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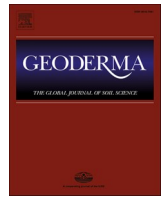
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## Redistribution of Zn towards light-density fractions and potentially mobile phases in a long-term manure-amended clayey soil

Thiago A. Formentini<sup>a,d,\*</sup>, Isabelle Basile-Doelsch<sup>b</sup>, Samuel Legros<sup>i,j</sup>, Daniel Borschneck<sup>b</sup>, Julia S. Venzon<sup>c</sup>, Adilson Pinheiro<sup>c</sup>, Cristovão V.S. Fernandes<sup>d</sup>, Fábio J.K. Mallmann<sup>e</sup>, Milton da Veiga<sup>f</sup>, Emmanuel Doelsch<sup>g,h</sup>

<sup>a</sup> Department of Soil and Environment, Swedish University of Agricultural Sciences, P.O. Box 7014, SE-750 07 Uppsala, Sweden

<sup>b</sup> Aix-Marseille Université, CNRS, IRD, Coll France, INRA, CEREGE, F-13545 Aix-en-Provence, France

<sup>c</sup> Environmental Engineering Program, Regional University of Blumenau (FURB), 89030-000 Blumenau, SC, Brazil

<sup>d</sup> Department of Hydraulics and Sanitation, Federal University of Parana (UFPR), 81531-980 Curitiba, Brazil

<sup>e</sup> Department of Soils, Federal University of Santa Maria (UFSM), 97105-900 Santa Maria, Brazil

<sup>f</sup> UNOESC, P.O. Box 250, 89620-000 Campos Novos, SC, Brazil

<sup>g</sup> CIRAD, UPR Recyclage et risque, F-34398 Montpellier, France

<sup>h</sup> Recyclage et Risque, Univ. Montpellier, CIRAD, Montpellier, France

<sup>i</sup> UPR Recyclage et Risque, CIRAD, 18524 Dakar, Senegal

<sup>j</sup> Recyclage et Risque, Univ. Montpellier, CIRAD, Montpellier, France

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### ABSTRACT

Zinc (Zn) occurs naturally in soils, but spreading Zn-rich livestock manure on agricultural soils may lead to hazardous Zn levels. Interactions between exogenous Zn (from animal manure) and the soil matrix must be assessed to predict its behavior. We conducted soil density fractionation using sodium polytungstate solutions (LST) to isolate and identify the soil constituents and investigated their associations with Zn within five density fractions by characterizing the total organic carbon (TOC), mineralogy and Zn speciation. We analyzed a clayey Hapludox soil from a field experiment that had received pig slurry applications over an 11-year period, causing a 2-fold increase in the Zn concentration within the 0–5 cm layer relative to the control soil. Two density fractions with contrasting compositions were found: (i) a light fraction ( $<1.9 \text{ g cm}^{-3}$ ), which accounted for only ~5% of the bulk soil mass but contained the highest OM concentration; and (ii) a mineral-rich fraction ( $2.5\text{--}2.7 \text{ g cm}^{-3}$ ), which had a low OM concentration but accounted for ~80% of the soil mass. Zn in the control soil (no pig slurry application) was mostly (79.6%) in the mineral-rich fraction. Pig slurry applications increased the amount of Zn extracted by the fractionation solution to 16.6% and Zn in the organic-rich fraction to 13.2%, although the Zn in the mineral-rich fraction was still dominant (57.3%). The fraction extracted by the fractionation solution, the  $<1.9 \text{ g cm}^{-3}$  fraction and the  $2.5\text{--}2.7 \text{ g cm}^{-3}$  fraction each accounted for roughly a third of the pig slurry-borne Zn in the amended soil. The results showed that 11 years of Zn-rich livestock manure application caused partial depletion of the clay sorption capacity of the soil, with the potential occurrence of Zn leaching in association with light density soil phases.

### 1. Introduction

Zinc (Zn) is a diet supplement given to livestock to prevent diseases and promote growth (Romeo et al., 2014). Sufficient levels of Zn are guaranteed by overdosing this element in livestock feed. Most of this Zn is not taken up by the animals and field application of Zn-rich livestock manure is common practice. This accounts for 51% and 78% of

exogenous Zn inputs in cultivated soils in China and France, respectively (Romeo et al., 2014). High Zn levels in livestock manure-amended soils may lead to phytotoxicity (Kumar et al., 2018), introduction into the food chain via food crops (Rai et al., 2019) and groundwater contamination (Hao et al., 2008). A complex and challenging combination of field and analytical approaches are required to assess the fate of Zn following continued livestock manure application onto soils.

\* Corresponding author at: Department of Soil and Environment, Swedish University of Agricultural Sciences, P.O. Box 7014, SE-750 07 Uppsala, Sweden.

E-mail address: [thiago.formentini@slu.se](mailto:thiago.formentini@slu.se) (T.A. Formentini).

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The “field trial challenge” concerns the need for realistic study conditions. Zn transformation may be a rather slow process in the environment yet vary over time according to soil and climate characteristics (Brazauskienė et al., 2008). For instance, Zn toxicity to barley growth was 30-times lower in a real Zn-contaminated soil as compared to a simulated Zn-spiked soil when assessed by the same method (Hamels et al., 2014). Long-term field-scale setups with continued and controlled livestock manure application and crop management are thus necessary—this means decades of fieldwork.

The “analytical challenge” concerns the speciation-dependent behavior of the contaminant. Zn may assume multiple physicochemical forms in association with the soil constituents, with substantial implications with regard to its environmental fate (e.g. Gelly et al., 2019; Tella et al., 2016). Assessing contaminant/soil associations is not straightforward for trace elements such as Zn bound within complex matrices such as soils. Extraction protocols or synchrotron-based spectroscopic techniques are commonly used analytical approaches (e.g. Scheinost et al., 2002).

Extraction methods reflect the contaminant extractability, often related to its potential mobility and bioavailability, yet they fail to distinguish contaminant-bearing phases (e.g. phyllosilicates, Fe oxides, organic matter or a combination of them) (Bacon and Davidson, 2008). Although extracted fractions are often named after their most likely bearing phases, direct assertions should be avoided (Bacon and Davidson, 2008). On the other hand, synchrotron-based techniques (notably X-ray absorption spectroscopy (XAS)) enable direct assessment of contaminant speciation and the molecular environment (e.g. Scheinost et al., 2002; Legros et al., 2010; Formentini et al., 2017). The application of XAS is, however, hampered by the need for synchrotron radiation.

Since extraction and synchrotron-based methods have limitations, we decided to explore alternative and complementary approaches. The primary goal of density fractionation is to decrease the soil heterogeneity and complexity (Doelsch et al., 2006; Basile-Doelsch et al., 2007; Levard et al., 2009; El-Mufleh et al., 2014). A given isolated soil fraction with a defined density range will ideally contain only the fraction of a given contaminant originally associated with that phase. Assessing the composition of each density fraction, together with the contaminant levels, should thus reflect the contaminant/soil associations. In this regard, density fractionation may generate more comprehensive findings than extraction methods, requiring simpler and less expensive equipment as compared to synchrotron-based techniques.

The present study aimed at assessing the environmental behavior of Zn following continual application of pig slurry (PS) as fertilizer on an agricultural soil. We used an original combination of field and analytical approaches to achieve this. We addressed the “field challenge” by analyzing a long-term field experiment that had received controlled PS amendments over an 11 year period. We tackled the “analytical challenge” in three complementary ways. First, we assessed the most likely Zn/soil associations in control and contaminated soils after fractionating them into five density fractions in which we characterized their organic and mineral content. Then we recorded and analyzed the Zn K-edge XAS spectra of the most representative density fractions. Finally, we compared our new observations with data we had previously reported for the same field experiment using well-established sequential extraction (Formentini et al., 2015) and XAS (Formentini et al., 2017) methods. To our knowledge, such an in-depth investigation has never been reported in the context of livestock manure-amended soils.

## 2. Materials and methods

### 2.1. Field experiment

This study was conducted on a basaltic Rhodic Hapludox soil (USDA, 2003) with 67.8% clay, 30.8% silt and 1.4% sand. The site was located in Campos Novos, SC, Brazil. Pig slurry was applied at  $200 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$  over 11 years, for a total of 22 amendments (two per year). A control soil

with no pig slurry application parallel to the amended soil was also monitored.

The 0–5 cm layer of the control soil contained  $51.7 \text{ kg Zn ha}^{-1}$  ( $100.4 \text{ mg Zn kg soil}^{-1}$ ). The same layer of the amended soil contained  $96.9 \text{ kg Zn ha}^{-1}$  ( $188.1 \text{ mg Zn kg soil}^{-1}$ ), i.e. a nearly 2-fold increase.  $94.6 \text{ kg Zn ha}^{-1}$  was added to the soil via 22 pig slurry amendments. Excess Zn accumulated in deeper layers, up to 30 cm, but at lower concentrations than at the surface (Formentini et al., 2015). Zn in the pig slurry consisted of nano-sized ZnS that underwent rapid oxidation after application on the soil (Formentini et al., 2017). Further description of the Zn distribution through the soil profile, soil characteristics, crop rotations, seeding and sampling may be found elsewhere (Formentini et al., 2017, 2015; Veiga et al., 2012). Note that the samples used for analysis in the present study were strictly the same as those used in the studies of Formentini et al. (2017) and Formentini et al. (2015).

### 2.2. Density fractionation

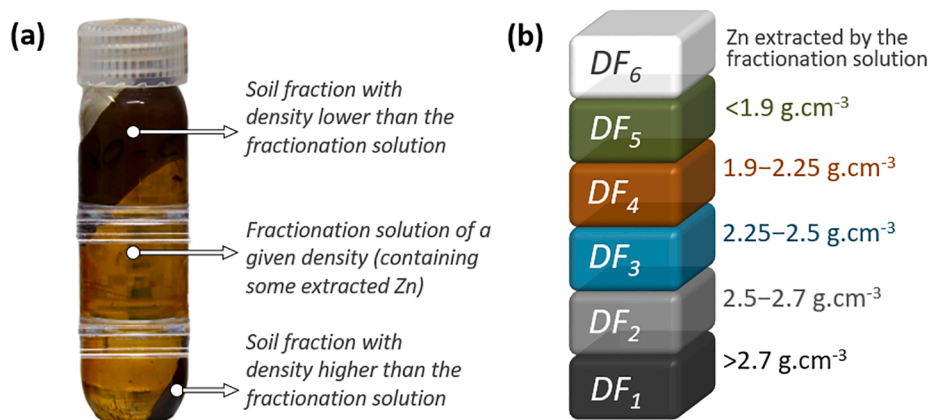
The density fractionation procedure was slightly modified from that of El-Mufleh et al. (2014). Soil density thresholds were selected on the basis of the theoretical density of minerals previously identified within the soil (Formentini et al., 2017) via X-ray diffraction (XRD). The chosen thresholds were 2.7, 2.5, 2.25 and  $1.9 \text{ g cm}^{-3}$ . Five density fractions were therefore obtained:  $>2.7 \text{ g cm}^{-3}$ ,  $2.5\text{--}2.7 \text{ g cm}^{-3}$ ,  $2.25\text{--}2.5 \text{ g cm}^{-3}$ ,  $1.9\text{--}2.25 \text{ g cm}^{-3}$  and  $<1.9 \text{ g cm}^{-3}$ . These density fractions (DF) were respectively denoted DF<sub>1</sub>, DF<sub>2</sub>, DF<sub>3</sub>, DF<sub>4</sub> and DF<sub>5</sub> (Fig. 1a). Moreover, a fraction not related to density was denoted DF<sub>6</sub> to account for the Zn that was extracted by the fractionation solutions. Note that DF<sub>6</sub> is not a solid fraction nor a density fraction. It is a liquid fraction containing colloidal particles and soluble ions extracted from the soil by the fractionation solution during the fractionation steps. This liquid fraction was taken into account to assess the recoveries of the fractionation procedure as well as to estimate the leachable amounts of Zn.

Fractionation solutions with densities equal to each of the density thresholds were prepared by diluting a sodium polytungstate (LST Fastfloat (Na<sub>6</sub>[H<sub>2</sub>W<sub>12</sub>O<sub>40</sub>])) commercial solution ( $d = 2.82 \text{ g cm}^{-3}$ ) in ultrapure water (Milli-Q Reference, Millipore). Dilutions were calculated and performed on a mass basis and the resulting densities were checked with a hydrometer. After dilution, NaOH was added to increase the pH of the fractionation solution from around 2.0 (pH of the commercial LST) to 5.8 (pH of the studied soil), which was required to reduce Zn losses to the solution due to sorption competition with excess protons.

Six grams of finely ground soil were weighed in 50 ml centrifuge tubes and mixed with 30 ml of the lightest fractionation solution. The centrifuge tubes containing this mixture were placed in an ice bath and sonicated to destroy soil aggregates. Sonication was performed using a Bioblock Scientific® Vibracell sonicator with a 13 mm probe tip, operating at 70% output for 1 min and 35% output for 8 min (El-Mufleh et al., 2014). The mixture was centrifuged and two density fractions were fractionated: one with a density lower than the fractionation solution and one with a density higher than the fractionation solution (Fig. 1a). The centrifuge time was adapted according to Stokes' law, with  $0.2 \mu\text{m}$  set as the smallest theoretical particle size to settle. The  $0.2 \mu\text{m}$  threshold was selected to provide reasonable centrifuge times (i.e. no more than a few hours).

The supernatant containing the soil density fraction with a density lower than the fractionation solution threshold was siphoned off and stored. The remaining soil was mixed with 30 ml of progressively heavier fractionation solutions. The sonication, centrifugation and siphoning steps were repeated for each fractionation step.

After the five density fractions were fractionated, ultrapure water was added to the stored supernatants to decrease the density of each fractionation solution to a value lower than the lowest density of soil particles in the concerned fraction (e.g. density of the fractionation solution decreased to  $2.4 \text{ g cm}^{-3}$  to collect the 2.5–2.7 fraction). After



**Fig. 1.** (a) Tube containing the bulk soil and the fractionation solution during one density fractionation step; (b) The five density fractions plus the fraction extracted by the fractionation solution obtained after all steps of the fractionation procedure.

centrifugation, the diluted fractionation solution was siphoned off. Finally, the soil density fractions were rinsed three times with ultrapure water, centrifuged and the supernatant was retained to determine the Zn contents that eventually dissolved during the rinsing steps. Soil density fractions were freeze-dried and weighed. The whole procedure was performed for each sample in triplicate.

### 2.3. Characterization of soil density fractions

The mineral composition of the soil density fractions was assessed using an X-ray diffractometer (X'Pert Pro, Panalytical) running at 40 kV and 40 mA, using Co K $\alpha$  radiation ( $\lambda = 1.79 \text{ \AA}$ ) with a linear detector (X'Celerator) and a secondary flat monochromator. Samples were ground in an agate mortar and placed on zero background silicon sample holders with drops of ethanol. For each sample, a counting time of 5.5 s per  $0.033^\circ$  step was used for  $2\theta$  in the  $4\text{--}80^\circ$  range. Phase identification was performed using the XRD data analysis software X'pert PRO High-score plus and its powder diffraction database ICDD-PDF2.

Total organic carbon (TOC) in bulk soils and density fractions was measured using an elemental analyzer (NA 1500, Fisons). Previous acid treatment was not necessary because the soils were carbonate-free. The elemental analyzer was calibrated with standard acetanilide (Säntis analytical). Reproducibility was checked with lab-internal marine and lacustrine standard sediments. The standard deviation was under 2%. The TOC distributions amongst soil density fractions of the control and amended soils were normalized to achieve 100% recovery in the soil fractionation procedure. The reasoning used for the normalization is discussed in the [Supporting Information SI-1](#).

For Zn analysis, bulk soils and density fractions were subjected to microwave-assisted acid digestion, according to the EPA 3051A protocol (USEPA, 2007) and then diluted in 2% HNO $_3$  (PlasmaPure Plus 69% HNO $_3$ , SCP Science; Milli-Q Reference Ultrapure Water, Millipore). Concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS, NexION 300X, PerkinElmer) with online addition of Rh-103 as internal standard element. The ICP-MS measurement accuracy was checked using certified reference samples [SLRS-5 river water, Canada; GBW07402 (GSS-2) and GBW07403 (GSS-3) soils, China]. All concentrations were in close agreement with certified values (i.e. in the 85–115% range). The Zn distributions amongst the control and amended soil density fractions were normalized to achieve 100% recovery in the soil fractionation procedure. The reasoning used for the normalization is discussed in the [Supporting Information SI-2](#).

### 2.4. XAS analysis

X-ray absorption spectroscopy (XAS) was used to assess the speciation of Zn in each soil density fraction of the control and amended soils.

Zn K-edge XAS spectra were recorded on the BM30B (FAME) beamline at the ESRF synchrotron (Grenoble, France). In order to prevent beam damage, samples were placed at liquid helium temperature and each scan was acquired at a different spot on the sample pellet. The spectra of each sample were an average of seven to nine scans, depending on the Zn concentration and the signal-to-noise ratio. Spectra were acquired in fluorescence mode with a 30-element solid-state Ge detector. Energy calibration was performed using a metallic Zn foil (absorption edge set at 9963.5 eV).

Normalization and data reduction were performed according to standard methods (Doelsch et al., 2006) using Athena software (Ravel and Newville, 2005). The EXAFS signal is a weighted average from the contributions of all Zn species present in the sample. Major Zn species could be individually retrieved via least square linear combination fitting (LCF) to known mixtures of Zn reference compounds. A library of spectra from Zn reference compounds (Le Bars et al., 2018; Legros et al., 2010; Jacquat et al., 2008) was used to identify Zn species in the soil fractions (SI-5). LCF was performed for each density fraction over a  $k$ -range of  $2.0\text{--}9.9 \text{ \AA}^{-1}$ . The residual factor of each LCF was calculated as follows:  $R = \frac{\sum(k^3\chi(k)_{exp} - k^3\chi(k)_{fit})^2}{\sum(k^3\chi(k)_{exp})^2}$ . At each step of the fitting, an additional reference spectrum was added to a best LCF fit but only kept in the result if the two following conditions were true: (i) the residual factor decreased by 20% or more and (ii) the additional reference had a contribution equal to or higher than 10% among Zn-species used for the fit. The uncertainty of this LCF method was estimated at  $\pm 15\%$  (Doelsch et al., 2006).

## 3. Results

### 3.1. Qualitative characterization of density fractions

[Fig. 2](#) shows the XRD characterization of soil density fractions. Only the control soil is shown as the diffractograms of equivalent fractions were very similar for the control and amended soils ([Supporting Information SI-3](#)). DF $_1$  contained the Fe and Ti oxides anatase ( $d = 3.89 \text{ g cm}^{-3}$ ), hematite ( $d = 5.26 \text{ g cm}^{-3}$ ), ilmenite ( $d = 4.79 \text{ g cm}^{-3}$ ), rutile ( $d = 4.25 \text{ g cm}^{-3}$ ) and some undistinguished pyroxenes. DF $_2$  contained quartz ( $d = 2.66 \text{ g cm}^{-3}$ ) and the phyllosilicates kaolinite ( $d = 2.63 \text{ g cm}^{-3}$ ) and vermiculite ( $d = 2.50 \text{ g cm}^{-3}$ ). The lower intensity peaks in the regions around  $40^\circ$ ,  $48^\circ$ ,  $65^\circ$  and  $75^\circ$  ( $2\theta$ , Co K $\alpha$ , dotted lines in [Fig. 2](#)), as compared to DF $_1$ , also indicated the presence of some residual hematite in DF $_2$ . DF $_3$  contained cristobalite ( $d = 2.33 \text{ g cm}^{-3}$ ) and gibbsite ( $d = 2.44 \text{ g cm}^{-3}$ ), as well as residual kaolinite, vermiculite and hematite. These three latter minerals were also present in DF $_4$  and DF $_5$ .

The fact that residual kaolinite, vermiculite and hematite were detected outside their expected density range may have been due to

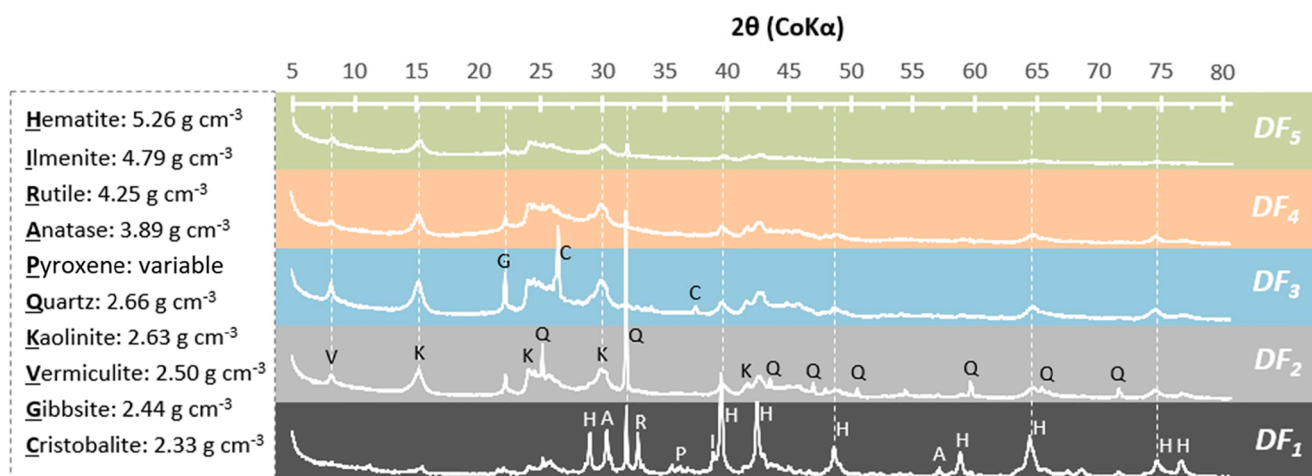


Fig. 2. XRD characterization of soil density fractions. DF<sub>1</sub>: >2.7 g cm<sup>-3</sup>, DF<sub>2</sub>: 2.5–2.7 g cm<sup>-3</sup>, DF<sub>3</sub>: 2.25–2.5 g cm<sup>-3</sup>, DF<sub>4</sub>: 1.9–2.25 g cm<sup>-3</sup> and DF<sub>5</sub> < 1.9 g cm<sup>-3</sup>.

incomplete breakage of the soil aggregates during the sonication step (Section 2.2). The diffusion bands centered around 24° (2θ) in DF<sub>2</sub>, DF<sub>3</sub>, DF<sub>4</sub> and DF<sub>5</sub> may indicate the presence of either OM and/or low-crystallized mineral phases (Bonnard et al., 2012). Organo-mineral aggregates within lighter density fractions were previously observed via SEM-EDX analyses (El-Mufleh et al., 2014) and OM oxidation combined with laser particle-size analyses (Badin et al., 2009) in stormwater sediments. Calculations revealed that organo-phylosilicate complexes may have densities as low as ~2 g cm<sup>-3</sup> (Bonnard et al., 2012).

### 3.2. Quantitative characterization of density fractions

#### 3.2.1. Soil mass distribution and concentrations of Zn and TOC

The mass of soil found in each fraction after density fractionation, as well as the TOC and Zn concentrations in bulk soils and density fractions are presented in Table 1. DF<sub>2</sub> accounted for 4.31 g (control soil) and 3.94 g (amended soil) out of the 6 g of soil subjected to density fractionation. The other fractions accounted for less than 0.4 g each in both the control and amended soils. The mass recovery of the density fractionation procedure was 88.5% (±5.3%) for the control soil and 85.5% (±0.6%) for the amended soil. Soil losses may have occurred due to soil leftover on the sonication probe after each sonication step and non-settling of soil particles smaller than 0.2 μm (centrifugation threshold).

The bulk TOC concentration was 43.2 mg g<sup>-1</sup> in the control soil and 50.5 mg g<sup>-1</sup> in the amended soil. After density fractionation, no TOC was detected in the heaviest fraction (DF<sub>1</sub>) of the control and amended soils. In fractions DF<sub>2</sub>, DF<sub>3</sub>, DF<sub>4</sub> and DF<sub>5</sub>, TOC concentrations progressively increased as the density range of the fractions decreased, reaching as much as 241.9 mg g<sup>-1</sup> (control soil) and 228.3 mg g<sup>-1</sup> (amended soil)

Table 1

Soil mass, TOC and Zn concentrations in bulk soils and density fractions. DF<sub>1</sub>: >2.7 g cm<sup>-3</sup>, DF<sub>2</sub>: 2.5–2.7 g cm<sup>-3</sup>, DF<sub>3</sub>: 2.25–2.5 g cm<sup>-3</sup>, DF<sub>4</sub>: 1.9–2.25 g cm<sup>-3</sup> and DF<sub>5</sub> < 1.9 g cm<sup>-3</sup>.

Density Fraction	Soil Mass <sub>(±SD)</sub> (g)		TOC Concentration <sub>(±SD)</sub> (mg g <sup>-1</sup> of fraction)		Zn Concentration <sub>(±SD)</sub> (mg kg <sup>-1</sup> of fraction)	
	Control Soil	Amended Soil	Control Soil	Amended Soil	Control Soil	Amended Soil
DF <sub>1</sub>	0.35 <sub>(0.036)</sub>	0.34 <sub>(0.046)</sub>	0.0 <sub>(0.0)</sub>	0.0 <sub>(0.0)</sub>	102.5 <sub>(3.2)</sub>	118.7 <sub>(20.4)</sub>
DF <sub>2</sub>	4.31 <sub>(0.171)</sub>	3.94 <sub>(0.038)</sub>	20.4 <sub>(1.4)</sub>	18.3 <sub>(0.1)</sub>	96.1 <sub>(12.2)</sub>	125.5 <sub>(9.0)</sub>
DF <sub>3</sub>	0.23 <sub>(0.033)</sub>	0.34 <sub>(0.089)</sub>	53.3 <sub>(4.1)</sub>	39.0 <sub>(2.1)</sub>	86.6 <sub>(14.0)</sub>	125.5 <sub>(9.3)</sub>
DF <sub>4</sub>	0.20 <sub>(0.039)</sub>	0.14 <sub>(0.026)</sub>	101.4 <sub>(17.1)</sub>	98.3 <sub>(11.6)</sub>	60.2 <sub>(6.3)</sub>	74.7 <sub>(6.2)</sub>
DF <sub>5</sub>	0.22 <sub>(0.014)</sub>	0.37 <sub>(0.007)</sub>	241.9 <sub>(0.8)</sub>	228.3 <sub>(1.6)</sub>	62.4 <sub>(3.1)</sub>	90.3 <sub>(3.7)</sub>
Bulk	6.00 <sub>(0.0)</sub>	6.00 <sub>(0.0)</sub>	43.2 <sup>1</sup> <sub>(0.3)</sub>	50.5 <sup>1</sup> <sub>(0.2)</sub>	105.8 <sup>2</sup> <sub>(12.3)</sub>	206.6 <sup>2</sup> <sub>(10.7)</sub>
Recovery	88.5% <sub>(5.3%)</sub>	85.5% <sub>(0.6%)</sub>	75.4% <sub>(7.5%)</sub>	70.9% <sub>(1.4%)</sub>	88.3%	57.5%

<sup>1</sup> Unit for bulk samples: mg g<sup>-1</sup> of bulk soil.

<sup>2</sup> Unit for bulk samples: mg kg<sup>-1</sup> of bulk soil.

in the lightest fraction (DF<sub>5</sub>).

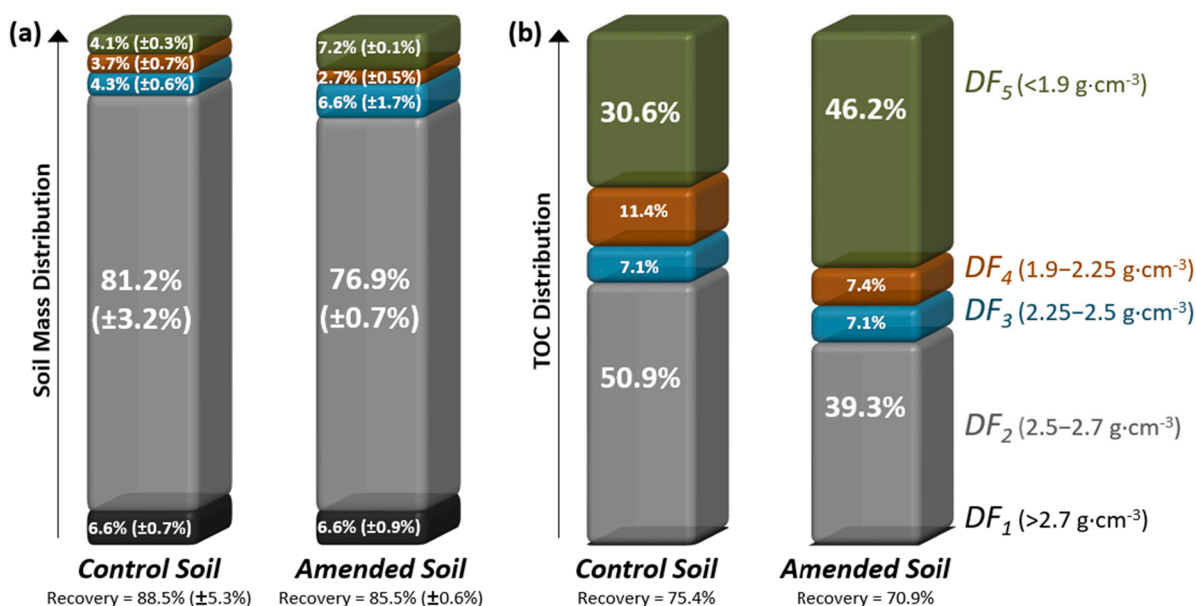
The TOC recovery was 75.4% for the control soil and 70.9% for the amended soil. TOC recoveries were therefore smaller than the mass recoveries in each soil. TOC may also have been lost (as compared to the soil mass) due to the greater proportion of OM in dissolved or colloidal particles that remained dissolved/suspended in the fractionation solution. This same trend (mass recovery > TOC recovery) has been observed elsewhere when applying density fractionation (Basile-Doelsch et al., 2007; Castanha et al., 2008; Wagai et al., 2015).

The Zn concentration in the bulk control soil was 105.8 mg kg<sup>-1</sup>, whereas that in the bulk amended soil was 206.6 mg kg<sup>-1</sup>. The Zn enrichment in the 0–5 cm soil layer, due to 11 years of continuous pig slurry spreading, was therefore 100.8 mg kg<sup>-1</sup>. This was in agreement with results reported by Mallmann (2013) and Formentini et al. (2015) in the same study area, as summarized in Section 2.1.

Within the density fractions, Zn concentrations were lower than in the corresponding bulk soils, most likely due to liquid extraction of Zn during the fractionation procedure. Indeed, Zn recovery in the density fractions was 88.3% for the control soil and 57.5% for the amended soil. In the control soil, Zn concentrations ranged from 60.2 mg kg<sup>-1</sup> of fraction (DF<sub>4</sub>) to 102.5 mg kg<sup>-1</sup> of fraction (DF<sub>1</sub>), whereas in the amended soil the Zn concentration ranged from 74.4 mg kg<sup>-1</sup> of fraction (DF<sub>4</sub>) to 125.5 mg kg<sup>-1</sup> of fraction (DF<sub>2</sub> and DF<sub>3</sub>). Overall, Zn concentrations increased in all fractions of the amended soils as compared to the same fractions of the control soil.

#### 3.2.2. Normalized soil mass and TOC distributions

After normalization to 100%, the heaviest DF<sub>1</sub> accounted for 6.6% (±0.7%) of the control soil mass (Fig. 3a). Most of the control soil was



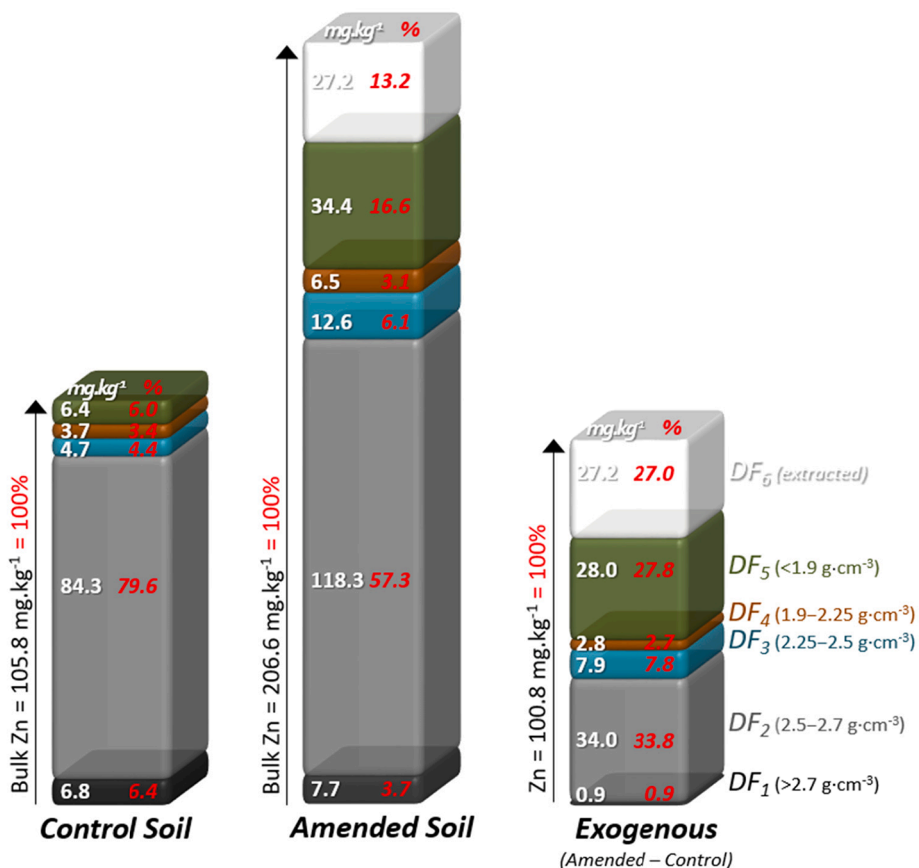
**Fig. 3.** (a) Percentage soil mass distribution amongst the density fractions (normalized to 100%); (b) Percentage TOC distribution amongst the density fractions (normalized to 100%).

accounted for in DF<sub>2</sub>: 81.2% (±3.2%). The three lightest fractions (DF<sub>3</sub>, DF<sub>4</sub> and DF<sub>5</sub>) jointly accounted for 12.1% (±1.0%) of the bulk soil, as follows: DF<sub>3</sub> = 4.3% (±0.6%); DF<sub>4</sub> = 3.7% (±0.7%); and DF<sub>5</sub> = 4.1% (±0.3%).

In the amended soil, the heavy DF<sub>1</sub> also accounted for 6.6% (±0.9%) of the soil mass (Fig. 3a). The contribution of DF<sub>2</sub> slightly decreased to

76.9% (±0.7%), as compared to the control soil. The three lightest fractions (DF<sub>3</sub>, DF<sub>4</sub> and DF<sub>5</sub>) jointly accounted for 16.5% (±1.8%) of the amended soil mass, as follows: DF<sub>3</sub> = 6.6% (±1.7%); DF<sub>4</sub> = 2.7% (±0.5%); and DF<sub>5</sub> = 7.2% (±0.1%).

After normalization (SI-1), the light DF<sub>5</sub> accounted for 30.6% of the TOC in the control soil. The contribution of DF<sub>5</sub> increased to 46.2% in



**Fig. 4.** Zn distribution among density fractions of control and amended soils and the resulting exogenous Zn distribution.

the amended soil. Conversely, DF<sub>2</sub> accounted for 50.9% of the TOC in the control soil, which decreased to 39.3% in the amended soil. Other density fractions jointly accounted for 18.5% (control soil) and 14.5% (amended soil) of the normalized TOC.

Therefore, the bulk TOC was roughly split between DF<sub>5</sub> and DF<sub>2</sub> in both soils. Note however that DF<sub>5</sub> presented much higher TOC concentrations than DF<sub>2</sub> (Table 1). On the other hand, DF<sub>2</sub> accounted for the largest proportion of the soil mass in both soils, i.e. nearly 20 times more than DF<sub>5</sub> (Fig. 3).

### 3.2.3. Normalized Zn distribution

Fig. 4 shows the Zn distribution among density fractions, normalized to 100% (SI-2). In the control soil (Zn = 105.8 mg kg<sup>-1</sup>), 79.6% of the Zn (i.e. 84.3 mg kg<sup>-1</sup> of bulk soil) was present in DF<sub>2</sub>. The contribution of other fractions was each below 7%. In the amended soil (Zn = 206.6 mg kg<sup>-1</sup>), Zn was mostly accounted for in DF<sub>2</sub> (118.3 mg kg<sup>-1</sup> of bulk soil, or 57.3%), DF<sub>5</sub> (34.4 mg kg<sup>-1</sup> of bulk soil, or 16.6%) and DF<sub>6</sub> (27.2 mg kg<sup>-1</sup> of bulk soil, or 13.2%).

Therefore, DF<sub>6</sub>, DF<sub>5</sub> and DF<sub>2</sub> presented the highest increases in Zn concentration when comparing the livestock manure-amended soil with the control soil. The fate of exogenous Zn (calculated as  $Zn_{amended} - Zn_{control} = 100.8 \text{ mg} \cdot \text{kg}^{-1}$ ) was as follows: DF<sub>6</sub> = 27.2 mg kg<sup>-1</sup> of bulk soil, or 27.0% of the exogenous Zn; DF<sub>5</sub> = 28.0 mg kg<sup>-1</sup> of bulk soil, or 27.8% of the exogenous Zn; and DF<sub>2</sub> = 34.0 mg kg<sup>-1</sup> of bulk soil, or 33.8% of the exogenous Zn. In other words, DF<sub>6</sub>, DF<sub>5</sub> and DF<sub>2</sub> each accounted for roughly a third of the exogenous Zn.

### 3.3. Zn speciation in density fractions (XAS)

Fig. 5 summarizes the Zn speciation findings, as assessed by synchrotron-based XAS, in the two fractions that accounted for most of the soil mass and TOC content in the control and livestock manure-amended soils: DF<sub>2</sub> and DF<sub>5</sub>. Supporting Information SI-4 contains the EXAFS spectra and Fourier transforms for each of the samples presented in Fig. 5, as well as the best LCF fits.

XAS confirmed that OM-bound Zn was only present in the OM-rich DF<sub>5</sub> fraction in both the control and amended soils. Moreover, Zn-

phyllsilicate and Zn-Fe (oxyhydr)oxide were the major Zn species in both soils. This was consistent with the findings of the density fractionation approach, which detected most of the Zn in the mineral-rich DF<sub>2</sub> fraction, mainly composed of phyllsilicates kaolinite and vermiculite and the Fe oxide hematite.

For both fractions, via XAS we were unable to detect a clear Zn speciation pattern in the amended soil compared to the control soil. Indeed, the increase of Zn concentration in the DF<sub>5</sub> of amended soil resulted mainly in an increase in the proportion of Zn-phyllsilicate species, whereas we observed an increase in the proportion of Zn-Fe (oxyhydr)oxide species in DF<sub>2</sub> of the amended soil. This means that both phyllsilicates and Fe (oxyhydr)oxide contributed to the sorption of exogenous Zn.

Note that the XAS measurements were performed in solid, freeze-dried samples of each density fraction. This therefore could not account for the amounts of Zn: (i) extracted by the fractionation solution (DF<sub>6</sub>), or (ii) calculated using the redistribution approaches presented in Fig. 4 and Supporting Information SI-2, because these amounts of Zn should have been present in the liquid phase. Consequently, the proportions of Zn bound to light or extractable matrices such as OM or aqueous LST (i.e. the fractionation solution) were likely highly underestimated by the XAS analysis, especially within the light DF<sub>5</sub>.

## 4. Discussion

### 4.1. Zn speciation in the control soil

In the control soil, 79.7% of the Zn was in the mineral-rich DF<sub>2</sub> (Fig. 4). A straightforward assumption could be that Zn was therefore associated with the minerals present in this mineral-rich/TOC-poor fraction. Nevertheless, despite the low TOC concentration in DF<sub>2</sub> (Table 1), the bulk TOC in the control soil was roughly split between DF<sub>5</sub> and DF<sub>2</sub> (Fig. 3). This could be explained by the fact that DF<sub>2</sub> accounted for a much larger proportion of the soil than DF<sub>5</sub> (Fig. 2). Therefore, without speciation data, it could be also hypothesized that Zn in DF<sub>2</sub> was associated with the OM present in this fraction.

The latter hypothesis was ruled out because the proportion of Zn in

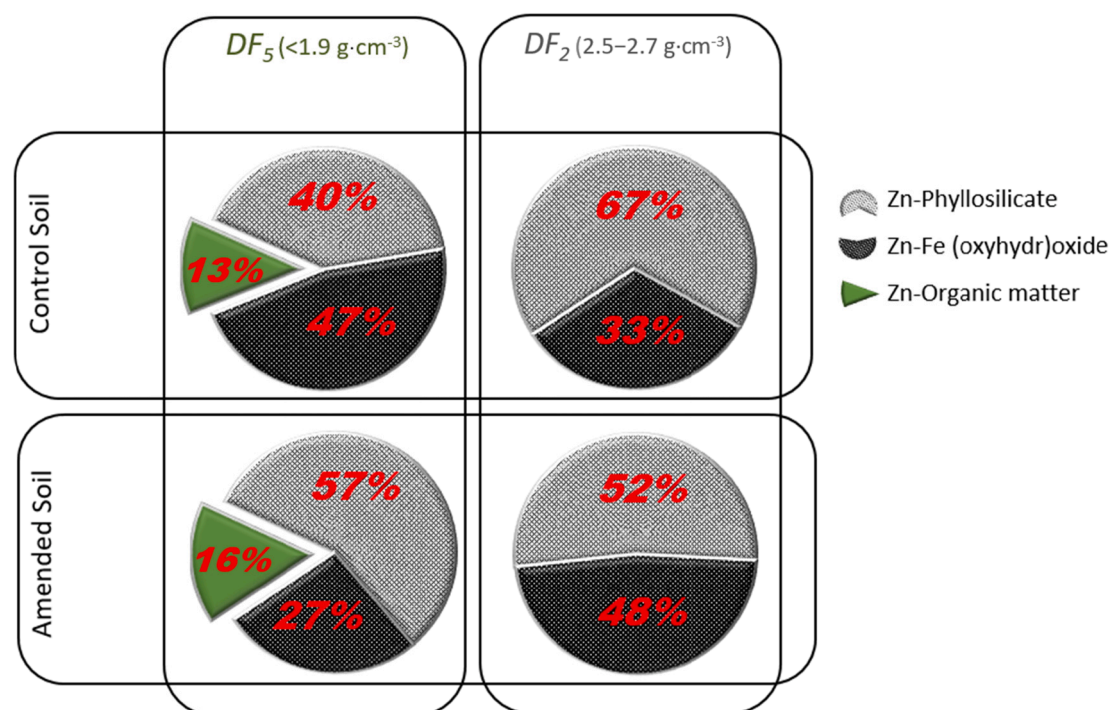


Fig. 5. Zn speciation within DF<sub>2</sub> and DF<sub>5</sub> of the control and amended soils.

the OM-rich DF<sub>5</sub> was only 6.0% in the control soil. Therefore, the TOC enrichment toward the light DF<sub>5</sub> caused by soil density fractionation did not lead to Zn enrichment toward this fraction. In other words, OM was not a major Zn pool in the control soil. Hence, most of the naturally occurring Zn (control soil) must have been associated with the minerals present in DF<sub>2</sub>: kaolinite, vermiculite and hematite. Quartz was omitted because it is known for being relatively inert and therefore holding very low trace element contents in soils (Gamble et al., 2000).

This is in agreement with the findings of two recent Zn-speciation studies we conducted in the same experimental field (exact same samples) using sequential extractions (SE) and X-ray absorption spectroscopy (XAS). After SE (Formentini et al., 2015), 76.9% of Zn in the control soil was in the residual fraction, which accounted for the Zn fraction related to the soil parent material, i.e. occluded in the crystalline structure of minerals. Indeed, XAS showed that Zn-kaolinite and Zn-hematite dominated the Zn speciation in the bulk control soil (Formentini et al., 2017). Moreover, the XAS data collected in the present study for DF<sub>2</sub> supported the association between Zn and the minerals present in DF<sub>2</sub> and indicated that no Zn was linked to organic species in DF<sub>2</sub> (Fig. 5).

#### 4.2. Zinc speciation in the amended soil

In the livestock manure-amended soil, Zn concentrations markedly increased in DF<sub>6</sub>, DF<sub>5</sub> and DF<sub>2</sub>, as compared to the same density fractions in the control soil (Fig. 4). In spite of the increase in concentration, the Zn proportion found in the mineral-rich DF<sub>2</sub> decreased from 79.6% (control soil) to 57.2% (amended soil). This was in agreement with the trends observed via SE (Formentini et al., 2015), which showed that Zn in the residual fraction (occluded Zn) decreased from 76.9% in the control soil to 43.5% in the amended soil.

Conversely, the Zn proportion found in the extracted DF<sub>6</sub> (13.2%) and in the OM-rich DF<sub>5</sub> (16.6%) increased in the amended soil as compared to the control soil (Fig. 4). This trend was also in line with previously reported SE and XAS results (Formentini et al., 2015, 2017).

The 13.2% Zn extracted by the fractionation solution (DF<sub>6</sub>) reported here in the amended soil was fairly close to the 6.2% Zn in the SE exchangeable fraction of the same soil (Formentini et al., 2015). Although the extractants had different compositions (density fractionation = aqueous LST; SE = aqueous NaNO<sub>3</sub>), both fractions behaved similarly: they accounted for virtually no Zn in the control soil and their Zn share had increased to around 10% in the amended soil. DF<sub>6</sub> thus likely represented the most labile and potentially mobile proportion of exogenous Zn in the soil. The proportion of Zn found in OM-rich DF<sub>5</sub> (16.6% in the amended soil) was very similar to the proportion found in the SE organic fraction and using XAS (both at 19.0%) (Formentini et al., 2015, 2017).

In spite of the low Zn recovery in the density fractions for the amended soil (57.5%), the close agreement between the results obtained by three different analytical techniques (DF, SE and XAS) is worth noting. This strengthens the relevance of the outcomes of these studies and paves the way to discussing the environmental impact of the Zn-rich livestock-manure spreading.

#### 4.3. Environmental impact

Zn accumulation within topsoil layers after long-term pig slurry application has been described worldwide regardless of soil properties and climatic conditions (e.g. Novak et al., 2004; Ogiyama et al., 2005; Berenguer et al., 2008; Couto et al., 2016; Jensen et al., 2016; Legros et al., 2013). All of these studies compared the Zn distribution in manure-amended versus control soil layers.

Only a few studies monitored Zn concentrations in the three compartments of the water–soil–plant system following pig slurry application, and also their results were contradictory. For instance, no uptake was observed for maize, alfalfa and sugar beet crops (Mantovi et al.,

2003) or *Stenotaphrum dimidiatum* grass (Legros et al., 2010), whereas 4% Zn uptake was reported for Indiangrass (*Sorghastrum nutans*) (McLaughlin et al., 2004) and 0.4–0.7% Zn uptake was reported for lettuce (*Lactuca sativa* L.) (Hodomihou et al., 2020). Likewise, Zn leaching has been detected (Hao et al., 2008; Xue et al., 2000) or not (Legros et al., 2010) following animal manure spreading. These discrepancies are usually explained by differences in the studied soils and/or crop species.

Based on our results, we propose another hypothesis to explain such differences. It is important to keep in mind that Zn speciation in pig slurry is not driven by organic matter. Indeed, XAS revealed that nanosized Zn sulphide (nano-ZnS) accounted for 100% of Zn speciation in pig slurry (Formentini et al., 2017). In oxic environments, Le Bars et al. (2018) recently demonstrated that the nano-scale of ZnS favoured its fast oxidation and dissolution. This could explain why nano-ZnS was not detected in the pig slurry-amended soil (Formentini et al., 2017). After pig slurry application on soil, the nano-ZnS oxidative dissolution released Zn that interacted with the different components of the soil matrix such as phyllosilicate, Fe (oxyhydr)oxide and organic matter. The sorption or complexation (i.e. retention) of the Zn released by soil components could explain the accumulation of exogenous Zn within the surface layers. Indeed, we previously demonstrated that the Zn mobility towards deeper layers in the studied soil was quite limited due to the high sorption capacity of the clayey soil (Formentini et al., 2015).

Zn accumulation, without leaching or crop uptake, will likely occur if the maximum soil retention capacity is not exceeded. Above this maximum soil retention capacity, we assumed that Zn mobility and/or phytoavailability could occur.

With the present study, we showed that, despite the very clayey soil texture (clays = 67.8%), the Zn sorption capacity of the soil seemed to have reached a limit within the surface layer (0–5 cm) after 11 years of livestock manure application. This was confirmed by the drop in the proportion of Zn found in the phyllosilicate-rich DF<sub>2</sub>, when comparing the amended soil with the control soil. Moreover, the increase in the amount of Zn extracted by the fractionation solution (DF<sub>6</sub>) and associated with organic species (DF<sub>5</sub>) in the amended soil was consistent with an increase in the potential mobility of Zn through the soil profile as a result of long-term livestock manure fertilization. It could therefore be expected that continued application of Zn-rich livestock manure would cause reduce the contaminant retention capacity of the soil in progressively deeper layers.

It would be interesting to quantify the maximum soil retention capacity of trace elements and to characterize its patterns over time. This could allow comparisons between studies, and help predict and limit the environmental impact of agricultural recycling

## 5. Conclusion

The density fractionation procedure decreased the soil heterogeneity and soil fractions with contrasting characteristics were obtained. The light DF<sub>5</sub> presented a high OM content and accounted for a minor amount of the bulk soil mass. Conversely, DF<sub>2</sub> accounted for most of the soil mass and mineral content, with a low OM concentration. Based on these contrasting features, the soil constituents and their most likely associations with naturally occurring and exogenous (pig slurry-borne) Zn were identified.

Zn extraction by the fractionation solution was higher than expected, especially in the livestock manure-amended soil. This was a relevant environmental finding despite the difficulties we encountered in further characterizing this fraction. The findings of the present study highlight that improvements are essential to control and better characterize the Zn extracted during the fractionation procedure.

When used alone, soil density fractionation may not provide a complete overview of the rather complex interactions between trace elements and soil constituents. Nevertheless, combining soil density fractionation with additional analytical approaches (e.g. SE, XAS and



XRD) can generate key insight into the Zn behavior and fate in soils. For instance, in the present study, we successfully applied this approach for the first time to investigate an agricultural soil that had received livestock manure amendments over an extended period of time.

The results obtained here reinforced and complemented our previous SE and XAS observations in the same field experiment. We conclude this analytical cycle by providing a comprehensive overview on Zn behavior in a clayey agricultural soil amended with pig slurry over 11 years. The present study revealed that the preponderance of a mineral-rich phase (DF<sub>2</sub> accounted for ~80% of the soil) was consistent with the high Zn retention. It also showed that Zn associated with OM (DF<sub>5</sub>) and extracted Zn (DF<sub>6</sub>) increased in the amended soil, suggesting ongoing depletion of the soil sorption capacity by the mineral matrix. There is a risk that an increase of Zn mobility and/or phytoavailability would occur following future spreading of pig slurry on the long-term amended soil. This potential risk should be carefully assessed in further studies.

### 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.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2021.115044>.

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