



## Stable isotopes analysis combined with X-ray absorption spectroscopy reveal the fate of organic waste-borne copper and zinc in amended soils

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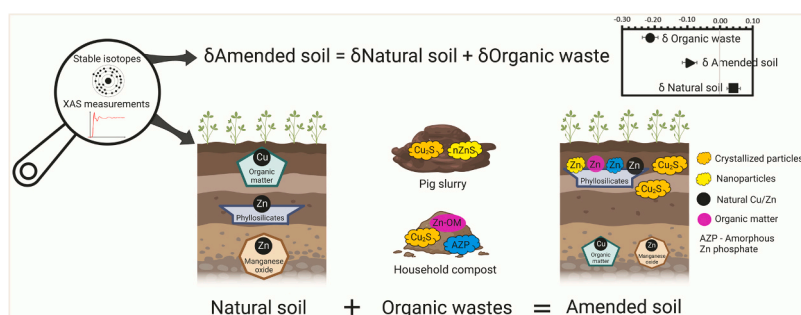
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### HIGHLIGHTS

- Soils acted as a closed system with respect to OW-borne Cu and Zn.
- The amended soil isotopic signatures were the result of the mixing of OW-borne and natural Cu and Zn.
- Persistence of OW-borne crystallized Cu (I)-sulfide in amended soils.
- Radical change in pig slurry- or household compost-derived Zn species in amended soils.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

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### ABSTRACT

Copper (Cu) and zinc (Zn), two potentially toxic trace elements, are commonly abundant in organic wastes (OWs) recycled in soils as fertilizer. Yet current knowledge on the long-term behavior and fate of Cu and Zn in soil following OW spreading is scant. We addressed this issue by studying the fate of OW-borne Cu and Zn in amended soils from four different long-term field experiments. By combining the stable isotope analysis and X-ray absorption spectroscopy, we identified changes in Cu and Zn concentrations, speciation and isotopic compositions in the amended soils only when OW had been applied at high rates over long periods. Under these conditions, we highlighted that: (i) all OW-derived Cu and Zn had accumulated in the topsoil layer regardless of the soil and OW type; (ii) the amended soil isotopic signatures were the result of the mixing of OW-borne and natural Cu and Zn; and (iii) Cu and Zn exhibited distinct speciation patterns in amended soils. Indeed, the unprecedented persistence of OW-borne crystallized Cu(I)-sulfide in the amended soils contrasted with the

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complete transformation of pig slurry-borne nanosized Zn-sulfide or household compost-derived amorphous Zn phosphate and Zn complexed by organic matter.

## 1. Introduction

Organic waste (OW) recycling to fertilize crops is a common practice as it offers a cost-effective and environmentally sustainable way to manage OW [1]. Despite its beneficial fertilization features, OW may also contain high concentrations of trace elements and contaminants [2, 3]. For instance, Cu and Zn are supplemented in animal feed because they are growth promoters [4,5]. However, only a small fraction is metabolized and most of the dietary Cu and Zn is excreted in their slurries [6,7]. Various studies have consequently highlighted a positive correlation between OW spreading and trace element concentrations in soil [8-10]. Trace element inputs in cultivated soils are dominated by Cu and Zn in many countries [8,9]. Copper and zinc are considered essential trace elements as they play an important role in soil fertility because they are involved in metabolic processes in both plants and soil micro-organisms [11-13]. However, at high concentrations as observed in soils following OW application, they may have toxic impacts on human health and freshwater ecosystems [14].

Current knowledge on mechanisms controlling the accumulation of OW-borne Cu and Zn in soil is scant. Sipos et al. [15] described Cu and Zn sorption in four natural soil samples with contrasting properties that had been spiked with a metal salt solution. They revealed that mechanisms responsible for metal sorption may vary depending on the trace element and soil properties. For example, alkaline soils were characterized by high Cu-carbonate precipitation whereas clay minerals were responsible for Cu and Zn sorption in clayey soils. However, the behavior of OW-borne Cu and Zn in soils may differ from that in soils spiked with Cu and Zn salts [16]. Organic wastes are complex mixtures of liquid and/or solid phases and thus Cu and Zn bearing phases may vary depending on the OW source (agricultural, urban, industrial) or OW treatment process (anaerobic digestion and composting) [17,18]. In a long-term field experiment, Formentini et al. [19,20] observed that the oxidative dissolution of unstable Cu-sulfide and Zn-sulfide species in pig slurry (PS) and subsequent sorption of Cu onto clay and Fe oxyhydroxide minerals in soil explained the accumulation of PS-borne Cu and Zn in an acidic Oxisol. Otherwise, in a lysimeter experiment, Hodomihou et al. [21] reported Zn accumulation in an amended soil after OW spreading, which the authors attributed to the persistence of OW-borne Zn phosphate species, and surprisingly they also noted similar accumulation patterns in a Fluvisol and Arenosol despite their contrasting properties. The findings of the above-mentioned studies highlight that the mechanisms controlling Cu and Zn accumulation in amended soils are complex and are yet to be fully understood. Indeed, trace element speciation in OWs and the soil properties seem to jointly contribute to Cu and Zn accumulation in amended soils.

Here we have attempted to bridge this scientific gap by combining isotopic measurement with X-ray Absorption Spectroscopy (XAS) analysis to describe the fate of OW-borne Cu and Zn in soils. Stable Cu and Zn isotope analysis is used since two decades to trace industry-related contamination in soils (e. g., [22-26] and to study the fate of anthropogenic metals in agricultural soils (e. g., [27-30]). However, the combined isotopes-speciation approach has previously been explored only in a few field studies. Aucour et al., [31,32,23]. For instance, Gelly et al. [32] studied the fate of Zn in smelter-impacted soils using an integrated stable isotope and speciation analysis approach. They reported heavy Zn isotope enrichment in the surface layer of the smelter-impacted soil, despite the prevalence of light Zn isotopes in historical emissions from smelter. These findings were attributed to post-depositional transformation of Zn speciation following its introduction in soil. It is worth noting that different isotope-fractionating post-depositional processes (dissolution/precipitation, sorption, complexation) can occur

simultaneously in soil following Cu and Zn introduction [33]. The direction of the isotopic shift can offer indication on the dominant process involved in Cu and Zn redistribution in soil, although different aspects of post-depositional transformations might be difficult to discriminate. The speciation changes observed using XAS analyses can help pinpoint the dominant mineral phase involved in these transformations and isotope fractionation, and thus avoid over-interpretation.

In this study, we systematically investigated the Cu and Zn concentration, isotope composition and speciation in OW and soil samples from four long-term field experiments. These experiments were chosen to represent different soil types (Nitisol, Luvisol, Fluvisol and Cambisol), different OWs (pig slurry, PS or household compost, HHC) and experimental modalities (low amounts of OW applied over short periods vs large amounts of OW applied over long periods). The aim of this study on the impact of long-term application of Cu- and Zn-rich OWs on agricultural soils was to: (i) examine the isotopic variability of OWs characterized by distinct Cu and Zn speciation, (ii) assess the possibility of tracing OW-borne Cu and Zn in amended soils using the combined stable isotope-speciation analysis approach, (iii) attempt to identify the mechanisms controlling the fate OW-borne Cu and Zn in soils characterized by contrasting properties.

## 2. Materials and methods

### 2.1. Sampling locations and experimental sites

We studied soil and OW samples from four different long-term field experiments. These experiments were chosen for several reasons: the pedoclimatic contexts differed between the sites (Fig. 1); the soil physicochemical properties differed between the sites; they allowed for a comparison between amended and control soils; they provided the opportunity to analyze the organic wastes applied, and; the sites were rigorously managed, thus enabling accurate mass balances to be drawn up. The use of different types of OW samples, PS and HHC, makes it possible to investigate the isotopic variability of OWs characterized by distinct Cu and Zn speciation and to potentially evaluate how OWs with different Cu and Zn chemical forms affect their fate and behavior in amended soils.

The samples used in this study were sourced from four different long-term field experiments (LTE's). The first site was on the French island of Réunion (Indian Ocean) and was part of the SOERE PRO research network (<https://valor-pro.hub.inrae.fr/presentation-de-l-observatoire-soere-pro>). The soil was characterized as Nitisol according to the WRB classification [34] and was amended with pig slurry, PS, at a yearly rate of 30 m<sup>3</sup>·ha<sup>-1</sup>·year<sup>-1</sup>. Control and PS amended soil samples were collected after 7 years of fertilization. The second site, located in Brittany, western France, was also part of the SOERE PRO research network. The soil identified as Luvisol was amended annually with 30.3 m<sup>3</sup>·ha<sup>-1</sup>·year<sup>-1</sup> of PS. Control and PS amended soil samples were collected after 8 years of fertilization. The third site was in Catalonia, northeastern Spain, where Fluvisol was amended with PS at a yearly rate of 60 m<sup>3</sup>·ha<sup>-1</sup>·year<sup>-1</sup>. Soil samples, both control and amended, were collected after 16 years of fertilization. The fourth experimental site was located at an experimental farm at the University of Copenhagen. The Cambisol was amended with household compost, HHC, at a yearly rate of 108 m<sup>3</sup>·ha<sup>-1</sup>·year<sup>-1</sup>. Control and HCC amended soil samples were collected after 17 years of fertilization. For each of these experimental sites, the applied organic waste was sampled fresh and stored in a freezer before analysis. A detailed description of each field experiment is given in the supplementary material.

## 2.2. Soil and organic waste characteristics

The characteristics of all selected soils and organic wastes are listed in Table 1.

## 2.3. Sample preparation for chemical and isotopic analysis

Soil samples were oven dried at 40 °C and ground to fine powder in a mechanical agate mortar. Total metal concentrations and isotopic compositions of the samples were determined by weighing 100–150 mg of ground sample followed by calcination at 450 °C for 4 h to remove the organic matter. Subsequently, the samples were digested in concentrated acids (3 ml HNO<sub>3</sub> and 1 ml HF). Clear solutions were evaporated to dryness, refluxed three times with concentrated HNO<sub>3</sub> to dissolve excess silicates, reacted with 3 ml concentrated HCl and finally dissolved in 1.5 ml 7 N HCl for ion chromatography. All acid reagents used were either distilled (HNO<sub>3</sub>, HCl) or Seastar© Baseline quality (HF).

## 2.4. Chemical and isotopic analysis

Total dissolved Cu and Zn concentrations were measured using a PerkinElmer NexIon 300 × Q-ICP-MS with <sup>103</sup>Rh as internal standard. Quality control for each run was achieved by measuring the EnviroMAT EP-L-3, the EnviroMAT ES-H-2 (SCP Sciences Courtaboeuf, France), and the SRLS-5 (Ottawa, Canada) reference materials. The limits of quantification were 0.05 µg.L<sup>-1</sup> for Cu, and 0.2 µg.L<sup>-1</sup> for Zn. The measurement uncertainty was estimated via repeated analyses of a soil standard GSS-3 sample (Institute of Geophysical and Geochemical Exploration, Langfang, China) and a cow manure sample (MARSEP sample 275, Evaluating Program, Wageningen University). The expanded uncertainty was determined by multiplying the calculated standard uncertainty by 2 to obtain 95 % confidence level.

For isotopic analyses, Cu and Zn were separated and purified using an anionic resin (AG-MP1, 100–200 mesh, chloride form), according to the procedure reported in Maréchal et al. [35]. For each sample, Cu and Zn recoveries were systematically verified by comparing the amount of Cu and Zn recovered in purified solutions after column chromatography with the amounts loaded on the columns. Samples with yields < 100 ± 10 % were discarded. The total procedural blank ranged from 20 to 70 ng for Cu and 20 to 300 ng for Zn for the entire process (dissolution and separation), which on average only accounted for 2.3 % and 3.8 %

of the total Cu and Zn masses, respectively, in the dissolved sample.

Cu and Zn isotopic compositions were determined at CEREGE (Aix-en-Provence, France), Envitop analytical facility, using a Thermo Fisher Scientific Neptune<sup>plus</sup> Multi-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS). For Cu and Zn, we used the combined standard-sample-standard bracketing method and the addition of an internal standard in the samples to correct for instrumental mass fractionation e.g. Maréchal et al., [35]. The Zn samples were doped with a Cu standard solution (VWR), while the Cu samples were doped with a Zn standard solution (NIST 683). We used AE633 and IRMM 3702 as bracketing standards for Cu and Zn isotopic analyses, respectively. Samples and standards were introduced in 1 % HNO<sub>3</sub> at 200 ppb Cu and 500 ppb Zn concentration. For each sample, the same sample solution was analyzed three times (i.e. analytical replicates), with the average values reported, and analysis repeatability was expressed as two standard deviations (2 s) calculated from the analytical replicates. Two samples were considered to be significantly different in their isotopic composition if their 2 s did not overlap. The Cu and Zn isotope ratios were expressed as δ<sup>65</sup>Cu and δ<sup>66</sup>Zn values relative to the AE633 and IRMM 3702 isotope reference materials, respectively, according to the following equations:

$$\delta^{65}\text{Cu}[\text{‰}] = \left( \frac{\left( \frac{^{65}\text{Cu}}{^{63}\text{Cu}} \right)_{\text{sample}}}{\left( \frac{^{65}\text{Cu}}{^{63}\text{Cu}} \right)_{\text{AE633}}} - 1 \right) * 1000 \quad (1)$$

$$\delta^{66}\text{Zn}[\text{‰}] = \left( \frac{\left( \frac{^{66}\text{Zn}}{^{64}\text{Zn}} \right)_{\text{sample}}}{\left( \frac{^{66}\text{Zn}}{^{64}\text{Zn}} \right)_{\text{IRMM3702}}} - 1 \right) * 1000 \quad (2)$$

Furthermore, we processed the USGS reference material NOD-P-1 (manganese nodule) and the results are presented in Fig. S1 in the supplementary material. The range of Zn isotopic compositions of NOD-P-1 obtained in this study (δ<sup>66</sup>Zn<sub>IRMM 3702</sub>: 0.50–0.51 ‰) were in close agreement with previously published data (δ<sup>66</sup>Zn<sub>IRMM 3702</sub>: 0.52–0.63 ‰, [22,36–39,32,40,41]). For the Cu isotopic composition, previously published data indicated high heterogeneity in the δ<sup>65</sup>Cu<sub>AE633</sub> values (0.28–0.53 ‰, [22,36,32,40,42,41]), and our results were at the low end of this range (δ<sup>65</sup>Cu<sub>AE633</sub>: 0.08–0.25 ‰). This high variability suggested that the NOD-P1 material might not be suitable for

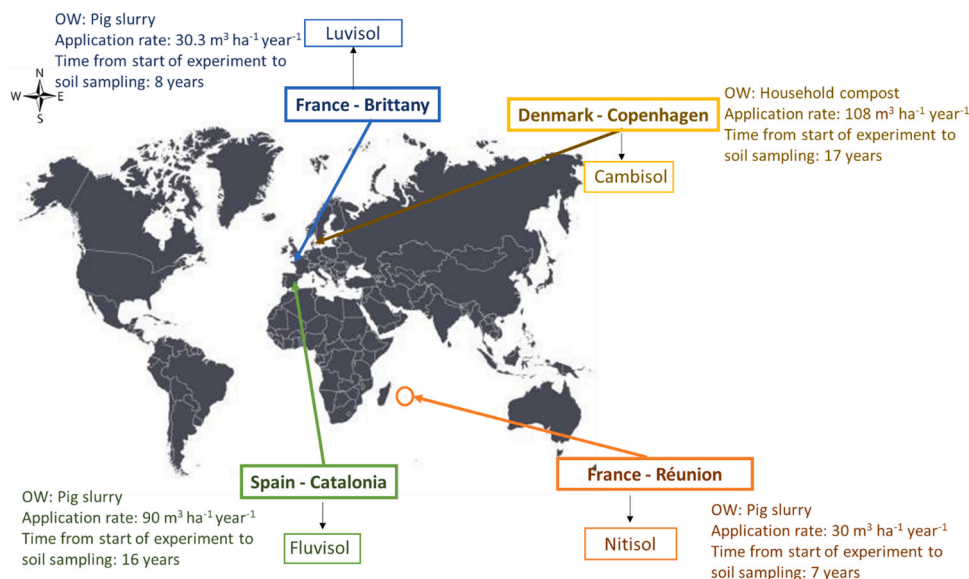


Fig. 1. Map showing sample locations and the corresponding information on the soil types, organic wastes applied as well as their application rates (cubic meter of fresh matter per hectare and per year) and the time between the start of the experiment to soil sampling.

**Table 1**  
Characteristics of organic waste and soil samples from the different long-term field experiments.

LTE	Field experiment ID	Location	OW application rate	Interval (OW spreading and soil sampling) <sup>a</sup>	Soil type	Samples	Depth (cm)	Cu (mg.kg <sup>-1</sup> ) <sup>b</sup>	Zn (mg.kg <sup>-1</sup> ) <sup>b</sup>
Nitisol	SOERE PRO Réunion	Réunion, France 20°53'24" S, 55°32'13" E	30 m <sup>3</sup> .ha <sup>-1</sup> .year <sup>-1</sup>	12 months	Nitisol	Pig slurry	0 - 10	256	969
						Control soil		86	219
Luvisol	EFFELE	Brittany, France 48°53'4.52" N, -1°48'52.25" W	30.3 m <sup>3</sup> .ha <sup>-1</sup> .year <sup>-1</sup>	12 months	Luvisol	Amended soil	0 - 30	90	228
						Pig slurry		134	709
Fluvisol		Catalonia, Spain 41°52'30" N, 1°09'13" E	90 m <sup>3</sup> .ha <sup>-1</sup> .year <sup>-1</sup>	6 months	Fluvisol	Control soil	0 - 15	20	60
						Amended soil		21	68
Cambisol	CRUCIAL	Copenhagen, Denmark 55°40'N, 12°18' E	108 m <sup>3</sup> .ha <sup>-1</sup> .year <sup>-1</sup>	1 month	Cambisol	Pig slurry	0 - 20	121	563
						Control soil		26	80
						Household compost		42	157
						Amended soil		116	314
						Control soil		12	59
						Amended soil		41	119

<sup>a</sup> The time interval between the last OW amendment and the soil sampling.

<sup>b</sup> The concentrations are expressed as mg kg<sup>-1</sup> of dry matter..

monitoring the Cu isotopic analyses procedure.

In addition to the NOD-P-1 reference material, we also processed full procedure replicates of the GSS-3 soil standard sample (Institute of Geophysical and Geochemical Exploration, Langfang, China) and the cow manure sample MARSEP sample 275, Evaluating Program, Wageningen University. The  $\delta^{66}\text{Zn}$  and  $\delta^{65}\text{Cu}$  values measured for GSS-3 were  $-0.05 \pm 0.06 \text{ ‰}$  (n full replicates = 3) and  $-0.08 \pm 0.05 \text{ ‰}$  (n = 2), while those measured for MARSEP sample 275 were  $-0.16 \pm 0.04 \text{ ‰}$  (n = 3) and  $0.56 \pm 0.06 \text{ ‰}$  (n = 4), respectively (Table S1; Fig. S1). These results could not be compared as no values for these standards have been published so far. However, the standards show good reproducibility and hence homogeneity and will therefore contribute to building our internal reference material isotopic database.

In addition to monitoring the quality of the analytical procedures, we examined the representability of our samples on a field scale. We collected and processed for isotopic analyses several samples from the same plot (i. e. field replicates) and the Cu and Zn isotopic compositions are consistent, within their 2 s (Fig. S2) uncertainty.

## 2.5. X-ray absorption spectroscopy (XAS)

Zinc K-edge and Cu K-edge X-ray absorption spectra for the OWs and soils were recorded on the FAME (BM30B) beamline at the European Synchrotron Radiation Facility (ESRF). All samples were freeze-dried, ground and moulded into pellets. They were analyzed at liquid helium temperature to improve the signal/noise ratio by decreasing the thermal motion of atoms and to prevent sample photoreduction damage. Measurements were carried out in fluorescence mode with a 13-element solid-state Ge detector. Each spectrum was the average of at least 3 and 12 scans for OWs and soils, respectively, depending on the noise level. For each scan, the X-ray beam was focused on a different position on the pellet to acquire a representative spectrum and limit the risk of beam-induced sample damage. Energy calibration was performed using Zn and Cu metallic foils (absorption energies defined at 9659 eV and 8979 eV, respectively) at the zero-crossing point of the second derivative spectrum. Normalization and data reduction were performed according to standard methods [43] using Fastosh [44] and Athena [45] software. Each spectrum can be divided into the X-ray absorption near edge structure (XANES) region and extended X-ray absorption fine structure (EXAFS) region. Both regions provided complementary information on the absorbing chemical element. XANES is sensitive to the oxidation state and coordination chemistry of the absorbing element. EXAFS is used to determine the distances, coordination numbers and nature of neighboring atoms surrounding the absorbing atom. The k to R space Fourier transforms of EXAFS spectra were obtained using Kaiser-Bessel apodization windows. This procedure resulted in

pseudo-radial distribution functions (RDF) uncorrected from phase shift functions. RDF peaks were therefore displaced by about 0.3 to 0.4 Å with respect to the crystallographic distances. In this study, fitting both XANES and EXAFS spectra for Cu and Zn was performed using a procedure previously described by [46]. Firstly, principal component analysis (PCA) of XANES and EXAFS spectra was performed separately on samples to determine the minimum number of components. Target transformation (TT) using a library of Cu and Zn reference compounds was subsequently performed to identify the probable components. Reference compounds with SPOIL values < 4 were selected. The selected reference compound contributions in the samples were determined using least square linear combination fitting (LCF) [47]. The LCF residual factor was calculated at each step of the fitting as follows:  $R = \frac{\sum(k^3\chi(k)_{\text{exp}} - k^3\chi(k)_{\text{fit}})^2}{\sum(k^3\chi(k)_{\text{exp}})^2}$ . An additional reference spectrum was added if: (1) the R factor decreased by 20 % or more, and (2) the contribution of the additional reference was  $\geq 10 \%$  among Cu or Zn species. The uncertainty of this LCF method was estimated at  $\pm 15 \%$  [43]. The sum of component weights in the best LCFs ranged from 84 % to 123 %. Differences in amplitudes due to over absorption during spectra acquisition or slight variations in the Cu and Zn species structures in the samples and reference materials may have led to the observed deviations from 100 % [48,49]. Linear combination results were normalized to 100 % for comparison of the relative speciation between samples. Details on the database of model compounds, PCA, TT (Tables S8-19) and LCF results (Figs. S3-7) are given in the supplementary material for Cu and Zn XANES and EXAFS, respectively.

## 2.6. Statistical analysis

The investigated variable and residue distributions were tested for normality using Shapiro-Wilk's test and for homoscedasticity using Levene's test. We used a nonparametric Kruskal-Wallis test as the Cu and Zn concentrations fitted a non-normal distribution. *p*-values < 0.05 were considered to be statistically significant (Table S2).

## 3. Results and discussion

### 3.1. Copper and zinc accumulation in organic waste amended soils

The studied long-term field experiments (LTEs) could be divided into two groups according to the application rate and experiment time: "low application rate/short experiment" (Nitisol and Luvisol) and "high application rate/long experiment" (Fluvisol and Cambisol).

In the low application rate/short experiment group, the applied pig slurry quantities were within the recommended application rates (20–40 m<sup>3</sup>.ha<sup>-1</sup>.year<sup>-1</sup>) to remain in compliance with the mandatory

170 kg ha<sup>-1</sup>.year<sup>-1</sup> organic N limit set by the EU under the Nitrate Directive [50]. The quantities of Cu derived from PS application corresponded to only 4 kg ha<sup>-1</sup> and 3 kg ha<sup>-1</sup> in the Nitisol and Luvisol samples over the entire period, respectively, whereas for Zn they respectively corresponded to 9 kg ha<sup>-1</sup> and 16 kg ha<sup>-1</sup> over the entire period. These low levels resulted in a small increase in Cu and Zn concentrations in amended soils as compared to their respective control soils (Fig. 2) and the statistical findings showed no significant difference in the mean Cu and Zn concentrations when comparing the control and amended soils (Nitisol and Luvisol, Table S2).

In the high application rate/long experiment group, large volumes of OW had been applied over long periods and the statistical tests revealed significantly higher amounts of Cu and Zn in OW amended soils compared to the respective control soils (Fluvisol and Cambisol, Table S2). Otherwise, the mass balance calculations showed that the Cu and Zn concentrations in amended soils were equal to the sum of Cu and Zn concentrations in their respective control soils and in the OW spreading inputs (Fig. 2). It could therefore be argued, considering the uncertainty (14 %), that all of the OW-derived Cu and Zn had accumulated in the soil, regardless of the type of OW (PS or HHC) and the soil type (Fluvisol and Cambisol) and the climatic conditions (Mediterranean and temperate). Our results were in line with previous findings in other soil types (Oxisol, Andosol, Arenosol, Fluvisol, Cambisol, Chernozem or Kastanozem) under temperate, Mediterranean and tropical climatic conditions [19,20,51,52-54]. Note that a direct comparison of the Cu and Zn concentrations between the studied field experiments is not feasible since soil samples were taken at different depths (Fig. 2). However, this does not change the patterns described for: “low application rate/short experiment” (Nitisol and Luvisol) and “high application rate/long experiment” (Fluvisol and Cambisol).

It is essential to gain insight into the potential mechanisms responsible for the retention of OW-derived Cu and Zn in soil to be able to predict their behavior and environmental impact following their input in soil. The use of stable isotopes and speciation analysis could highlight the impact of the repeated application of Cu- and Zn-rich OWs on agricultural soils, as discussed hereafter.

### 3.2. Copper and zinc isotopic signatures and speciation in the organic wastes

The pig slurry (PS) samples had  $\delta^{65}\text{Cu}$  values ranging from 0.01 ± 0.01 ‰ to 0.26 ± 0.02 ‰, which reflected heavy Cu isotopic enrichment (Fig. 3a, c); while the  $\delta^{66}\text{Zn}$  values ranged from -0.07 ± 0.02 ‰ to -0.03 ± 0.02 ‰, indicating modest light Zn isotopic enrichment (Fig. 3b, d). In contrast, household compost (HHC) had unfractionated  $\delta^{65}\text{Cu}$  values (0.02 ± 0.00 ‰) and  $\delta^{66}\text{Zn}$  values reflecting light Zn isotopic enrichment (-0.17 ± 0.06 ‰). The observed Cu and Zn isotopic signatures were similar to those previously reported for those noted in pig slurry, dairy manure and liquid cow manure samples [29,30]. The analyzed PS sample findings and previously published isotopic data

indicated a tendency towards higher  $\delta^{65}\text{Cu}$  values and greater Cu isotopic variability in animal manure samples as compared to Zn.

Livestock feed additives represent the main source of Cu and Zn found in PS. Only a small proportion of these elements is metabolized, while a major part (95 %, [5,6]) is excreted. Processes contributing to Cu and Zn transformation in the livestock digestive system can induce isotopic fractionation [55,56]. However, this isotopic effect may remain undetectable in the large pool of Cu and Zn excreted in faeces, i.e. pig slurry. Alternatively, the redistribution of Cu and Zn between the solid and liquid phases during PS storage in anaerobic lagoons or during OW composting could prompt isotopic fractionation. The isotopic signal produced would be detected if substantial amounts of Cu and Zn were to leave the system during the storage or composting treatment. However, there is a priori no Cu and Zn leaching or loss from anaerobic lagoons or composting units, which are designed to avoid any leakage of potentially environmentally harmful components. The isotopic effects of the storage or the treatment would therefore not be reflected in the OW. In other words, isotopic signatures measured in pig slurry or compost samples reflect the isotopic signature of the Cu and Zn sources, i.e. feed additives or raw OW before composting.

Cu- and Zn-sulfide accounted for 100 % of the Zn and Cu species in the studied pig slurry samples (Fig. 4), with a single exception regarding pig slurry from Nitisol samples, where 29 % Cu complexed by organic matter was detected. Note that Cu was monovalent (Cu(I)) in all of the Cu-sulfide cases mentioned in this study. In household compost, Cu and Zn speciation differed drastically from that in PS, in which there was a dominant proportion of oxidized species. Cu complexed by organic matter accounted for 68 % of the Cu speciation, while Zn species consisted mainly of amorphous Zn phosphate (49 %) and Zn complexed by organic matter (31 %). The prevalence of reduced Cu and Zn species in PS was in agreement with the findings of previously reported PS studies [57,19,20,58,59]. Reducing conditions during PS storage favoured the preservation of Cu- and Zn-sulfide species formed in the livestock digestive tract [60,61]. The prevalence of oxidized Cu and Zn species in household compost samples was in line with those previously reported in compost [17,62,21,18,63,64]. During composting, oxidative dissolution of Cu and Zn species (initially present in the non-composted OW) release Cu<sup>2+</sup> and Zn<sup>2+</sup> that are then likely to be complexed by organic compounds and/or precipitated with phosphate.

Speciation is the main parameter controlling Cu and Zn behaviour in soils. In the following sections, we thus discuss the impact of OW application on Cu and Zn speciation patterns and links with the isotopic compositions in the amended soils.

### 3.3. Amended soil isotopic signatures and speciation are not modified by low OW application rates over the short term

No differences in  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  values in control and amended soils were observed in LTEs where low OW volumes had been applied over short periods (Nitisol and Luvisol). The mixing calculations

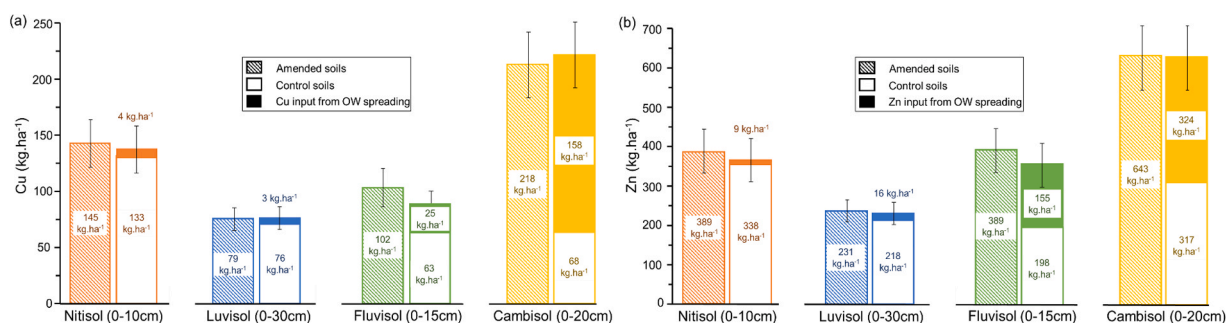
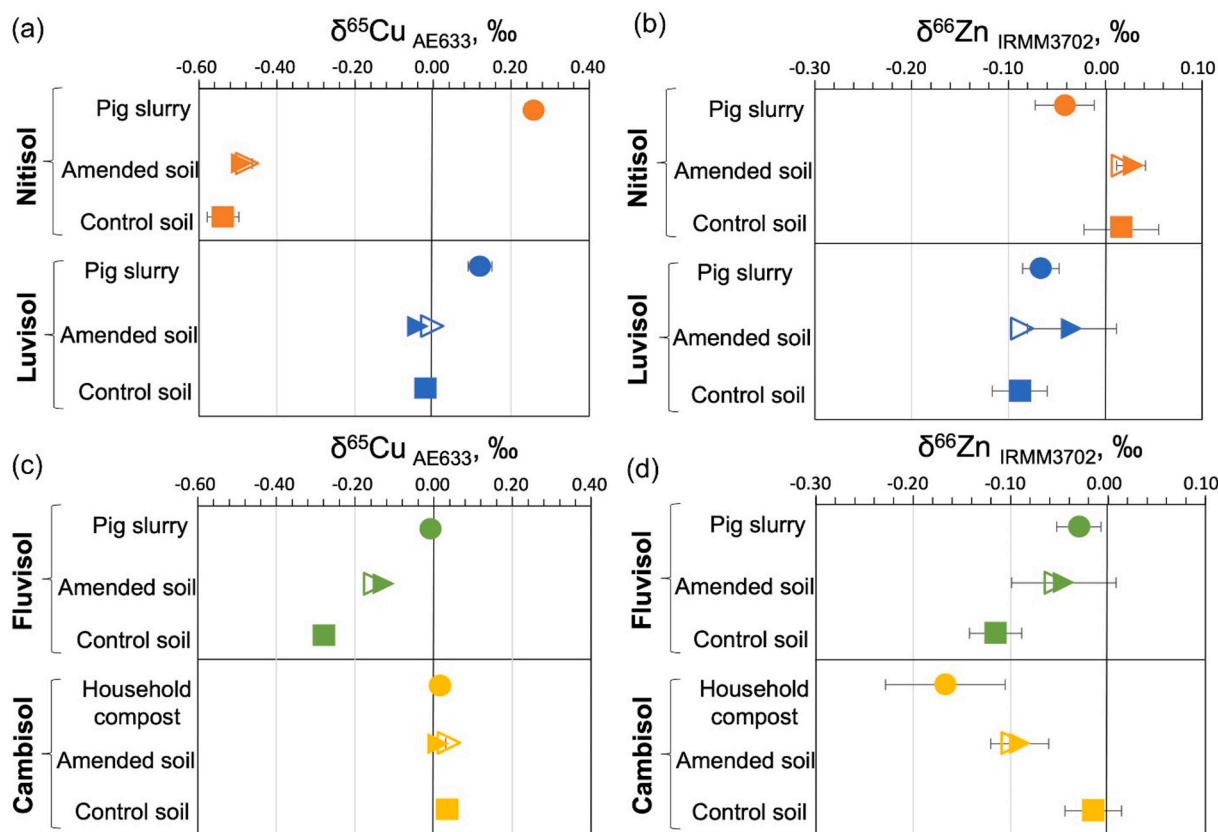


Fig. 2. Total amount of Cu (a) and Zn (b) in the topsoil layer of control and amended soils from different long-term field experiments. Empty bars represent the control soils, filled bars represent OW-derived Cu and Zn inputs and bars with diagonal hatching represent the amended soils. Error bars represent the uncertainty (14 % for Cu and Zn).



**Fig. 3.** (a, b): Copper and zinc isotopic compositions in organic wastes and soils under the low application rate/short experiment and (c, d) high application rate/long experiment conditions. Empty symbols represent the expected isotopic signatures in amended soils obtained from mixing calculations, whereas filled symbols represent the actual isotopic values we obtained through sample isotopic analysis. Error bars represent  $2\sigma$  calculated from the repeated analyses of the same sample solution. For  $\delta^{65}\text{Cu}$ , most of the error bars are smaller than the size of symbols.

(Fig. 3a, b and Table S3) showed that, although the Cu and Zn isotopic signatures of PS were fractionated and distinct from that noted in the control soils, the overall Cu and Zn input from OW was insufficient to modify the isotopic signature of the amended soils.

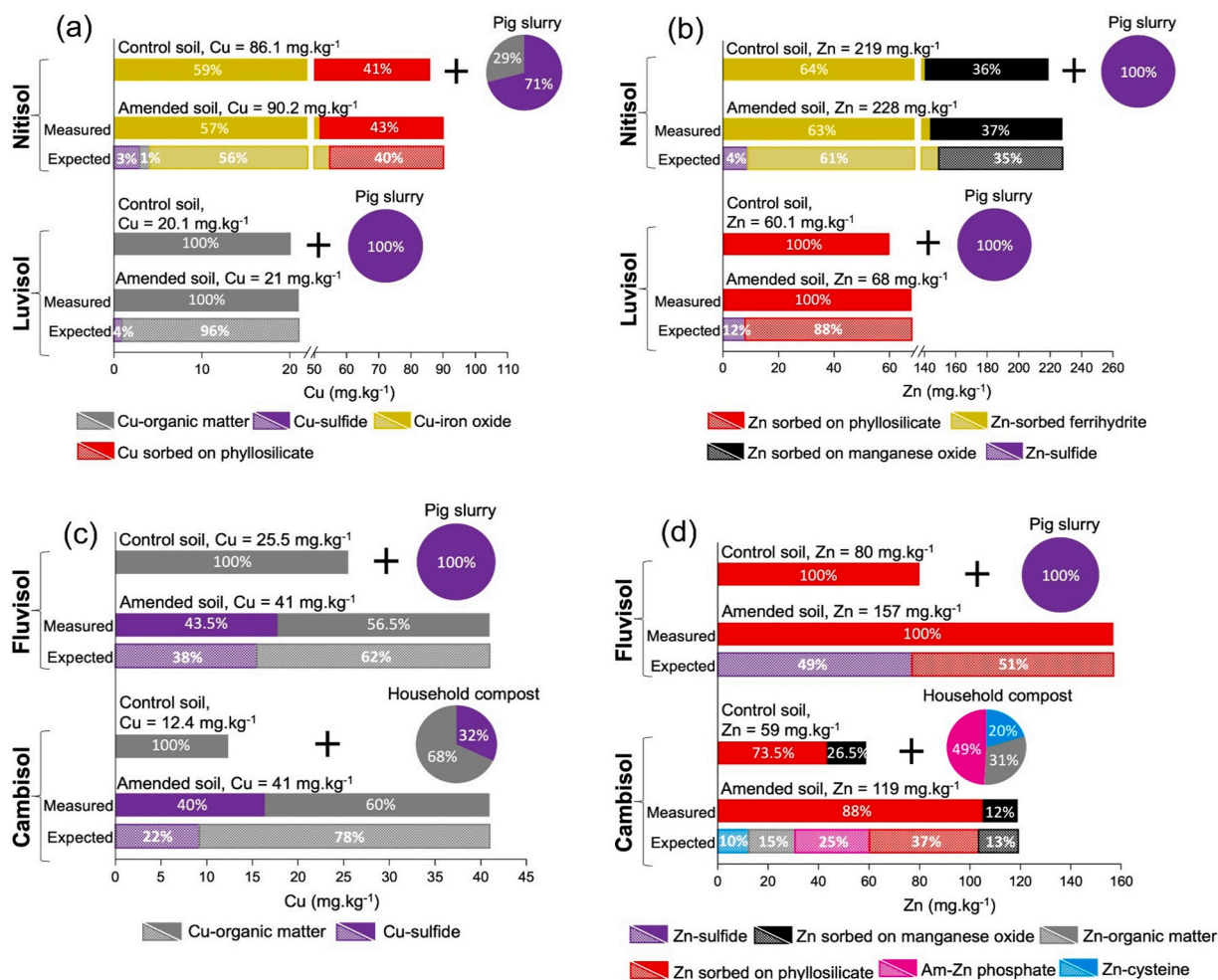
The Nitisol and the Luvisol control soils had different  $\delta^{65}\text{Cu}$  values (Fig. 3a, b). Their isotopic compositions reflected the isotopic signatures inherited from their parental materials and subsequently modified via weathering and pedogenetic processes involving transfer and redistribution of the elements between the solid and aqueous phases (i.e. dissolution of primary minerals, leaching and precipitation of secondary phases).

The speciation data were consistent with the isotopic data. Indeed, no change in Cu and Zn speciation was observed between the Nitisol and Luvisol control and amended soils. Cu sorbed on phyllosilicate accounted for 41–43 % and Cu-incorporated iron oxide for 57–59 % of the Cu speciation, respectively, in the control and amended Nitisol samples (Fig. 4a). In the Luvisol, Cu complexed by organic matter was the only Cu species detected in the control and amended soils (Fig. 4a). Similar to Cu, no change in Zn speciation was observed between the control and amended soils. For the Nitisol, the control and amended soils contained 36–37 % Zn sorbed on manganese oxide and 63–64 % Zn sorbed on ferrihydrite, respectively (Fig. 4b). Regarding the Luvisol, the Zn species was in the form of Zn sorbed on phyllosilicates in both the control and amended soils (Fig. 4b). Yet Cu and Zn speciation of Cu and Zn in PS contrasted the pattern noted in the soils. Indeed, Cu-sulfide and Zn-sulfide were the major species in PS (79–100 %). The mass balance calculations indicated that Cu- and Zn-sulfide represented around 10 % of Cu and Zn speciation in their respective amended soils (Table S4-5). The contribution of Cu- and Zn sulfide from OW in soil was probably not detected since the uncertainty for the LCF procedure was assumed to be

around 15 % with a detection limit of about 10 % [18,43]. This assumption was further supported by visual comparison of the spectral features of the LCF XANES and EXAFS results for amended soils with (= 10 %) and without Cu- and Zn-sulfide (Fig. S8-9). No differences were visually observed when 10 % Cu- and Zn-sulfide was added LCF (Fig. S8-9). Hence, the isotopic signature and speciation of the amended soils were not modified by low OW application rates over the short term.

#### 3.4. Amended soil isotopic signatures and speciation significantly altered by high application rates over the long term

Soil is an open system where transformations are driven by flux of matter and energy [65]. In case a given element is partially lost from soil horizon, the isotope fractionation associated with processes of its redistribution between soil components can be recorded and preserved even on a bulk soil scale. Particularly with respect to excessive amounts of exogenous Cu and Zn, one would expect their mobilization to be associated with isotopic fractions. On the other hand, changes in Cu and Zn isotope composition of soils can result from mixing between different sources. We observed a shift in  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  values in the amended soils compared to the control soils in the LTEs where large OW volumes had been applied over long periods (Fluvisol and Cambisol, Fig. 3c, d). For the Fluvisol, the isotopic compositions in the amended soil samples ( $\delta^{65}\text{Cu} = -0.13 \pm 0.01 \text{ ‰}$ ;  $\delta^{66}\text{Zn} = -0.05 \pm 0.05 \text{ ‰}$ ) indicated a shift towards heavier  $\delta^{65}\text{Cu}$  and  $\delta^{66}\text{Zn}$  isotopic values compared to the control soils (Fig. 3c, d). This suggests that there was Cu and Zn input from PS, for instance, with a heavier isotopic signature. For the Cambisol, no difference in the Cu isotopic signature was observed between the HHC ( $\delta^{65}\text{Cu} = 0.02 \pm 0.00 \text{ ‰}$ ) control soil ( $\delta^{65}\text{Cu} = 0.04 \pm 0.01 \text{ ‰}$ ) and amended soil ( $\delta^{65}\text{Cu} = 0.01 \pm 0.02 \text{ ‰}$ ). Isotope tracing was thus not



**Fig. 4.** (a, b): Copper and zinc speciation in the control and amended soils and organic waste from the low application rate/short experiment and (c, d) the high application rate/long experiment. Dotted bars represent the expected Cu and Zn speciation in the amended soils based on the mass balance calculations, whereas solid bars represent the actual Cu and Zn speciation we obtained via XAS analysis of the samples.

possible despite the high total Cu input (158 kg/ha, Fig. 2a). On the other hand, Zn showed a slight shift towards a lighter Zn isotopic composition in the amended soil ( $\delta^{66}\text{Zn} = -0.11 \pm 0.06 \text{‰}$ ) compared to the control soil ( $\delta^{66}\text{Zn} = -0.03 \pm 0.07 \text{‰}$ ), suggesting that there had been input of Zn with a negative isotopic signature, such as in HHC (Fig. 4d). The mixing calculations confirmed that Cu and Zn input from pig slurry or HHC spreading was the main process controlling the shifts in Cu and Zn isotopic compositions in both experiments (Fluvisol and Cambisol; Table S3). Despite the fact that the isotopic mixing calculations were based only on one OW sample per experiment, the results corroborated the concentration-based mass balance (Section 3.1) showing that the amended soils behaved as a closed system with respect to Cu and Zn. Furthermore, this agreement suggests that there was negligible interannual variability in the OW isotopic signatures.

In the Fluvisol, PS-borne Cu-sulfide, which was responsible for a 2-fold increase in the Cu concentration in the amended soil samples, represented 44 % of the Cu speciation in the amended soil. In the Cambisol, Cu-sulfide accounted for 40 % of the Cu speciation in the amended soil samples, whereas it accounted for 32 % of the Cu speciation in HHC. ZnS, which accounted for 100 % of the Zn speciation in PS, was no longer detected in the amended Fluvisol samples (Fig. 4d). Similarly, the Zn species detected in HHC (amorphous Zn phosphate and Zn bound to cysteine and organic matter) were not detected in the amended Cambisol samples. The distinction between the Cu and Zn speciation patterns is clearly shown in Fig. 4c, d when comparing the measured and expected speciation patterns of the amended soils. OW-

borne Cu and Zn species therefore exhibited different behaviors following OW soil application.

The Cu species pattern reported here, i.e. persistence of Cu-sulfide in amended soils, differed markedly from that recently reported by Formentini et al. [19]. In their study, Cu-sulfide, which accounted for 100 % Cu speciation in PS, was not observed in soils that had been subject to PS applications over an 11-year period. These discrepancies may be explained by the contrasting Cu-sulfide species identified in the PS, i.e. only nanosized Cu-sulfides were identified by Formentini et al. [19] whereas we observed a majority of crystallized Cu-sulfides (Table S6). Due to their small size, nanoparticles are less stable and dissolve faster than larger and crystallized particles [66,67]. This could explain why Cu-sulfide was detected in amended soils upon which crystallized Cu-sulfides had been applied. The presence of nanosized or crystallized Cu-sulfides in the same type of OW, i.e. PS, has yet to be elucidated. Recently, it was shown that anoxic storage of OWs and anaerobic digestion contributed to nanosized Cu-sulfide particle ageing, thereby inducing the transformation of nanosized Cu-sulfide into crystallized Cu-sulfide [17]. It could thus be assumed that varying PS storage times under anaerobic conditions were responsible for the presence of the two distinct Cu-sulfide species detected in the three studies [[19,20] and this study]. In HHC, Cu(II) bound to organic matter was the major Cu species detected (68 % of Cu speciation). This oxidation state was favored by the oxidizing conditions prevailing during the aerobic composting process. However, crystallized Cu-sulfide was detected as a minor species (32 % of Cu speciation), thus confirming recent findings in

several different composts [17] and aged biosolids [62]. As previously mentioned, we hypothesized that the presence of crystallized Cu-sulfide in HHC accounted for the persistence of this species in the amended soil.

In contrast, PS- or HHC-derived Zn species were not detected in the amended soil samples, thereby highlighting the instability of these Zn species following OW application. The apparent loss of nanosized ZnS, i. e. the main species identified in PS (Table S7), has already been described in PS-amended soil [20]. Recently, Le Bars et al. [68] confirmed that: (i) nanosized ZnS dissolution was fast in a clayey soil, i. e. 82 % dissolution within a month, and (ii) released Zn was not available due to sorption on clay minerals. Similar behaviour of PS-borne ZnS was observed here in the Fluvisol, i. e.; fast dissolution of ZnS after PS application and sorption of released Zn by clay minerals. The behaviour of ZnS species contrasted with that of Cu-sulfide. Indeed, Cu-sulfide was persistent in amended soils, whereas Zn-sulfide was no longer detected. In soil, Hesterberg et al. [69] also observed that Zn-sulfide oxidized more rapidly than Cu-sulfide. Galvanic cathodic protection was put forward to explain this behaviour: Cu-sulfide with a higher oxidation phase-boundary potential acts as a cathode to Zn-sulfide, hence accelerating the oxidation of the latter. For the Cambisol, HHC-derived Zn species, i. e. amorphous Zn phosphate and Zn bound to organic matter, were not detected in the amended soils. The mineralization of organic matter from HHC [63], combined with the instability of amorphous Zn phosphate, are the most probable factors explaining the release of Zn, subsequently sorbed by clay minerals.

The described speciation changes are evidence that OW-borne Cu and Zn is redistributed within the amended soil through dissolution, adsorption and precipitation, which are stable isotope fractionating processes (e.g. Wiederhold [70]). In addition, the nature and changes in the bonding environment can be recorded via isotopic fractionation (e.g. Schauble [71] and predicted by ab initio calculations (e.g. Fujii, Albarède [72-74]). Previous experimental studies have documented (e.g. Aucour et al., [31,75] isotopic variability linked to different speciation patterns. However, in both LTE conditions where high amounts of OW were applied over a long period (Fluvisol and Cambisol), and all OW-borne Cu and Zn was retained in the horizon (no loss), the isotopic shifts observed in the amended soils could be explained by binary mixing between the OW-borne Cu and Zn and the Cu and Zn originally present in the control soil. On a bulk soil sample scale, the isotopic effect associated with mixing dominates the isotopic signal and fractionations related to the individual processes cannot be detected. Recent studies suggest that isotopic analyses on a smaller than bulk soil scale are necessary to assess the isotopic variability related to the redistribution of OW-borne Cu and Zn within amended soil. For example, Fekiacova et al. [76], who used stable Fe isotopes to study the impact of artificial drainage on a Retisol, reported no isotopic variability on a bulk soil scale but major isotopic contrasts between the different soil constituents. The authors explained that these contrasts reflected interactions of Fe with different mineral phases constituting the soil components.

To summarize, our results showed that high amounts of OW should be applied repeatedly over many years in order to generate modifications in Cu and Zn concentrations, speciation and isotopic compositions in amended soils. Further studies are needed to explore the threshold—in terms of years and applied OW quantities—beyond which the changes become detectable in amended soils. Furthermore, isotope tracing of the OW-borne Cu and Zn in amended soils require down-scaling and isotope analyses on a smaller than bulk soil scale in order to gain insight into fractionations linked to processes involving metal redistribution between different soil components (e.g. sorption on clay minerals (e.g. Guinoiseau et al., [77,78] and complexation by organic matter (e.g. Bigalke et al., [79,80]).

#### 4. Conclusion

We studied organic waste and soil samples from four long-term field experiments representing different soil types and experimental

modalities. We combined concentration, isotope composition and speciation analyses to assess the long-term fate of OW-borne Cu and Zn in the amended soils.

The speciation of OW-borne Cu and Zn was dominated by reduced species, i. e. Cu(I)- and Zn-sulfide in the pig slurry and oxidized species in the household compost, in agreement with previously published observations. Their isotope compositions showed heavy Cu enrichment in PS and slight light Zn enrichment in HHC, and we propose that the described isotopic signatures reflect the isotopic signatures of the source, i. e. feed additives or raw OW before composting.

In the field experiments where low volumes of OW (up to 30 m<sup>3</sup>·ha<sup>-1</sup>·year<sup>-1</sup>) had been applied for a short time (7–8 years), the Cu and Zn concentrations, isotope signatures and speciation in the amended soils showed no significant changes. In contrast, in the field experiments where the soils had received large amounts of OW (up to 108 m<sup>3</sup>·ha<sup>-1</sup>·year<sup>-1</sup>) over long periods (16 and 17 years), the Cu and Zn concentrations significantly increased, regardless of the soil type and applied OW. The mass balance calculations provided evidence that the soils acted as a closed system with respect to Cu and Zn. The isotopic shifts observed in the amended soils could be explained by binary mixing between OW-borne Cu and Zn and Cu and Zn originally present in the soil. Even though speciation analyses revealed significant changes reflecting Cu and Zn redistribution in the amended soils, the isotopic fractionations linked to these processes could not be detected on a bulk soil scale.

Furthermore, Cu and Zn exhibited contrasting speciation patterns in OW-amended soils. We documented, for the first time, the persistence of OW-borne Cu(I)-sulfides in the amended soils and we propose that the crystalline nature of Cu(I)-sulfide could explain this persistence. In contrast, Zn species identified in PS and HHC were completely transformed after OW spreading on the soil and this behavior could be explained by the nanosized nature of the ZnS in PS and the instability of amorphous Zn-phosphate species and Zn complexed by organic matter in HHC.

#### Environmental implication

Organic wastes (OWs) are commonly recycled in agricultural soil as fertilizer. They also contain high concentrations of potentially toxic Cu and Zn trace elements. OW recycling results in a consequential increase in Cu and Zn concentrations in soil, thereby raising environmental concerns regarding the potential ecotoxicity and introduction of these trace elements in the human food chain. Predicting the toxicity and environmental fate of OW-derived Cu and Zn requires an understanding of their chemical speciation. Our detailed characterization of Cu and Zn speciation in OW and soils enhances insight into the environmental fate of contaminants in amended soils.

#### CRediT authorship contribution statement

**Abraham Pappoe:** Writing – original draft, Investigation, Formal analysis, Data curation. **Zuzana Fekiacova-Castanet:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Emmanuel Doelsch:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Denis Testemale:** Validation, Investigation. **Jakob Magid:** Validation, Investigation. **Thierry Morvan:** Validation, Investigation. **Pierre Deschamps:** Methodology. **Frédéric Feder:** Validation, Investigation. **Abel Guihou:** Validation, Supervision, Methodology. **Angela D. Bosch-Serra:** Validation, Investigation.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence



the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136039.

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