the soil type, the proportion %*r*-DOM higher than 35% and of  $\log K_{\text{Cu}}$  equal to 2.16 (while the  
435 other  $\log K_{\text{Cu}}$  values were less than 2.16) optimized in WHAM was higher with organic fertilization than  
436 without fertilization or with mineral fertilization, except for the proportion of  $\log K_{\text{Cu}}$  equal to 2.16 in the  
437 cambisol, which was equivalent to the proportion with organic and mineral fertilization (Fig. S8). This  
438 suggests that organic fertilization over a decade tended to increase the binding properties of DOM  
439 compared to no fertilization or mineral fertilization by increasing both the DOM binding site density and  
440 the DOM binding affinity towards Cu.

441 To the best of our knowledge, previous studies on the impact of organic fertilization on DOM binding  
442 properties towards soilborne metals were focused on the determination of the Cu binding properties of  
443 fulvic and humic acids extracted from OF, OF-amended soils and non-amended soils (Bolan et al., 2011).  
444 The comparison of our results with these data appeared particularly relevant as geochemical models  
445 such as WHAM have been developed from characterization of the ion binding properties of humic  
446 substances (Tipping 1998). It is noteworthy that this approach has led to conflicting results in the  
447 literature. In partial agreement with our results, Sebastia et al. (2007) found that fulvic acids in a soil  
448 amended with straw or conifer compost over 15 years bound Cu more strongly than fulvic acids in the  
449 non-amended soil, while the binding site density of fulvic acids and their Cu binding affinity respectively  
450 decreased and increased in the amended soil. Plaza et al. (2005 and 2006) found, however, that fulvic

451 and humic acids in soils amended with either pig slurry or sewage sludge bound Cu less strongly than  
452 fulvic and humic acids in the corresponding non-amended soils, with a consistent decrease in both the  
453 binding site density and Cu binding affinity of fulvic and humic acids in the amended soils.

454 Whatever the reason for the discrepancy between the findings of Sebastia et al. (2007) and Plaza et al.  
455 (2005 and 2006), the fact that these authors opted to study the binding properties of soil DOM by  
456 focusing on humic substances was recently the focus of intensive debate in the literature (Lehmann and  
457 Kleber, 2015; Olk et al., 2019), and this strategy is questionable in the light of recent literature findings.  
458 Firstly, Groenenberg et al. (2010) and Ren et al. (2015) showed that fulvic and humic acids represented  
459 less than 50 and 5% of soil DOC, respectively. This strongly suggests that humic substances only partially  
460 contribute to DOM binding properties, thus indicating that the nature and binding properties of non-  
461 humic substances in DOM would require further investigation. Secondly, humic substances are usually  
462 extracted from soils with a procedure involving alkaline, acid, and then purification steps (see e.g.  
463 Sebastia et al., 2007), which is much harsher than the soft procedure that we used to extract DOM from  
464 the soil solution. It is highly suspected that the procedure used to extract soil humic substances may  
465 alter the chemical nature of functional groups able to bind metals and even more so the molecular  
466 structure of humic substances, although the extent of such alterations is still under debate (Lehmann  
467 and Kleber, 2015; Olk et al., 2019), finally resulting in metal binding properties different from those of  
468 less disturbed humic substances in extracted soil solution. In agreement with that view, Botero et al.  
469 (2017) recently showed that the last step of the soil humic acid extraction procedure (i.e. HF/HCl  
470 treatment followed by dialysis) was able to alter the  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Pb}^{2+}$  concentration by one order of  
471 magnitude. This alteration was substantial enough to necessitate a specific optimization of the binding  
472 properties of humic acids in the model used to predict metal speciation. While the extent of the bias  
473 induced by the extraction procedure used for humic substances requires further investigation, it would

474 be more relevant to study DOM binding properties towards metals directly in extracted soil solutions as  
475 we did to maximally reduce potential analytical artefacts.

476 Note finally that we were unable to adequately predict (i.e. within  $\pm 0.5$  pCu<sup>2+</sup> unit) measured Cu<sup>2+</sup>  
477 activity in three solutions extracted from the arenosol and one solution extracted from the andosol for  
478 which Cu<sup>2+</sup> was underestimated by up to 1 pCu<sup>2+</sup> unit (Fig. 4c). To adequately predict Cu<sup>2+</sup> activity with  
479 WHAM in these four soil solutions, it was necessary to set the %*r*-DOM between 5 and 30% while  
480 maintaining the logK<sub>Cu</sub> at 1.84 (Fig. S9). Such low %*r*-DOM values are below the range determined  
481 analytically for 401 DOM, including standard and reference fulvic acids, humic acids, and natural organic  
482 matter (Ritchie and Perdue, 2003). This could possibly be explained by the fact that the DOM  
483 composition in these four soil solutions exhibited a high proportion of molecules with very low Cu  
484 binding properties, such as sugars. This hypothesis is supported by the particularly high percentage (i.e.  
485 65-80%) of non-humic substances (including sugars) usually recovered in soil solutions exhibiting the  
486 lowest DOM concentration (Groenenberg et al., 2010; Ren et al., 2015), and secondly by the fact that the  
487 four soil solutions were collected from the two trials in fields with permanent fodder crop cover. Fodder  
488 crops are known to exude a large amount of organic molecules in their rhizosphere, with the bulk of  
489 them being composed of free sugars (Jones et al., 2009). This hypothesis underlines the need to further  
490 investigate the contribution of non-humic substances to metal binding in soil solution.

491

#### 492 **4. Conclusion**

493 Organic fertilization over a decade induced, as expected, ongoing soil Cu and Zn contamination  
494 compared to no fertilization or mineral fertilization. But we also showed that organic fertilization  
495 substantially increased some major soil physical-chemical properties, namely pH, organic C

496 concentration and the concentration and aromaticity of DOM along with its Cu binding properties. The  
497 similar alterations in the physical-chemical properties observed for the three studied soil types suggest  
498 that the effects of organic fertilization might generally apply to a broad range of initially acidic soils.

499 Considering the low affinity of Zn for DOM, Zn availability in OF-amended soils was closely related to the  
500 increase in soil pH. The increase in soil pH induced by organic fertilization decreased the total Zn  
501 concentration and  $Zn^{2+}$  activity in soil solution. Accordingly, and despite a marked increase in soil Zn  
502 contamination, DGT-available Zn was equal or lower in soils amended with OF compared to soils  
503 receiving either no fertilizer or mineral fertilizers.

504 Considering the high affinity of Cu for DOM, Cu availability in OF-amended soils was closely related to the  
505 concomitant increase, in soil solution, of the pH and DOM concentration, aromaticity and binding  
506 properties towards Cu. The increase in DOM concentration and aromaticity induced by organic  
507 fertilization increased the total Cu concentration in soil solution. By contrast, the increase in pH and  
508 DOM binding properties induced by organic fertilization decreased  $Cu^{2+}$  activity in soil solution.  
509 Accordingly, and despite a marked increase in soil Cu contamination, DGT-available Cu was equal or only  
510 slightly higher in OF-amended soils compared to soils receiving either no fertilizer or mineral fertilizers.

511 Our results consistently suggested that alterations in the soil physical-chemical properties regulate Cu  
512 and Zn availability in soils receiving long-term organic fertilization by exerting a protective effect that  
513 offsets the concomitant increase in the soil Cu and Zn contamination level. This protective effect of  
514 organic fertilization was, however, less clear for Cu than for Zn due to the opposite effect of DOM on the  
515 total Cu concentration and  $Cu^{2+}$  activity in soil solution. From an ecotoxicological perspective, it remains  
516 questionable whether such a protective effect would also be able to regulate Cu and Zn bioavailability  
517 and toxicity to organisms living in soils receiving long-term OF applications.

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530

531 **Appendix A. Supplementary material**

532 Supplementary material to this article can be found online at <https://doi...>

533

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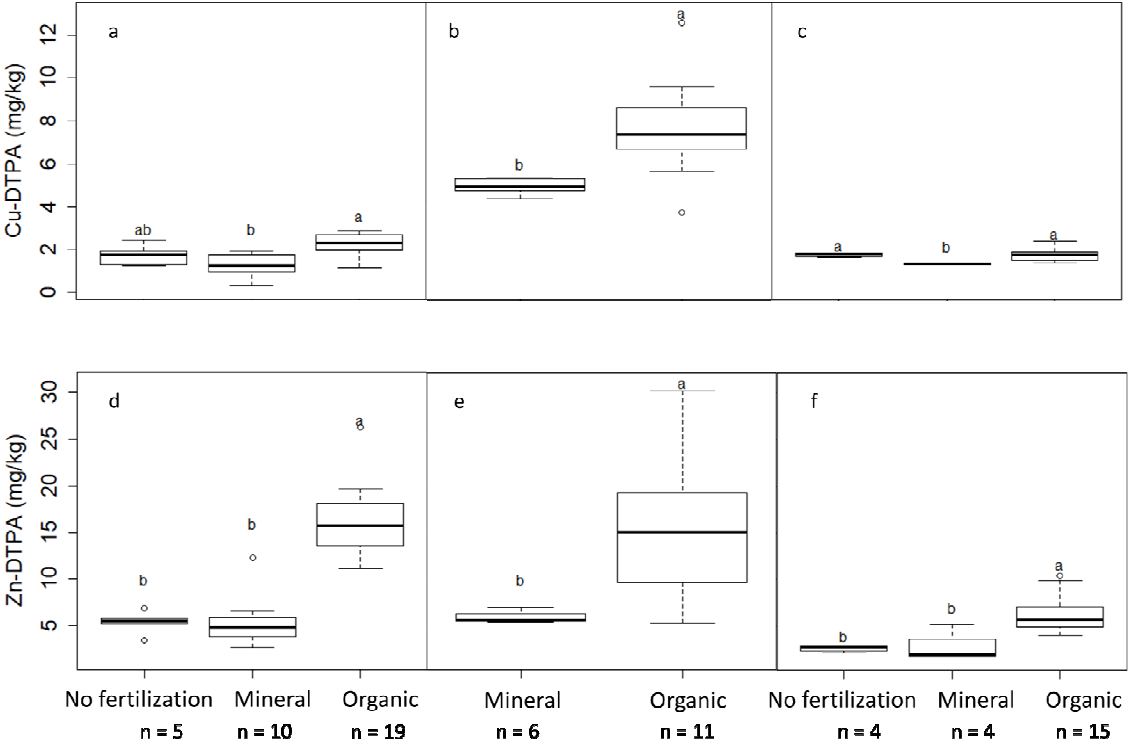
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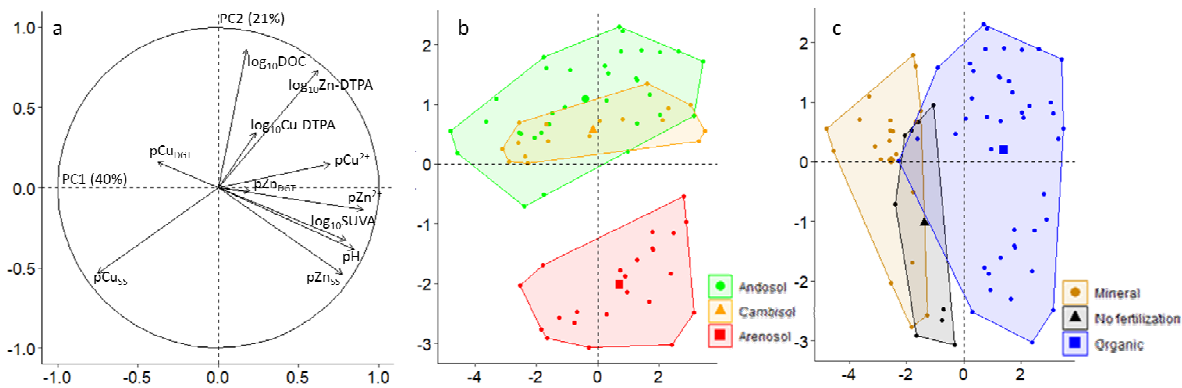
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**Figure 1** DTPA-extractable concentration of copper (Cu) and zinc (Zn) in the andosol (a and d), the cambisol (b and e) and the arenosol (c and f) as a function of the fertilization type (No fertilization, Mineral or Organic). Different letters indicate a significant difference at the 0.05 level. The values represented from the bottom to the top indicate the minimum, first quartile, median, third quartile, and maximum values. Where there are outliers, they are represented by circles and the related vertical dashed bars represent the whiskers, i.e. 1.5-times the interquartile range above the third quartile or below the first quartile.

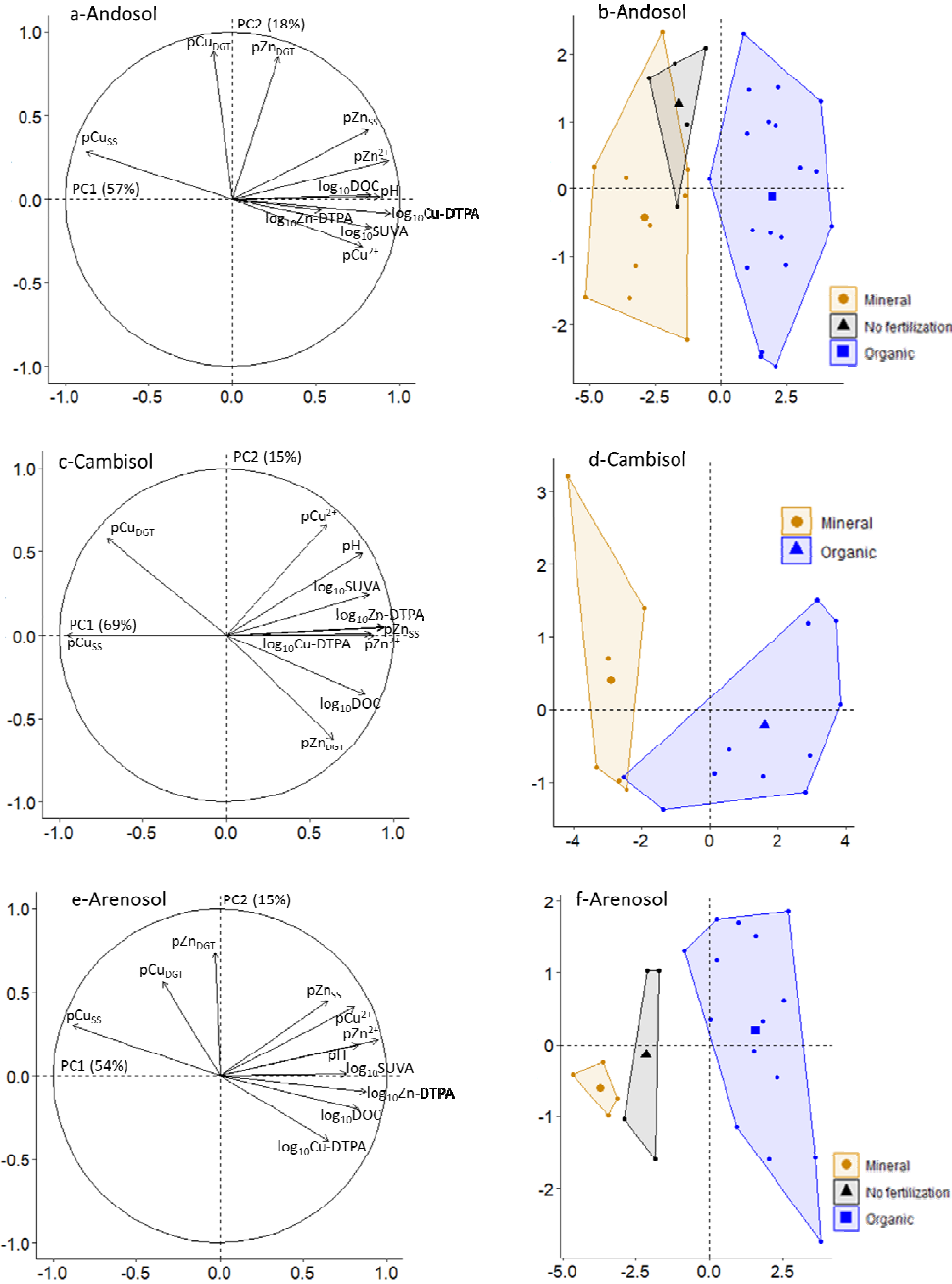


**Figure 2** Principal component analysis (PCA) of the dataset on the soil solution chemistry and copper (Cu) and zinc (Zn) contamination and availability proxies in soils. The correlation plot (a) shows the contribution of each variable to the two first principal components (PC1 and PC2). Soil samples ( $n = 74$ ) were clustered (b) as a function of soil types (andosol, cambisol, and arenosol) or (c) as function of fertilization types (No fertilization, Mineral, or Organic). Data points with a larger size in (b) and (c) correspond to the barycenter of each cluster. The quantitative variables considered in the PCA are pH, the dissolved organic carbon concentration ( $\log_{10}\text{DOC}$ ), the specific UV-absorbance of the soil solutions at 254 nm ( $\log_{10}\text{SUVA}$ ), the DTPA-extractable Cu and Zn concentration ( $\log_{10}\text{Cu-DTPA}$  and  $\log_{10}\text{Zn-DTPA}$ ), the DGT-available Cu and Zn concentration ( $\text{pCu}_{\text{DGT}}$  and  $\text{pZn}_{\text{DGT}}$ ), the total Cu and Zn concentration in soil solution ( $\text{pCu}_{\text{SS}}$  and  $\text{pZn}_{\text{SS}}$ ), and the free ionic Cu and Zn activity in soil solution ( $\text{pCu}^{2+}$  and  $\text{pZn}^{2+}$ ).

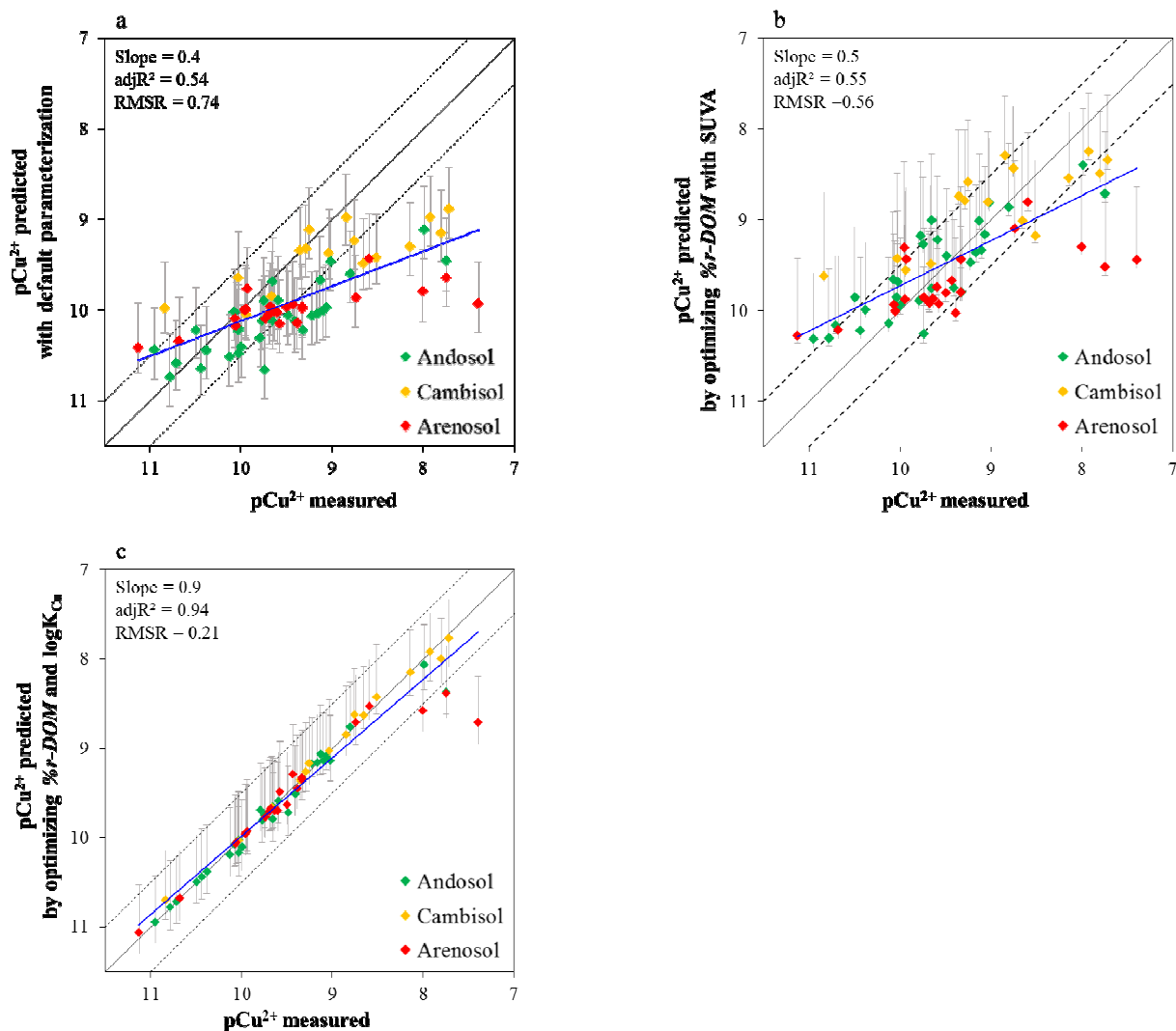




**Figure 3** Principal component analysis (PCA) on the soil solution chemistry and copper (Cu) and zinc (Zn) contamination and availability proxies in (a) the andosol (a and b;  $n = 34$ ), (b) the cambisol (c and d;  $n = 17$ ) and (c) the arenosol (e and f;  $n = 23$ ). The correlation plots (a, c, and e) show the contribution of each variable to the two principal components (PC1 and PC2). Soil samples were clustered (b, d, and f) as a function of fertilization types (No fertilization, Mineral, or Organic). Data points with a larger size in (b), (d), and (f) correspond to the barycenter of each cluster. The quantitative variables considered in the PCA are pH, dissolved organic carbon concentration ( $\log_{10}\text{DOC}$ ), the specific UV-absorbance of the soil solutions at 254 nm ( $\log_{10}\text{SUVA}$ ), the DTPA-extractable Cu and Zn concentration ( $\log_{10}\text{Cu-DTPA}$  and  $\log_{10}\text{Zn-DTPA}$ ), the DGT-available Cu and Zn concentration ( $\text{pCu}_{\text{DGT}}$  and  $\text{pZn}_{\text{DGT}}$ ), the total Cu and Zn concentration in soil solution ( $\text{pCu}_{\text{SS}}$  and  $\text{pZn}_{\text{SS}}$ ), and the free ionic Cu and Zn activity in soil solution ( $\text{pCu}^{2+}$  and  $\text{pZn}^{2+}$ ).



**Figure 4** Free copper activity ( $pCu^{2+}$ ) predicted with WHAM as a function of  $pCu^{2+}$  measured in the soil solution samples ( $n = 70$ ).  $pCu^{2+}$  was predicted by (a) using WHAM default parameters with the percentage of dissolved organic matter reactive towards metal cations ( $\%r\text{-DOM}$ ) equal to 65% and the copper binding constant ( $\log K_{Cu}$ ) equal to 2.16 for all soil solutions, (b) optimizing the  $\%r\text{-DOM}$  with the SUVA measured in soil solutions, or (c) optimizing the  $\%r\text{-DOM}$  and the  $\log K_{Cu}$  values for each soil solution within a range of physically meaningful values (for b and c, see the *Material and Methods* section for rationale). Vertical error bars stand for the  $\pm 2$  standard deviations in predicted  $pCu^{2+}$  when considering the analytical uncertainty on the pH, total Cu concentration in soil solution, and DOM. The blue line, the thick solid line, and the dashed lines represent the regression line, the 1:1 line, and the 1:1 line  $\pm 0.5 pCu^{2+}$  unit, respectively. The slope and the adjusted coefficient ( $\text{adj}R^2$ ) of the regression and the root mean square residual (RMSR) between measured and predicted  $pCu^{2+}$  are calculated.



**Table 1** Soil physical-chemical properties of the three soil types up to 8 years of either no fertilization, mineral fertilization, or organic fertilization. Mean values are given with their standard deviation in parentheses.

Fertilization		Units	pH-H <sub>2</sub> O <sup>a</sup>	Organic C <sup>b</sup>	CEC	Total Cu <sup>c</sup>	Total Zn <sup>c</sup>
				g/kg	cmol <sub>e</sub> /kg	mg/kg	mg/kg
		<i>n</i> <sup>d</sup>					
Andosol	No fertilization	5	5.6 (±0.3)	108 (±6)	12 (±2)	84 (±3)	164 (±10)
	Mineral fertilization	10	5.1 (±0.4)	109 (±9)	9.8 (±5.1)	79 (±6)	175 (±16)
	Organic fertilization	19	6.2 (±0.4)	121 (±8)	23.1 (±5.7)	84 (±5)	187 (±16)
Cambisol	Mineral fertilization	6	5.4 (±0.2)	34 (±2)	10.1 (±0.1) <sup>e</sup>	81 (±2)	242 (±4)
	Organic fertilization	11	6.2 (±0.5)	37 (±3)	19.6 (±2.7) <sup>e</sup>	85 (±6)	261 (±13)
Arenosol	No fertilization	4	6.7 (±0.1)	10.3 (±0.5)	5.2 (±0.3)	73 (±1)	136 (±6)
	Mineral fertilization	4	5.9 (±0.1)	10.4 (±0.5)	3.7 (±0.8)	69 (±2)	134 (±2)
	Organic fertilization	15	7.3 (±0.2)	13.6 (±2)	7.9 (±1.3)	71 (±2)	141 (±5)

<sup>a</sup>Soil:liquid ratio 1:5

<sup>b</sup>NFISO 10694

<sup>c</sup>NFISO 14869-1

<sup>d</sup>*n* = number of soil samples

<sup>e</sup>Data from Djae et al. (2017), *n* = 2 for mineral fertilization and *n* = 4 for organic fertilization

**Table 2** Multiple linear regression explaining the total copper (Cu) and zinc (Zn) concentration in soil solution ( $pCu_{SS}$  and  $pZn_{SS}$ ) as a function of the DTPA-extractable Cu or Zn concentration in soil ( $\log_{10}M_{DTPA}$ ), pH,  $\log_{10}$  of the dissolved organic carbon concentration ( $\log_{10}DOC$ ), and  $\log_{10}$  of the specific UV absorbance of soil solutions at 254 nm ( $\log_{10}SUVA$ ) in the three soil types considered together or independently. Exponents indicate the order of contribution of explanatory variables to the regression. Non-significant regression coefficients and intercepts (i) were labelled as ns ( $p \geq 0.05$ ).

		$\log_{10}M_{DTPA}$	pH	$\log_{10}DOC$	$\log_{10}SUVA$	i	adj R <sup>2a</sup>	n <sup>b</sup>
$pCu_{SS}$	3 soils	-0.3 <sup>3</sup>	ns	-0.41 <sup>1</sup>	-0.61 <sup>2</sup>	8.4	0.8****	73
	Andosol	ns	-0.19 <sup>2</sup>	-0.95 <sup>1</sup>	ns	8.7	0.78****	34
	Cambisol	ns	ns	-0.55 <sup>2</sup>	-0.95 <sup>1</sup>	8.6	0.9****	16
	Arenosol	ns	ns	-1.01 <sup>1</sup>	-0.28 <sup>2</sup>	8.4	0.87****	23
$pZn_{SS}$	3 soils	-0.15 <sup>3</sup>	0.68 <sup>1</sup>	ns	0.41 <sup>2</sup>	2.1	0.68****	73
	Andosol	ns	0.63 <sup>1</sup>	ns	ns	2.6	0.58****	33
	Cambisol	0.86 <sup>1</sup>	ns	ns	ns	5.6	0.52****	17
	Arenosol	ns	0.66 <sup>1</sup>	ns	ns	2.6	0.57****	23

<sup>a</sup>adj R<sup>2</sup> = adjusted R<sup>2</sup> ; <sup>b</sup>n = number of samples

**Table 3** Multiple linear regression explaining the activity of free ionic Cu and Zn in soil solution ( $pCu^{2+}$  and  $pZn^{2+}$ ) as a function of the pH,  $\log_{10}DOC$ ,  $\log_{10}SUVA$ , and  $pCu_{SS}$  or  $pZn_{SS}$  in soil solution ( $pM_{SS}$ ) in the three soil types considered together or independently. Exponents indicate the order of contribution of explanatory variables to the regression. Non-significant regression coefficients and intercepts (i) were labelled as ns ( $p \geq 0.05$ ).

		pH	$\log_{10}DOC$	$\log_{10}SUVA$	$pM_{SS}$	i	adj $R^2$ <sup>a</sup>	n <sup>b</sup>
$pCu^{2+}$	3 soils	1.98 <sup>1</sup>	1.51 <sup>2</sup>	ns	ns	-5.3	0.64***	74
	Andosol	1.6 <sup>1</sup>	1.64 <sup>2</sup>	ns	ns	ns	0.77***	34
	Cambisol	2.85 <sup>1</sup>	ns	ns	ns	-8.8	0.75***	17
	Arenosol	3.03 <sup>1</sup>	ns	ns	ns	-10.2	0.5***	23
Predicted $pZn^{2+}$	3 soils	0.33 <sup>3</sup>	0.68 <sup>2</sup>	ns	1.14 <sup>1</sup>	-3.5	0.97***	71
	Andosol	0.41 <sup>3</sup>	0.68 <sup>2</sup>	ns	1.20 <sup>1</sup>	-4.3	0.98***	31
	Cambisol	0.21 <sup>2</sup>	ns	0.27 <sup>3</sup>	1.18 <sup>1</sup>	-2.4	0.99***	17
	Arenosol	0.3 <sup>3</sup>	0.6 <sup>2</sup>	ns	0.92 <sup>1</sup>	-1.6	0.99***	23

<sup>a</sup>adj  $R^2$  = adjusted  $R^2$ ; <sup>b</sup>n = number of samples

