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

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Abstract

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

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

1. Introduction

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

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

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

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

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

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

2. Material and Methods

2.1. Soil and field trial characteristics

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

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

Amongst the yearly sampled soils, seventy-four soil samples (andosol, n = 34; cambisol, n = 17; arenosol, n = 23) were selected over time under all fertilization modalities in each field trial to ensure that the collected soil samples would exhibit a Cu and Zn contamination, pH and organic matter concentration gradient that could influence the Cu and Zn availability (Table 1 and Fig. S1). For each field trial, we selected one or more samples for each fertilization modality in each sampling year considered, to ensure that the gradients observed could not be attributed to the comparison of samples from different sampling year. All analytical determinations and modelling calculations were performed without replication on composite samples prepared with an equal amount of soil from the three blocks.

The time-cumulated rate of Cu and Zn inputs by mineral and OF were calculated ($F_{M\text{-}rate}$, mg Cu or Zn kg^{-1} soil) as follows:

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

where n is the number of years since the beginning of fertilization, r is the yearly rate of OF or mineral fertilizer applied (kg dry matter m^{-2} y^{-1}), M is the Cu or Zn concentration in the OF or mineral fertilizer (mg Cu or Zn kg^{-1} dry matter), dp is the soil depth (m), and ρ is the bulk soil density (kg m^{-3}).

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

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

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

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

2.3. DTPA extractions from soils

Soil extraction with diethylenetriaminepentaacetic acid (DTPA) was performed according to Lindsay and Norvell, (1978). Briefly, soil extractions were conducted with a solution composed of 5 mM DTPA, 10 mM $CaCl_2$ and 100 mM triethanolamine buffered at pH 7.3, with a soil:solution ratio of 1:2. The soil-solution mixture was stirred for 2 h and then centrifuged at 1000 *g*, and the supernatant was filtered with ashless medium speed filter paper with an 8 µm pore size. Solutions were analyzed with a flame atomic absorption spectrophotometer. The DTPA extraction procedure was validated by using an uncertified internal reference soil from Réunion when the extracted Cu and Zn recovery was within the 90-110 % range and the coefficient variation on the three replicated internal reference was within 10%.

2.4. DGT soil analysis

Each soil sample was analyzed with the DGT technique using devices equipped with a Chelex binding layer. Soils were incubated at 100% of their water holding capacity at 25°C for 24 h and the devices were then deployed on the soil surface for 24 h. DGT devices were finally removed from the soil samples and the Chelex layer was eluted in 1.2 ml of 1 M HNO_3 for 24 h. Cu and Zn concentrations in eluates were determined by inductively coupled plasma-mass spectrometry (ICP-MS, ICAP Q, Thermo Scientific). DGT-available Cu and Zn was expressed as the Cu and Zn concentration at the interface between the soil and the DGT surface (C_{DGT}, mol l^{-1}) as follows:

$$C_{DGT} = \frac{m.\Delta g}{M.D.A.t} \qquad (2)$$

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

2.5. Soil solution extraction and analysis

The solution of each soil sample was extracted with 2.5 mM $Ca(NO_3)_2$ and 3.5 mM KNO_3 and analyzed as described by Djae et al. (2017). Each soil solution was subdivided and subsamples were stabilized with either NaN_3 to avoid a shift in metal speciation while preventing DOM degradation for measurement of Cu^{2+} activity and the specific ultraviolet absorbance (SUVA) at 254 nm (A_{254}) or HNO_3 for measurement of the dissolved organic carbon (DOC) and total major cation concentration. Total concentrations of major and trace cations (Ca, Mg, K, Na, Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn) were determined by ICP-MS. A solid-solution partitioning coefficient (K_D in l kg^{-1}) for Cu and Zn (K_{DCu} and K_{DZn}) was then calculated according to Sauvé et al. (2000):

$$K_D = \frac{M_{DTPA}}{M_{solution}} \qquad (3)$$

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

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

$$SUVA = \frac{A_{254}}{b.DOC} \qquad (4)$$

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

2.6. Modeling Cu^{2+} and Zn^{2+} activity

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

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

2.7. Data processing and analysis

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

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

3. Results and Discussion

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

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

As soilborne Cu and Zn availability is considered to be low in Réunion soils (Doelsch et al., 2008), we assumed that the DTPA-extractable Cu and Zn concentration, which represented less than 10 % of the total Cu and Zn, would be more sensitive to cumulative inputs of OF-derived Cu and Zn in soil than the total Cu and Zn concentration. In agreement, OF significantly increased the DTPA-extractable Cu and Zn concentration in soils compared to the non-fertilized treatment or to mineral fertilization (Fig. 1). This result is consistent with the expected moderate but chronic Cu and Zn contamination of soils under OF and this pattern could be better highlighted through DTPA extraction than by measuring the total concentration.

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

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

By performing PCA separately on each soil type, the effects of the fertilization types on the solution chemistry and on the Cu and Zn availability proxies in soils were even more clearcut (Fig. 3). Both PC1 and PC2 explained 75%, 84% and 69% of the variance for the andosol, cambisol and arenosol, respectively (Fig. 3a, c, and e). Regardless of the soil type, PC1 was mainly determined via the pH, DOC, SUVA, Cu-DTPA, Zn-DTPA, pCu_{SS}, pZn_{SS}, and pZn^{2+} findings, while pCu_{DGT}, pZn_{DGT} and pCu^{2+} contributed to PC1 or PC2, or to both depending on the soil type. For each soil, the fertilization types were clearly dissociated in two (Fig. 3d) or three (Fig. 3b and f) clusters along the PC1 axis. These results highlight the substantial and similar relative influence of the fertilization type on the solution chemistry and Cu and Zn availability proxies in all of the soils, with a clear distinction between mineral and organic fertilization.

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

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

Organic fertilization increased the soil organic C concentration (Table 1) as well as (by almost two-fold) the DOC concentration and SUVA in the soil solution for the three soil types compared to mineral or no fertilization (Figs. S4b&c, S5b&c, and S6b&c). The increase in DOC concentration following OF application was consistent with previous findings (Cambier et al., 2014; Seo et al., 2018; Wright et al., 2008) and was attributed to the input of organic matter whose solubilization was further favored by the increase in soil pH (Bolan et al., 2011). Araújo et al. (2019) similarly found an increase in SUVA in the solution of a soil that had received dairy manure amendments for 3 years, which suggests an increase in DOM aromaticity. We thus conclude that a decade of organic fertilization increased the soil DOM concentration and aromaticity.

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

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

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

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

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

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

Although we did not perform any kinetic measurements, changes in the total Cu and Zn concentration and activity in soil solution were indicators of Cu and Zn lability in the soil solid phase and in the soil solution, respectively. The total Cu concentration increased while Cu^{2+} activity decreased in the solution of the three soil types with organic fertilization, thus indicating that Cu lability was more or less the same whatever the fertilization type. In addition, the negative correlation between the SUVA and DGT-available Cu in the cambisol, while DGT-available Cu was positively correlated with the total Cu concentration in soil solution, suggests that Cu bound to aromatic DOM, such as phenolic compounds, was weakly labile, in agreement with previous findings obtained under laboratory conditions (Amery et al., 2010; Kalis et al., 2006; Welikala et al., 2018). The total Zn concentration and Zn^{2+} activity decreased in the solution of the three soil types with organic fertilization, implying that Zn lability decreased with organic fertilization. However, DTPA-extractable Zn substantially increased in the three soil types with organic fertilization (Figs. 1d, e&f), which may have counteracted the decrease in Zn lability, resulting in a similar DGT-available Zn concentration regardless of the fertilization type.

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

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

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

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

Whatever the soil type, WHAM parameterization by manually optimizing the *%r-DOM* and then the $\log K_{Cu}$ in each soil solution within physically meaningful ranges substantially improved the prediction of measured Cu^{2+} activity compared to predictions conducted with default parameterization or SUVA optimization of WHAM (adjR^2 = 0.94 and RMSR = 0.21; Fig. 4c). The optimized *%r-DOM* and logK_{Cu} values came within the variation range we set for these two parameters, as previously observed for the WHAM prediction of Cu^{2+} activity in 72 soil samples exhibiting a very broad range of physical-chemical properties (Djae et al. 2017; Li et al. 2017). These results suggest that organic fertilization induced substantial changes in DOM binding properties, which must be accounted for to adequately predict Cu speciation in soil solution using geochemical models.

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

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

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

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

4. Conclusion

Organic fertilization over a decade induced, as expected, ongoing soil Cu and Zn contamination compared to no fertilization or mineral fertilization. But we also showed that organic fertilization substantially increased some major soil physical-chemical properties, namely pH, organic C concentration and the concentration and aromaticity of DOM along with its Cu binding properties. The similar alterations in the physical-chemical properties observed for the three studied soil types suggest that the effects of organic fertilization might generally apply to a broad range of initially acidic soils.

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

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

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

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Appendix A. Supplementary material

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

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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.

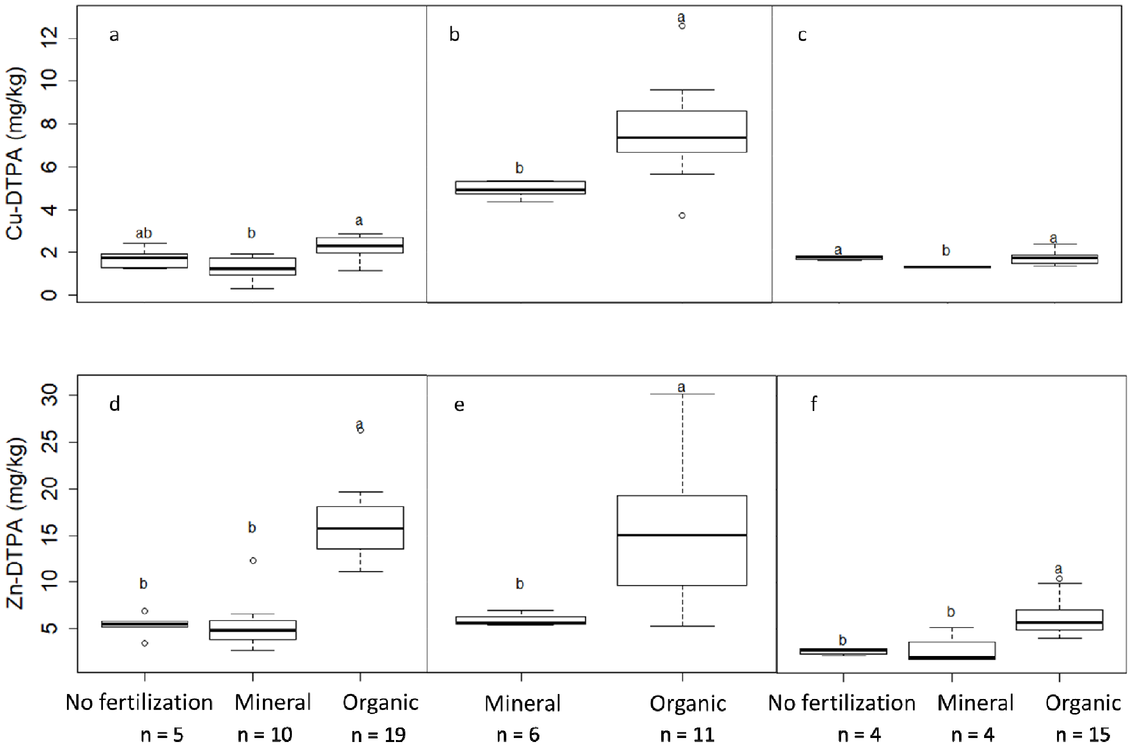


Figure 2 Principal component analysis (PCA) of the dataset on the soil solution chemistry and copper (Cu) and zinc (Zn) contamination and availability proxies in soils. The correlation plot (a) shows the contribution of each variable to the two first principal components (PC1 and PC2). Soil samples (*n* = 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 ($\log_{10}$DOC), the specific UV-absorbance of the soil solutions at 254 nm ($\log_{10}$SUVA), the DTPA-extractable Cu and Zn concentration ($\log_{10}$Cu-DTPA and $\log_{10}$Zn-DTPA), the DGT-available Cu and Zn concentration (pCu_{DGT} and pZn_{DGT}), the total Cu and Zn concentration in soil solution (pCu_{SS} and pZn_{SS}), and the free ionic Cu and Zn activity in soil solution (pCu^{2+} and pZn^{2+}).

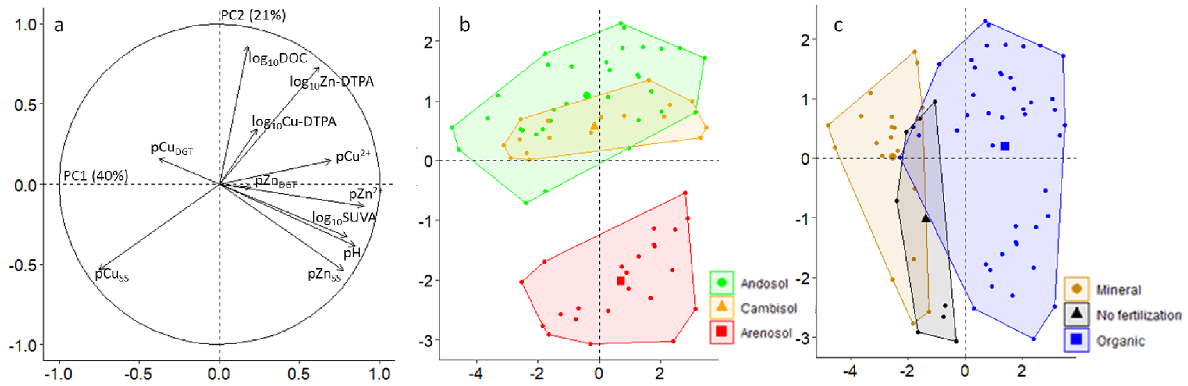


Figure 3 Principal component analysis (PCA) on the soil solution chemistry and copper (Cu) and zinc (Zn) contamination and availability proxies in (a) the andosol (a and b; *n* = 34), (b) the cambisol (c and d; *n* = 17) and (c) the arenosol (e and f; *n* = 23). The correlation plots (a, c, and e) show 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 ($\log_{10}$DOC), the specific UV-absorbance of the soil solutions at 254 nm ($\log_{10}$SUVA), the DTPA-extractable Cu and Zn concentration ($\log_{10}$Cu-DTPA and $\log_{10}$Zn-DTPA), the DGT-available Cu and Zn concentration (pCu_{DGT} and pZn_{DGT}), the total Cu and Zn concentration in soil solution (pCu_{SS} and pZn_{SS}), and the free ionic Cu and Zn activity in soil solution (pCu^{2+} and pZn^{2+}).

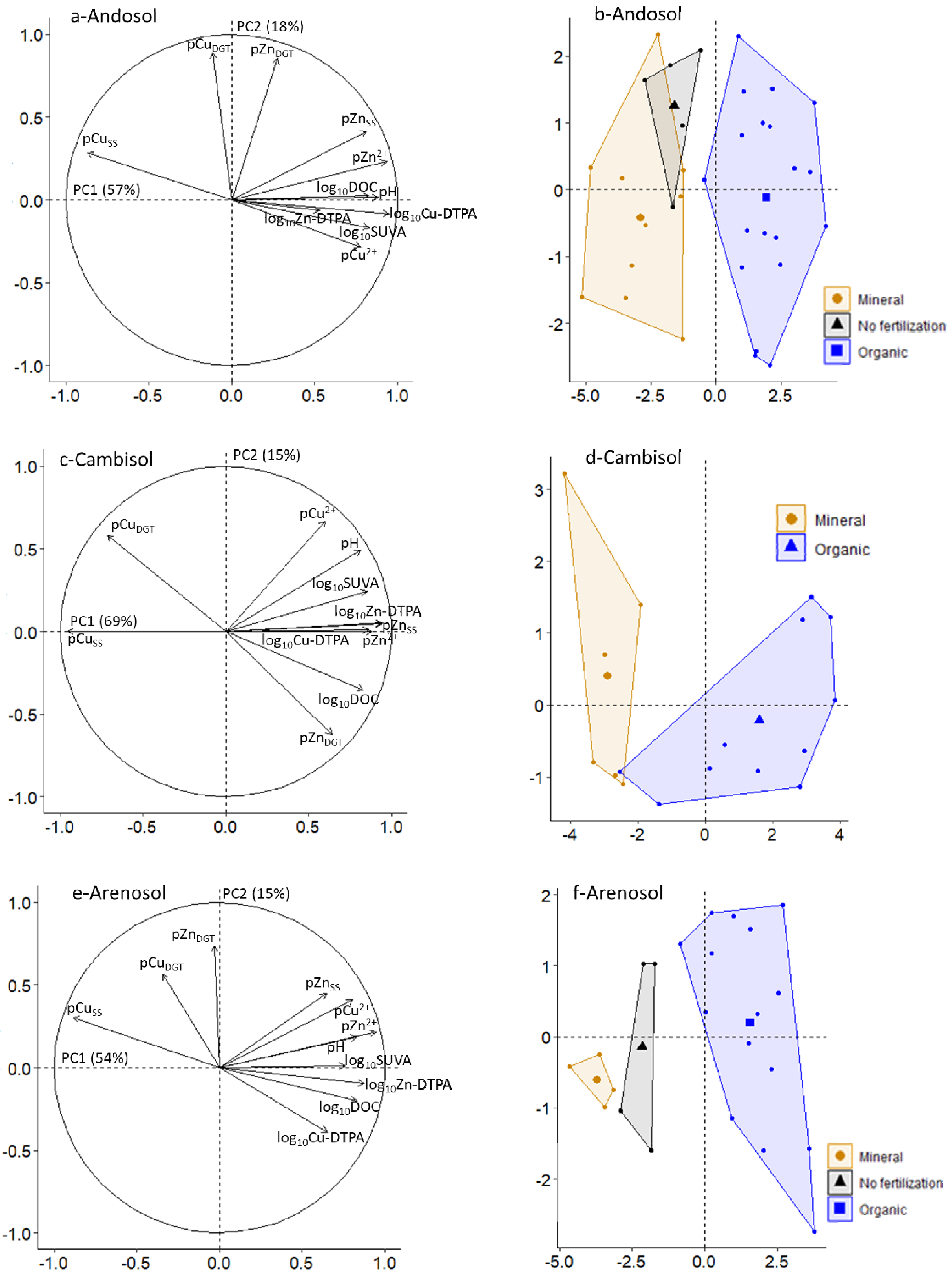


Figure 4 Free copper activity (pCu^{2+}) predicted with WHAM as a function of pCu^{2+} measured in the soil solution samples (n =70). pCu^{2+} was predicited by (a) using WHAM default parameters with the percentage of dissolved organic matter reactive towards metal cations (*%r-DOM*) equal to 65% and the copper binding constant ($logK_{Cu}$) equal to 2.16 for all soil solutions, (b) optimizing the *%r-DOM* with the SUVA measured in soil solutions, or (c) optimizing the *%r-DOM* and the $logK_{Cu}$ 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 ± 2 standard deviations in predicted pCu^{2+} when considering the analitycal 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 ± 0.5 pCu^{2+} unit, respectively. The slope and the adjusted coefficient ($adjR^2$) of the regression and the root mean square residual (RMSR) between measured and predicted pCu^{2+} are calculated.

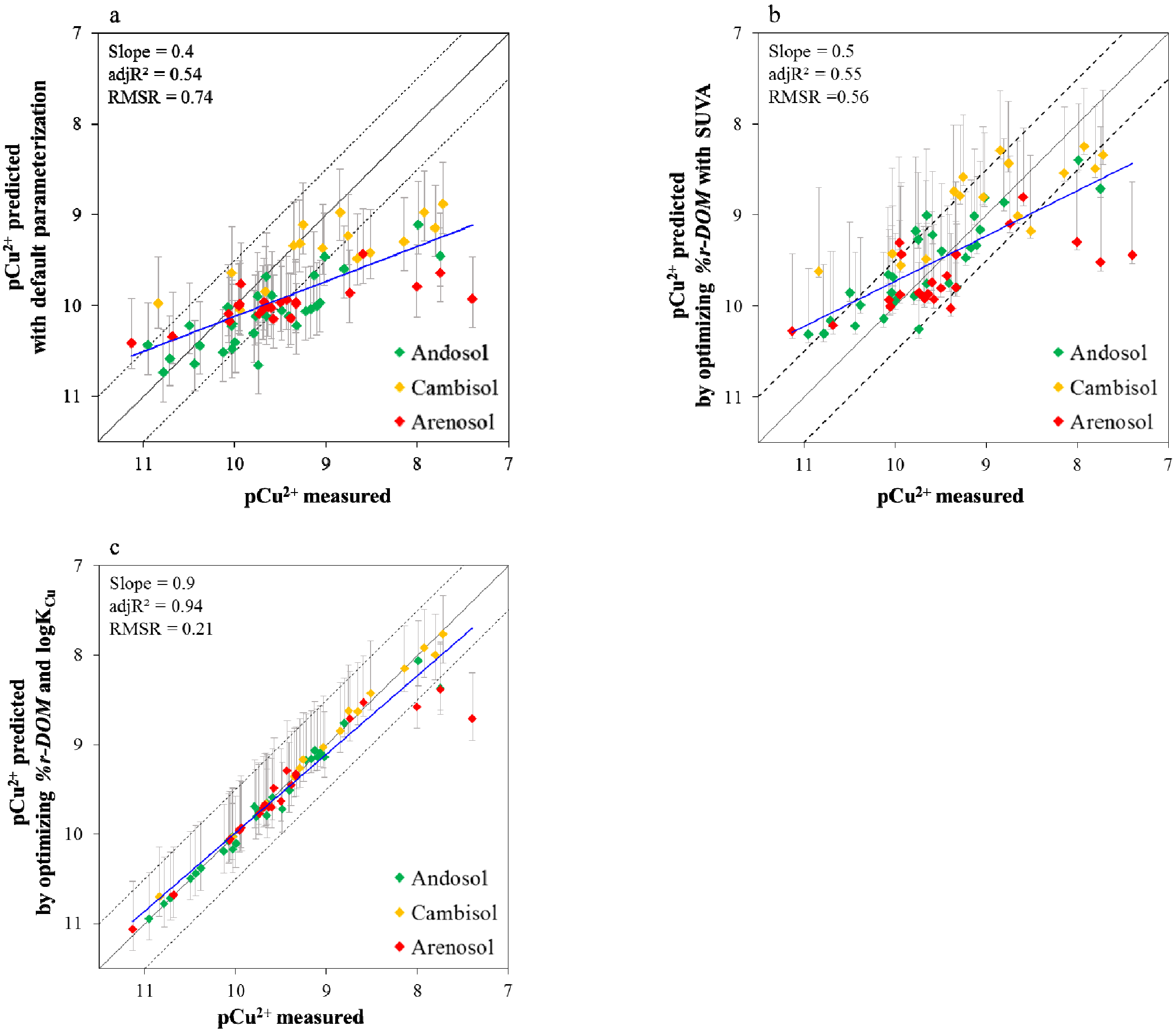


Table 1 Soil physical-chemical properties of the three soil types up to 8 years of either no fertilization, mineral fertilization, or organic fertilization. Mean values are given with their standard deviation in parentheses.

	Fertilization		pH-H_2O[a]	Organic C[b]	CEC	Total Cu[c]	Total Zn[c]
		Units		g/kg	cmol$_+$/kg	mg/kg	mg/kg
		n[d]					
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)[e]	81 (± 2)	242 (± 4)
	Organic fertilization	11	6.2 (± 0.5)	37 (± 3)	19.6 (± 2.7)[e]	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)

[a] Soil:liquid ratio 1:5

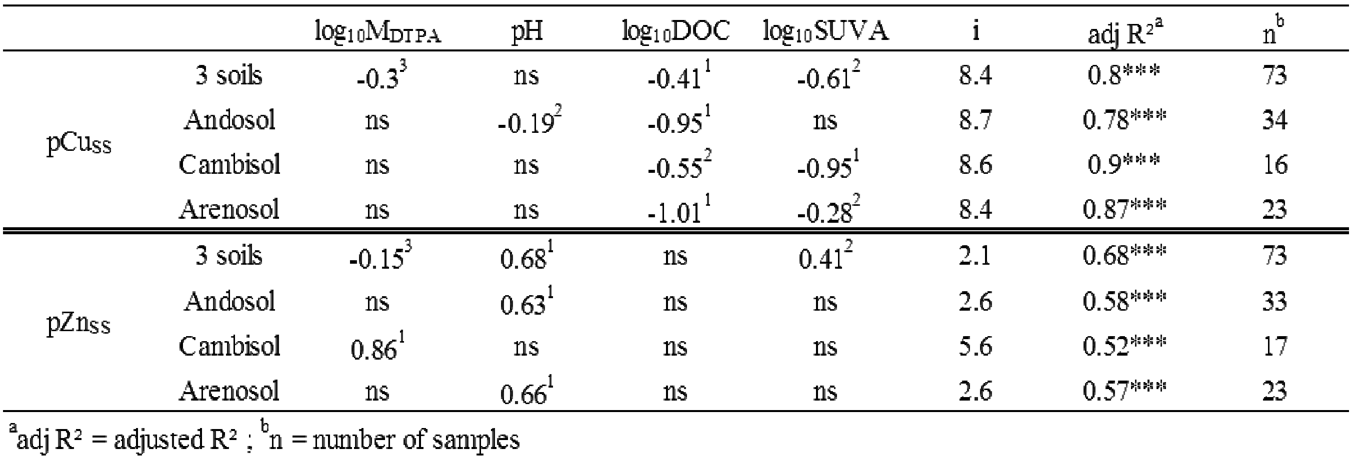
[b] NF ISO 10694

[c] NF ISO 14869-1

[d] *n* = number of soil samples

[e] Data from Djae et al. (2017), n = 2 for mineral fertilization and n = 4 for organic fertilization

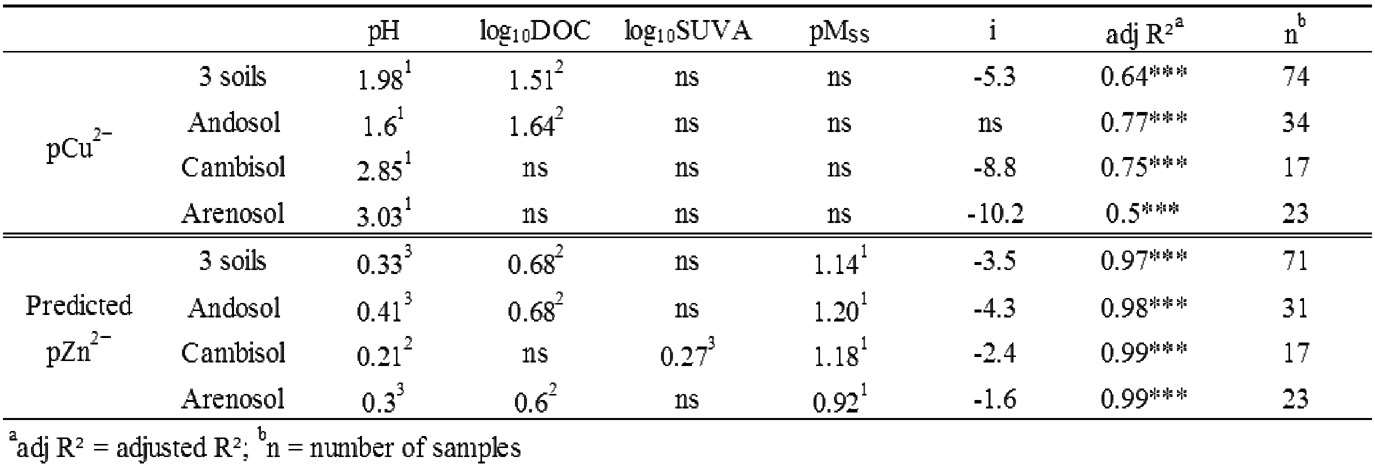
Table 2 Multiple linear regression explaining the total copper (Cu) and zinc (Zn) concentration in soil solution (pCu_{SS} and pZn_{SS}) as a function of the DTPA-extractable Cu or Zn concentration in soil ($\log_{10}M_{DTPA}$), pH, $\log_{10}$ of the dissolved organic carbon concentration ($\log_{10}DOC$), 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^{2}[a]	n[b]
pCu_{SS}	3 soils	-0.3^{3}	ns	-0.41^{1}	-0.61^{2}	8.4	0.8***	73
	Andosol	ns	-0.19^{2}	-0.95^{1}	ns	8.7	0.78***	34
	Cambisol	ns	ns	-0.55^{2}	-0.95^{1}	8.6	0.9***	16
	Arenosol	ns	ns	-1.01^{1}	-0.28^{2}	8.4	0.87***	23
pZn_{SS}	3 soils	-0.15^{3}	0.68^{1}	ns	0.41^{2}	2.1	0.68***	73
	Andosol	ns	0.63^{1}	ns	ns	2.6	0.58***	33
	Cambisol	0.86^{1}	ns	ns	ns	5.6	0.52***	17
	Arenosol	ns	0.66^{1}	ns	ns	2.6	0.57***	23

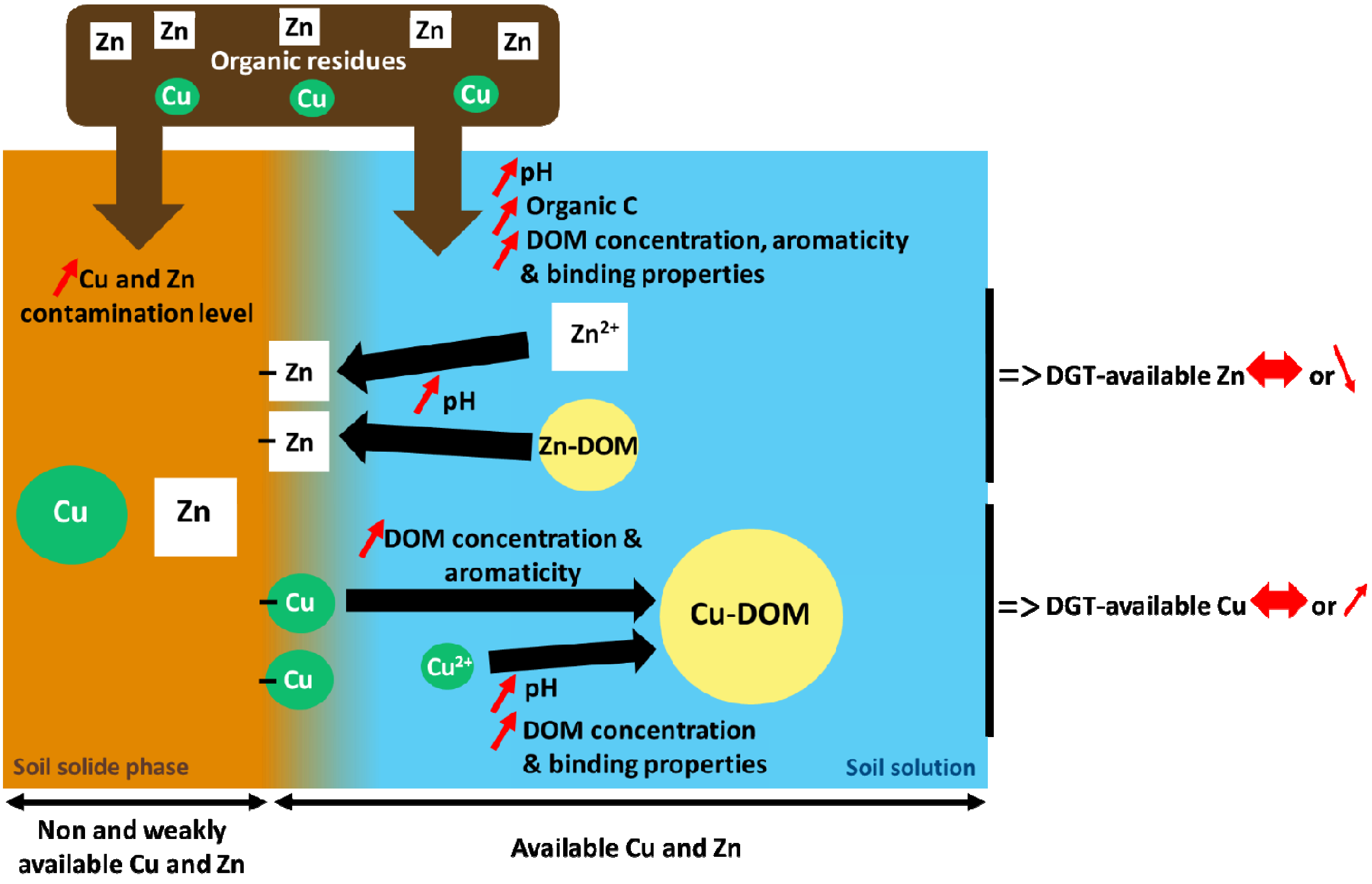
[a] adj R^{2} = adjusted R^{2} ; [b] n = number of samples

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^{2a}	n^b
pCu^{2-}	3 soils	1.98^1	1.51^2	ns	ns	-5.3	0.64***	74
	Andosol	1.6^1	1.64^2	ns	ns	ns	0.77***	34
	Cambisol	2.85^1	ns	ns	ns	-8.8	0.75***	17
	Arenosol	3.03^1	ns	ns	ns	-10.2	0.5***	23
Predicted pZn^{2-}	3 soils	0.33^3	0.68^2	ns	1.14^1	-3.5	0.97***	71
	Andosol	0.41^3	0.68^2	ns	1.20^1	-4.3	0.98***	31
	Cambisol	0.21^2	ns	0.27^3	1.18^1	-2.4	0.99***	17
	Arenosol	0.3^3	0.6^2	ns	0.92^1	-1.6	0.99***	23

[a]adj R^2 = adjusted R^2; [b]n = number of samples


Zn
Zn
Zn
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Zn
Organic residues
Cu
Cu
Cu
pH
Organic C
DOM concentration, aromaticity
& binding properties
Cu and Zn
contamination level
Zn^{2+}
Zn
pH
Zn
Zn-DOM
=> DGT-available Zn ⟷ or ↘
Cu
Zn
DOM concentration &
aromaticity
Cu
Cu-DOM
Cu
Cu^{2+}
pH
DOM concentration
& binding properties
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Soil solution
Non and weakly
available Cu and Zn
Available Cu and Zn