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Using isotope dilution assays to understand speciation changes in Cd, Zn, Pb and Fe in a soil model system under simulated flooding conditions

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ABSTRACT

Flooded soils are systems with complex chemistry and understanding the mechanisms that control the mobility and bioavailability of metals in these soils is important for their management. This work uses stable metal multi-element isotopic dilution combined with sequential extraction assays to help understand the changes in solid and solution speciation of Cd, Fe, Pb and Zn in a contaminated soil following submergence. However, it is necessary to ensure that the isotopic dilution principles, originally developed for aerobic soils, are not compromised; in particular due to the presence of non-labile colloids in the solution phase. In particular, no studies examining the validity of these assays in systems where rapid pH and Eh changes are occurring due to fermentation reactions have been published. Thus sucrose (0.42% and 1.26% added C) was used as a carbon source to stimulate bacterial mediated fermentation reactions allowing changes in Cd, Zn, Fe and Pb isotopic exchangeability, speciation and solution chemistry to be examined after 10, 20 and 42 days of submergence. Without the addition of added C, submergence for 42 days only produced minor changes in the speciation of the metals in solid or solution phases. However, the presence of easily labile carbon produced significant responses depending on the quantity of C added. Assessments of whether fermentation products caused over-estimation of the isotopically exchangeable pool of metals (E-values) were made by measuring concentrations with and without a resin purification step. Results showed generally good agreement over a pH range of 4–7 for Pb, Cd, Zn and Fe and demonstrate that fermentation by-products do not induce the formation of non-exchangeable metal colloids. E-value concentrations were compared with fractions extracted using a modified Tessier sequential extraction. With no carbonate phases present in the soils, the E-values for Cd, Zn, Fe and Pb compared favourably with the concentrations of metal present in the combined solution, exchangeable and specifically adsorbed fractions. This provided additional evidence that the conditions for the isotopic dilution assays were not violated as these fractions should be isotopically exchangeable. Combining results from the different treatments and stages of the reduction process, strong pH dependence was found for the isotopically exchangeable and the solution pools of Cd, Zn and Pb.

1. Introduction

Trace metal solubility in contaminated submerged soils is controlled largely by the extent of change in the redox status experienced; this being a result of the duration of the flooding period and the nature of the carbon source that is utilised by the microbial system. Increased mobility of toxic metals can increase the potential for metal transfer through ecosystems and food chains (Schröder et al., 2005). Thus the processes that control metal solubility and bioavailability require improved understanding. Following submergence a recognised thermodynamic sequence of microbially mediated redox reactions occurs. Initially, oxidation of organic matter by microbial respiration consumes O_2 but once this has been used, the subsequent electron acceptors are NO_3^- (which is reduced to N_2), followed by the reduction of Mn^{4+} to Mn^{2+}, Fe^{3+} to Fe^{2+}, SO_4^{2-} to H_2S, and CO_2 to CH_4 (Ponnamperuma, 1972). The use of the different electron receptors during microbial organic C decomposition results in soil pH and Eh changes that change the solubility and mobility of metals through a series of complex interactions (Kögel-Knabner et al., 2010). For redox sensitive trace elements (e.g. As, Cu, Se, Cr) a change in oxidation state leads to the release of more toxic species e.g. As(III) and Cr(VI), or the precipitation of new mineral phases (e.g. Cu^0, CuS) (Hofacker et al., 2013). The dissolution of Fe and Mn oxides may increase the concomitant solubility (e.g. Rennart et al., 2010) of a number of non-redox-sensitive trace elements e.g. Zn, Co, Cd or Pb, whilst the increase in soil pH or the precipitation of new mineral phases such as carbonates or sulphides may decrease it (Barrett and McBride, 2007; Gambrell, 1994; Ponnamperuma, 1972;

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Weber et al., 2009). Although the precipitation of sulphide (Cu, Cd, Pb) colloids will decrease a metal's bioavailability, their formation can increase their transport through the soil profile (Weber et al., 2009). For Zn, the precipitation of $ZnCO_3$ is often cited as a reason behind low Zn uptake by rice in paddy fields (Chuan et al., 1996; Impa and Johnson-Beebout, 2012). However, Van Laer et al. (2010) demonstrated that solution Zn increased in smelter contaminated soils as a result of Fe oxide dissolution and competition between Fe^{2+} and Zn^{2+} for sorption sites on organic matter, this despite an increase in soil pH of 1.8 units, from an initial value of ~5.4.

The change in the reactive pool of trace metals in submerged soils has been studied using a range of extraction methods. For example, Römkens et al. (2009) determined the "reactive" pool of Cd, Ni and Zn in top soils from paddy fields in Taiwan using operationally defined soil extractions i.e. 0.05 M EDTA or 0.43 M HNO_3. However, Isotope Dilution (ID) assays provide a more robust mechanistic description of the size of the reactive pool of metals, as they measure the pool of metal that is in equilibrium in the soil-pore water system, but until now, its use has been relatively limited in flooded soil. Early work by Tiller et al. (1972) examined the use of Zn isotopes in determining E and L-values in paddy field soils. More recent examples include those by Stroud et al. (2011) who assessed changes in As lability in soils from Bangladesh, but reducing conditions such as those occurring in paddy fields were not successfully achieved. The use of multi-element ID assays, in particular, has the potential to add new insights into the complex interactions that affect the reactivity of different metals simultaneously in submerged soils. Recently, Marzouk (2012) used multi-element isotopic dilution to assess changes in the E-value of Fe, Cd, Zn and Pb in mine spoil-rich soils from the UK subjected to alternate aerobic/anaerobic 15-day long cycles.

As a result of the large variation in geochemical conditions produced when a soil undergoes submergence, methodological considerations need to be considered with respect to the fundamental rules of ID assays; these being (i) that the background electrolyte should not mobilise non-labile metal, (ii) that the metal-complexes in the solution phase remain labile so that complete isotope equilibrium may occur and (iii) that the isotope spike does not precipitate. For example, recent work by Izquierdo et al. (2016) demonstrated the fixation of the Zn isotope spike within 5 days leading to overestimates of the E-value. Thus to fully utilise ID assays in examining the behaviour of trace elements in submerged soils a greater understanding of how different redox conditions affect the assay is required.

Two major influences that may affect the efficacy of the ID assay are (i) the nature of the carbon substrate that the microbial biomass use and the metabolites produced and released into solution and (ii) the submergence time. These parameters could affect the isotopic exchangeability in solution or cause tracer removal before the exchange with the native isotope occurs, thus compromising the results. Bell (1969) demonstrated that changes in Eh, pH and gas evolution varied between different carbon sources. For example, whilst reducing conditions can result in the release of dissolved organic carbon (Buettner et al., 2014), volatile fatty acids (VFA) such as acetate, propionate and butyrate can be formed during fermentation reactions when sugars are decomposed (Krylova et al., 1997; Chidthaisong et al., 1999). Fermentation by-products such as acetate have different binding properties for trace metals than humic (HA) and fulvic acids (FA) and the potential for violating one of the fundamental ID principals exists; the formation of non-labile complexes in the solution phase. However, the fermentation phase is one where the use of E-values could be of great value because of the associated rapid pH and Eh changes. Acetate has been identified in many wetland environments including peatlands (Duddlestone et al., 2002), lake and river sediments (Nozhevnikova et al., 2007). However, it is in salt marsh sediments (Hines, 1994) and paddy field soils (Klüber and Conrad, 1998; Yao et al., 1999) where acetate concentrations are often highest and can reach millimolar levels.

This study uses a model system to examine the processes and impacts on measured E-values and the solubility of Pb, Fe, Cd and Zn when a contaminated floodplain soil was amended with different concentrations of carbohydrate (sucrose), and subjected to an extended period of submergence (42 days). The environment produced will be high in sugar metabolites (e.g. acetate), similar to those that may occur during the fermentation phase of the reduction cycle, and will provide an indication of the effectiveness of ID assays where the solution organic complexes are not dominated by HA and FA. This was achieved by comparing the established ID methodology with one that involves a resin purification step (Lombi et al., 2003), whereby labile metal is concentrated on an exchange resin, and is then eluted before measurement. These changes in measured reactive metal concentrations were examined in association with sequential extraction assays, which provide complementary information regarding changes in mineral phases and help explain changes in the E-value. Whilst the model system used in this work to generate fermentation products do not wholly replicate field conditions, it demonstrates the potential of ID assays in understanding the complex interactions in submerged soils.

2. Materials and methods

2.1. Soil collection and general soil characterisation

The topsoil sample (0–15 cm depth) used in the incubation experiments was collected from the River Trent floodplain at Barton in Fabis, close to the city of Nottingham (UK). The site was rough pasture land within 5 m of the riverbank and the sample consisted of a ~1.5 kg bulk sample produced from five sub-samples collected at the corners and the centre of a 5 m^2 grid. The wet soil was sieved to <2 mm and kept at 4 °C until used. Approximately 30 g were oven dried (105 °C), sub-sampled and ground in an agate ball-mill to produce a homogeneous powder for acid digestion and total element analysis. Sample digestion was undertaken by weighing 0.25 g of soil into a Savillex™ vial where concentrated analytical grade HF, HNO_3 and $HClO_4$ were added, with a subsequent stepped heating program up to 170 °C overnight; the purpose being the digestion of silicate and oxide phases. The dry residue was re-constituted after warming with Milli-Q water, HNO_3 and H_2O_2, to 25 mL of 5% v/v HNO_3 and stored in HDPE bottles. Reference materials (GSS-6 and BCR-2), triplicate samples and blanks were all prepared in a similar manner to check accuracy of the analytical and digestion method. Soil pH was determined in 0.01 M $CaCl_2$ at a liquid/solid ratio = 2.5 L/kg. Loss on ignition (LOI) was undertaken at 450 °C as a measure of soil organic matter. Particle size analysis was determined using a laser diffraction particle size analyser after organic matter had been destroyed using H_2O_2 until no further reaction with fresh H_2O_2. Estimates of oxalate extractable Fe oxides in the soil were determined using 0.2 M ammonium oxalate and 0.125 M oxalic (Schwertmann, 1973). Total free Fe oxides in soils were extracted using 25% (w/v) Na-citrate and of 10% (w/v) Na-dithionite (Olsen and Roscoe, 1982). Inorganic carbon measurements were undertaken on the assayed soils before and after incubation using a Shimadzu TOC-Vc analyser, the purpose being to check for redox-induced carbonate precipitation in microcosms during the flooding period.

2.2. Soil incubation and sampling

2.2.1. Experimental setup

Incubation of soils under anaerobic conditions was undertaken using 60 mL square Nalgene HDPE bottles as individual microcosms. To each microcosm, the equivalent of 5 g oven dry soil was added along with 30 mL of 0.01 M $CaCl_2$. This gave a head of liquid of between 2.5 and 3 cm. Three main treatments were used, these being (i) Control soils, assayed as collected (ii) Low C soils amended with 1% sucrose (wt, dry soil basis; 0.42% C) and (iii) High C soils amended with 3% sucrose (1.26% C). Sucrose was chosen as it is likely to produce a range of metabolites including acetic and butyric acids, and isopropyl alcohol (Bell, 1969), both through fermentation reactions and the direct conversion to acetate by homoacetogenic bacteria. Microcosms were sealed under an N_2 environment and incubated at 20 °C in a MACS VA500 + VA airlock variable atmosphere workstation with an atmosphere of 95% N_2 with 5% H_2. Sampling was undertaken after 10, 21 and 42 days. Table 2 provides an outline of experimental treatments, sampling times and the number of microcosms used at each sampling.

The short term response of soils to re-oxygenation was also examined. This was to understand the potential for reversibility in the soluble and isotopically exchangeable concentrations of metal during a return to aerobic conditions Thus, 36 additional microcosms each with High C application (3% sucrose) were incubated as described above. Then, at each sampling date, 12 of the microcosms were bought into aerobic conditions by removing them from the N_2 incubator and uncapping them to allow exposure to O_2 in for 5 days, hand shaking occasionally. In addition, 12 control microcosms were incubated in an aerobic environment for the whole duration of the experiment. These were open to O_2 and shaken every 2–3 days during the experimental period to maintain aerobic conditions. For solution characterisation, 12 microcosms of each treatment (Anaerobic Control, Low C and High C) were used at each sampling. Eight replicates were used for E-value determinations and the remaining 4 replicates were used to measure changes in pH, Eh and metal in solution $[M_{Sol}]$.

With respect to their effects on soil, easily labile sources of carbon such as sucrose are likely to produce a more rapid response in Eh and pH than less labile sources (Bell, 1969). We compared all the incubated microcosms with soil samples assayed shortly after collection ($t = 0$ days) to determine initial $[M_E]$ and $[M_{Sol}]$. To assess changes in the sorptive surfaces that metals bind to after changes in redox status, sequential extractions (SE) were undertaken on one set of samples from each treatment after 42 days of flooding.

2.2.2. Sampling - solution and isotopic dilution assays

At each sampling, pH and Eh (mV) were measured in the 4 microcosms sampled for solution characteristics for each treatment. The redox potential was measured using a Hanna HI3210 ORP platinum electrode and values were converted to the standard hydrogen electrode. After measurement, the supernatant solutions were filtered through 0.20 μm filters and split up in a number of aliquots for characterising changes in solution variables including multi-elements, Fe^{2+}, DOC and inorganic carbon.

Multiple-element ID techniques were used to measure labile (isotopically exchangeable) Cd_E, Fe_E, Pb_E and Zn_E simultaneously (Marzouk, 2012). Enriched ^{108}Cd (66.3%), ^{204}Pb (99.94%), ^{70}Zn (99.53%) and ^{57}Fe (99.63%) were obtained from ISOFLEX as metal or oxide form and dissolved to obtain stock solutions in 2% HNO_3. At each sampling time, 4 microcosms were spiked with 0.5 mL of a working solution containing ^{108}Cd, ^{204}Pb and ^{70}Zn and ^{57}Fe. Spike enrichment aimed to double the natural abundance of the selected isotopes in 5 g of dry soil. Another 4 replicates were used to measure the natural isotopic abundances of these elements. The spiked and unspiked suspensions were re-equilibrated in the anaerobic chamber by hand-shaking at regular intervals for further 3 days and filtered through 0.20 μm filters. The filtrates were divided into two portions. The first 5 mL aliquot was acidified to give a final concentration 1% HNO_3 for determination of isotopic ratios and analysed immediately by ICP-MS; the results are reported as E-values. A second 5 mL aliquot was used for the determination of non-isotopically exchangeable metal in colloidal form, following the method developed by Hamon and McLaughlin (2002) and Lombi et al. (2003). The method uses a cation exchange resin that adsorbs labile metals present in solution as free ions, soluble metal complexes, and metals exchangeably adsorbed onto sub-micron colloids, whilst non-isotopically exchangeable colloidal metals will remain in solution (Lombi et al., 2003). These filtered solutions were shaken for 2 h with 0.10 mg Ca-Chelex®-100 and then the supernatant solution was discharged. After washing the resin twice with Milli-Q water, the metals were eluted from the resin by adding 5 mL 0.5 M HNO_3. The supernatant solutions were analysed for the isotopic ratios and the results are reported as M_{Er} values.

2.2.3. Sequential extractions

The procedure used was based on the 5 step sequential extraction procedure described in detail by Tessier et al. (1979). The concentration of metals allocated in different operationally defined fractions were: exchangeable (F1) extracted with $MgCl_2$; specifically adsorbed/carbonate bound (F2), extracted with NaOAc; the reducible or oxide bound (F3), extracted with $NH_2OH \cdot HCl$; the oxidisable or organic and sulphide bound (F4), extracted with H_2O_2, NH_4OAc and HNO_3, and the residual phase (F5), digested with a HNO_3:HF:$HClO_4$ mixture. Prior to drying at 30 °C for 3 days, samples had the equilibrating solution removed, before allowing the samples to dry under N_2 conditions. The concentrations of dissolved metal $[M_{Sol}]$ analysed. These are plotted and discussed as an independent fraction (F0) for comparative purposes. All samples were filtered through 0.20 μm before analysis with the exception of F5. For the F2 step we examined whether the metal was likely to be specifically bound or carbonate bound by analysing samples of each treatment for inorganic C (see Section 2.1). Mass balance closures (recovery rates) between 93 and 110% were obtained for the 4 studied metals across all the treatments.

2.3. Analytical procedures

2.3.1. Analyses of solutions

The concentrations of elements in the total digests, sequential extractions, ID extracts and solution extracts were analysed using an Agilent 7500 quadrupole ICP-MS instrument, using a mixture of "He collision cell" and "no-gas" modes. Response suppression/enhancement effects were corrected by using a mixed internal standard containing Sc, Ge, In, Rh, Te and Ir. The instrument was calibrated using a series of synthetic chemical solutions diluted from multi-element stock solutions (SPEX Certprep™). The calibration was validated using synthetic chemical standards from a different source. The calibration and quality control standards were inserted every 20 samples to check possible drift over the run. Data were corrected for blank contribution and possible interferences by running a number of blanks and synthetic chemical solutions of Ba, Ce, Gd, Nd, Pb and Sm. Solution aliquots were analysed for non-purgeable organic carbon (NPOC) concentrations using a Shimadzu TOC-Vcph analyser; solution pH and HCO_3^- concentration were measured using a Radiometer Analytical TIM865 titration manager. The concentrations of $[Fe^{2+}]$ were determined after stabilisation with 2–2 Dipyridyl using a UV-VIS (Perkin Elmer Lambda 35) spectrometer detector at 520 nm wavelength.

2.3.2. Isotopic dilution analyses

Prior to analysis all samples were diluted using 1% HNO_3/0.5% HCl to give a count rate for the most abundant isotope as close to, but below 800 kcps to produce optimum counting statistics within the linear range of the pulse counting detector. The issue of mass bias in the isotope ratios was addressed by determining the measured isotope ratio for single element solutions (Cd, Fe, Zn) and NIST SRM981 (Pb) regularly throughout the analytical session and producing interpolated correction factors of sample isotope ratios with reference to the accepted isotope ratios (IUPAC). Quality control for isotope ratios was performed using synthetic chemical solutions diluted from multi-element stock solutions (SPEX Certprep™).

The E-value was determined using Eq. (1):

$$\text{E-value} = \left(\frac{M_{nat}}{W}\right)\left(\frac{C_{spike}V_{spike}}{M_{spike}}\right)\left(\frac{\text{Itracer} - {}^{ref}IA_{spike}R_{ss}}{{}^{ref}IA_{soil}R_{ss} - {}^{tracer}IA_{soil}}\right) \tag{1}$$

where M is the average atomic mass of the metal in natural soil and in the spike, C is gravimetric metal concentration (mg L^{-1}), V is the volume of added spike (L), W is the weight of soil (kg), $^{tracer}IA$ denotes isotopic abundance of the isotopes used for spiking the soil (^{108}Cd, ^{57}Fe, ^{204}Pb and ^{70}Zn) in the spike or soil, ^{ref}IA is the isotopic abundance of the isotopes used as reference (^{111}Cd, ^{56}Fe, ^{208}Pb and ^{66}Zn) in the spike or unspiked soil, and R_{ss} is the ratio of the isotopic abundances of the isotopic tracer over the reference isotope in the spiked soil supernatant. The values reported in this work are the average of 4 replicates, with coefficients of variation in the 0.4–8% range (typically 1–4%) for Cd, Pb and Zn, and 2–20% (typically 4–8%) for Fe.

3. Results and discussion

3.1. General soil characteristics

Relevant soil characteristics of the studied soil are given in Table 1. Total Cd, Pb and Zn were 2.3, 237 and 303 mg kg^{-1} respectively. The concentrations of M_E in control aerobic soils were 1.3 mg kg^{-1} for Cd ($Cd_E = 57\%$ of Cd_{Total}), 98 mg kg^{-1} for Pb ($Pb_E = 38\%$ of Pb_{Total}) and 97 mg kg^{-1} for Zn ($Zn_E = 32\%$ of Zn_{Total}). The total Fe concentration in soil was approximately 2.5% and Fe_E was <0.1% of Fe_{Total}. Inorganic C was below the detection limit (<0.01%) in the starting soil and after the various treatments, indicating that the proportion of metals associated with carbonate phases in the aerobic soil is likely to be negligible.

3.2. Changes in soil pH, redox potential and DOC with flooding

3.2.1. Redox potential

The aerobic control soils, where O_2 was maintained throughout the experiment did not experience any change in redox status after 42 days flooding (Fig. 1). The anaerobic control soils were also largely irresponsive to 42 days flooding. The redox potential declined very slightly from +428 mV to +405 mV towards the end of the experiment, suggesting that soil redox changes were starting to develop. For the Low C treatment, Eh values decreased to +212 mV after 10 days and +41 mV towards the end of the experiment. In the High C soils, Eh initially fell to +2 mV after 10 days, recovering to ~+200mv after 21 days before decreasing again to +130 mV (Fig. 1). The decrease in redox potentials in the organic C treated soils indicate that O_2 was rapidly consumed. Typically, Eh values <+200 mV suggest that electrochemical reactions involving Mn and Fe oxide reduction are operative (Ponnamperuma et al., 1967, 1969). Changes in the redox potential with time after the High C amendment demonstrate a typical response of submerged soils (Ponnamperuma, 1972). This involves an initial Eh drop during the first few days, followed by an increase in Eh before a subsequent slow decrease. It is likely that a similar response in the Low C amended soils would have occurred, but because of the timing of the samplings it was missed. The presence of easily decomposable organic C present can sharpen and accelerate this initial redox potential minimum, depending on its type and concentration. Yamane and Sato (1968) found that carbohydrates such as glucose and soluble starch deepened the drop in the Eh whereas Bell (1969) reported a short-lived rapid decrease followed by a period of rising Eh shortly after flooding in a glucose amended soil. The initial Eh drop is associated with the release of H_2 and CO_2 as well as the products of fermentative metabolism of glucose (Bell, 1969; Yamane and Sato, 1968).

Table 1
General characteristics of the studied soil.

Particle size %	Clay	Silt	Sand
	21	16	63
Total metal mg kg^{-1}	Cd	Pb	Zn
	2.3	237	303
Iron %	Total	Free oxides (dithionite)	Free amorphous oxides (oxalate)
	2.5	1.7	0.4
pH (0.01 M $CaCl_2$)	6.7		
LOI %	7.8		

3.2.2. Soil pH

Soil pH changed marginally in aerobically incubated soils, increasing from 6.2 to 6.4. A minor change was also observed for the anaerobically incubated unamended soils; decreasing from 6.2 to 5.9 after 10 days, before returning to the initial value and remaining relatively constant for the rest of the experiment (Fig. 1). Concomitant with changes in the redox potential, the pH in amended soils changed over time. After 10 days incubation, soil pH fell by 0.6 and 1.1 units for the Low C and High C amendments respectively (Fig. 1), reflecting accumulation of CO_2 along with other acidic fermentation products of carbohydrate decomposition (Ponnamperuma, 1972). After this minimum ($pH = 5.6$ for Low C soils and $pH = 5.2$ for High C soils), soil pH slowly rose over time due to the majority of reducing reactions consuming H^+. The pH values in the Low C amended soils recovered whilst a smaller and slower rise in pH values was found for the High C amended soils reaching $pH = 5.5$ at the end of the experiment. Compared to the Low C amendment, the typical responses between pH and Eh in the High C treatment appear slightly out of phase (i.e. between 10 and 20 days both pH and Eh increase together). This is most likely a result of the sampling times selected. The Eh value at 20 days may actually be decreasing from a higher value reached between 10 and 20 days.

3.2.3. DOC

Changes in NPOC concentrations reflected the quantity of added C and the changes in Eh (Fig. 1). Little change in NPOC concentrations were found for untreated control soils, which remained ~6 mg L^{-1}. Concentrations of ~400 mg L^{-1} and 1200 mg L^{-1} were found for the Low and High C additions after 10 days and remained constant throughout the experiment. The large increases in NPOC may contain volatile fatty acids such as acetate, formed directly from sugar monomers by homoacetogenic bacteria. These were observed as films of oily substances on the surface of the microcosm solutions. Potentially if all the carbon added in the sucrose was converted to acetate this would generate concentrations of 350 and 1050 mg L^{-1} of acetate for the Low and High C amendments respectively i.e. the equivalent to 700 and 2100 mg $L^{-1}C$ respectively. However, it is likely that some of the consumed sucrose would be lost as CO_2 through microbial respiration as demonstrated by Kelliher et al. (2005). The continual release of Fe^{2+} after both amendments (see Section 3.4.2) would suggest that any acetate produced would remain throughout the incubation period as methanogenesis may not start until the inorganic electron acceptors had been exhausted (Yao and Conrad, 1999).

3.3. Changes in metal fractionation with flooding (sequential extractions)

Little change in metal distribution was found for unamended soils incubated under aerobic or anaerobic conditions for 42 days, reflecting the minor changes in the pH and redox conditions (Fig. 2). However, after C addition a number of changes occurred in the binding phases of metals due to the change in redox status. Overall, these consisted of a transfer of metals from less mobile phases, i.e. the oxide bound (F3) and specifically adsorbed (F2) fractions to the more bioavailable fractions, i.e. the soluble (F0) and exchangeable (F1) phases. Undetectable inorganic C levels in microcosms across all the treatments suggest that the fraction extracted with acetate (F2) is mostly specifically adsorbed and unlikely to contain carbonate associated metal in significant amounts.

3.3.1. Iron and manganese

Over 80% Fe was in the residual phase (F5) in untreated aerobic and anaerobic soils (Fig. 2). Lower residual Fe (70–75%) in the C amended soils and decreasing concentrations of Fe in the oxide fraction (F3)

Table 2
An outline of the experimental treatments, times and number of microcosms used at each sampling. Of the 12 microcosms, 8 are used in the isotopic dilution assay (4 unspiked, 4 spiked) and 4 are used to measure the pH, Eh and solution characteristics.

Name	Treatment	Sampling time (days)			
		0	10	21	42
Aerobic control	Shaken every 2 days in O_2 – no carbon addition	12			12
Anaerobic control	Anaerobic – no carbon addition		12	12	12
Low C	Anaerobic – 1% (w/w) sucrose		12	12	12
High C	Anaerobic – 3% (w/w) sucrose		12	12	12
High C & re-oxygenated	Anaerobic – 3% (w/w) sucrose re-oxygenated for 5 days prior to sampling		12	12	12
Sequential extractions	Control, Low C, High C, High C reoxygenated				4 of each treatment

indicate that anaerobic conditions caused the reductive dissolution of Fe oxy-hydroxides. This resulted in an increase in the solution phase (F0) in amended soil with up to 15% of the Fe_{Total} present as $[Fe^{2+}{}_{Sol}]$. Whilst some authors (e.g. Ponnamperuma, 1972; Ponnamperuma et al., 1967; Kashem and Singh, 2004) have suggested that Fe carbonate minerals (such ash siderite) may form under submergence, the lack of inorganic C in the analysed samples suggest that these minerals were not formed. Thus, the small increases found in the F2 phase after C amendments probably represents an increase in specifically adsorbed Fe^{2+}. Around 3% Fe was bound to the sulphide/organic matter (F4) phase and remained unchanged across the treatments. Manganese in untreated soils was primarily bound to the oxide phase (75%) whilst only 20% was found in the residual phase. These can easily dissolve under anaerobic conditions, leading to large changes in the Mn fractionation as shown in Fig. 2. Over 70% of the Mn originally associated with oxides (F3) was released and transferred to the mobile pools (F0–F1) during flooding. The High C amendment solubilised more Mn than the Low C amendment, with the result that $[Mn_{Sol}]$ accounts for up to 50% of the Mn_{Total}.

3.3.2. Zinc

Zn was the least reactive trace metal studied, with >35% found in the F4 + F5 extraction steps. These fractions remained unchanged after 42 days regardless of the organic C content added to the system. Around 40% of Zn was associated with Mn and Fe oxides (F3), similar to other contaminated soils in the catchment (Li and Thornton, 2001). Submergence in the presence of added C increased both the exchangeable (F1) and soluble phases (F0) (Fig. 2), with the Zn mainly coming from the oxide bound fraction (F3), as well as a smaller proportion being found in the specifically adsorbed fraction (F2).

3.3.3. Lead

Around 50% of the Pb_{Total} present was originally bound to oxides (F3) whilst only 20% of the Pb_{Total} was found in the residual phase (F5). Reductive dissolution of Fe and Mn oxides released much of the oxide bound Pb (F3) which was then mostly re-adsorbed, thus increasing the specifically adsorbed Pb in F2. The organically bound and residual reservoirs remained largely unchanged and only a marginal increase in the exchangeable fraction was found. Fig. 2 shows that the concentrations in the solution and exchangeable pools (F0 + F1) are low regardless of the redox status. Other authors also found that Pb is typically poorly exchangeable in soils (Atkinson et al., 2011).

3.3.4. Cadmium

Around 30% of Cd_{Total} was found in the soluble and exchangeable phases (F0 + F1, Fig. 2) in the original soil and <10% in the residual fraction (F5), which is in agreement with other studies on contaminated soils (Kashem et al., 2007; Li and Thornton, 2001). In the amended soils Mn and Fe oxide dissolution following submergence released much of the Cd originally hosted in this phase (F3). The proportion of specifically adsorbed Cd (F2) also decreased, whilst greater Cd was found in the exchangeable fraction probably as a result of the lower pH values enhancing desorption processes. As a result, the proportion of Cd hosted in F0 + F1 increased up to 70%, with the exchangeable Cd being dominant. Flooding did not have an impact on residual and sulphide/organically bound Cd (~15%), which remained largely unaltered.

3.4. Changes in metal isotopic exchangeability and solubility with flooding

3.4.1. Comparison of ID assays with and without resin purification

To test for non-labile metal complexes in the solution phase we compared M_E with M_{Er} concentrations (Fig. 3). Results show a generally good agreement between the % M_E and M_{Er} values. However, for Pb in the unamended treatment, the resin-treated spiked soil solutions were found to be enriched in native ^{208}Pb with respect to the ^{204}Pb tracer when compared to their non-resin counterparts. This effectively resulted in overestimates of E_r concentrations. Whilst the chelating resin is meant to selectively sorb exchangeable metal from the solution, it is possible that colloids can be adsorbed (Lombi et al., 2003). Our observations suggest that the resin may have sorbed either DOC or Fe colloids that contained non-labile Pb complexes. The purification process i.e. resin exchange and subsequent elution effectively transferred and pre-concentrated this non-labile fraction in the eluent, shifting the isotopic ratio values closer to natural ratios and producing unrealistically high M_{Er}-values. Similar observations were reported for the unamended Fe_E measurements where Fe_{Sol} concentrations are relatively low. Whilst similar enrichment in non-exchangeable metal-colloid complexes is also likely for other metals, any impact on the isotopic ratio of the eluent is reduced because of the higher concentrations of metal in the equilibrating solution.

Fig. 4 shows the ratios of M_E/M_{Er} plotted against pH for each element, also showing a good agreement between M_E and M_{Er} across the pH range and especially at pH < 6. For Zn, particularly for the unamended treatment there is a tendency that at pH > 6, an increase in the M_E/M_{Er} is found, suggesting the presence of non-labile colloidal Zn in the soil solutions. Lombi et al. (2003) reported greater amounts of non-exchangeable Zn bound to colloids with increasing pH, occurring mostly at pH > 6. For Cd, M_E was also similar to M_{Er} without showing a particular trend with pH. The greater scatter could be due to the lower concentrations of $[Cd_{Sol}]$ in comparison with other metals and has previously been observed by Marzouk (2012). For Pb and Fe, M_E/M_{Er} are also close to the 1:1 line across the studied pH range with the exception of unamended microcosms due to the reasons discussed above. Our findings demonstrate that large amounts of fermentation-derived organic compounds in solution such as acetates do not enhance the formation of non-labile metal-bearing organic colloids which would produce overestimated E-values. In the subsequent discussion, for the purposes of comparing results the non-resin measurements will be used.

3.4.2. Iron and manganese

As observed in the sequential extractions, Fe and Mn speciation in aerobically and anaerobically incubated unamended soils were largely

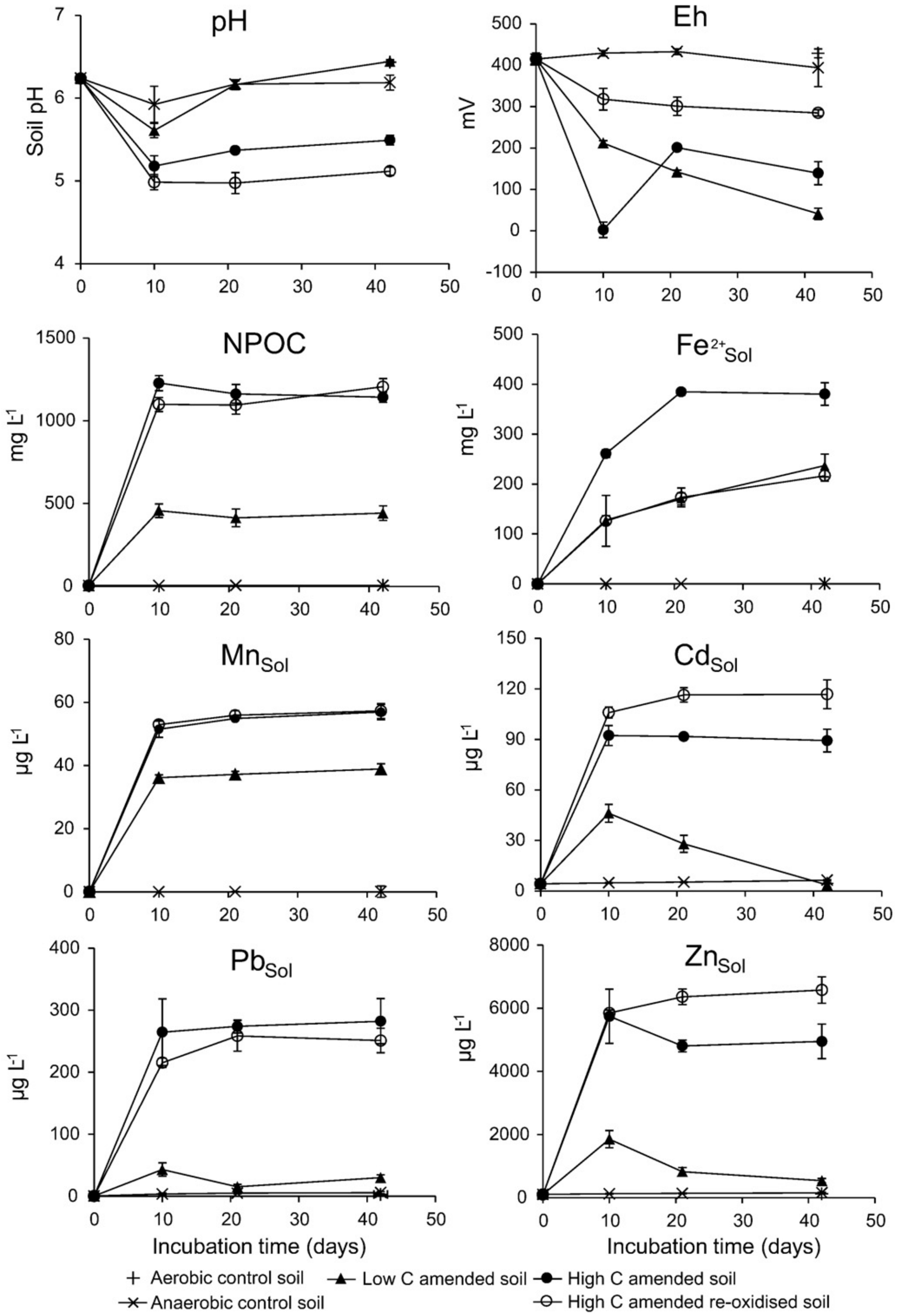

Fig. 1. Changes in pH, Eh, DOC and concentration of Fe^{2+}, Mn, Cd, Pb and Zn in solution during 42 days incubation under aerobic and anaerobic conditions. Error bars denote ±1 SD.

insensitive to 42 days of flooding (Figs. 1 and 5). Thus, Fe remained virtually inert (<0.1% as Fe_E) and the concentrations of $[Fe^{2+}{}_{Sol}]$ and $[Mn_{Sol}]$ remained <0.05 mg L^{-1} throughout the experiment. The addition of a carbon source fuelled the growth of bacteria populations, thus enhancing to different degrees the reductive dissolution of Mn and Fe oxyhydroxides via anaerobic metabolism. A sharp initial increase in $[Fe^{2+}{}_{Sol}]$ (130 mg L^{-1}) was observed after 10 days submergence of Low C soils, coinciding with the drop in the redox potential and the onset of reducing conditions. After this period, further decreases in redox potential brought more $[Fe^{2+}{}_{Sol}]$ into solution (240 mg L^{-1} at 42 days). High C amended soils followed a similar trend with a 2-fold increase in $[Fe^{2+}{}_{Sol}]$ concentrations (Fig. 1). A consistent and steady increase in Fe_E was found over the 42 days flooding period, rising from <0.1% to 14% in Low C amended soils and 21% in High C amended soils (Fig. 5). A strong correlation between $[Fe_E]$ and $[Fe^{2+}{}_{Sol}]$ ($r = 0.91$, $p < 0.001$) across the different treatments was found.

A broad relationship of increasing Fe_E with decreasing redox potential was found across the treatments (Fig. 6). However, within this broad

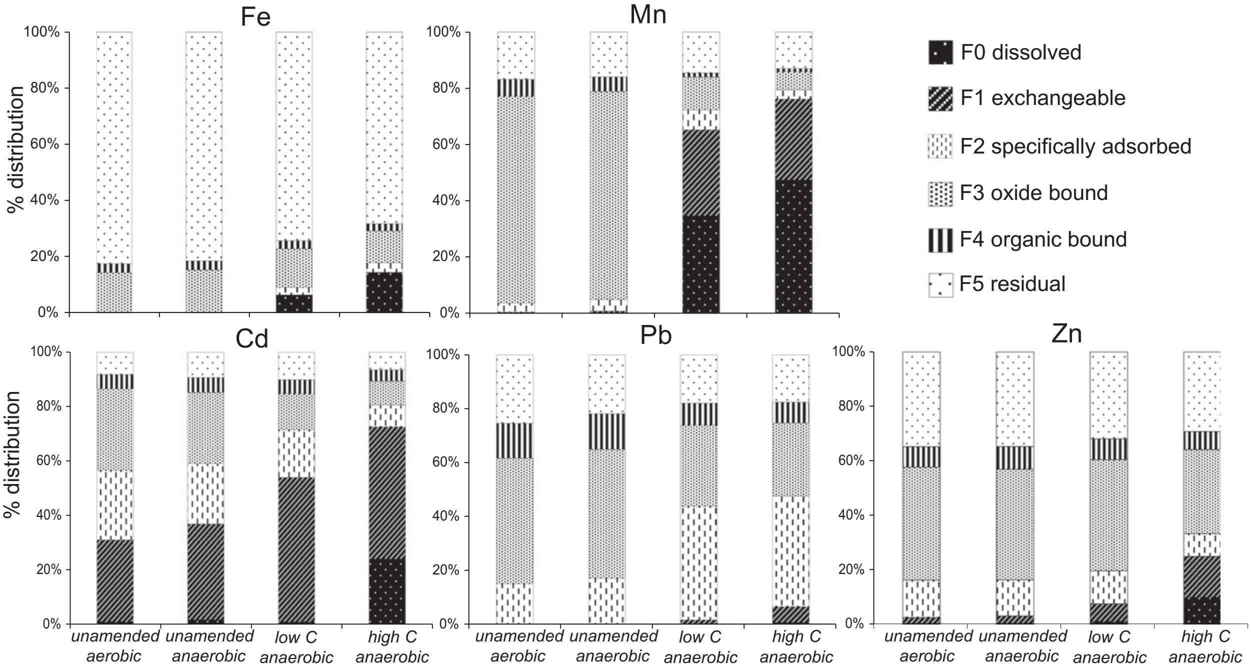

Fig. 2. Distribution of metals among the six fractions for amended and unamended soils based on modified sequential extraction after Tessier et al. (1979).

trend, stronger relationships can be found. For example, it can be seen that Fe_E increases with decreasing Eh for the Low C treatment. This relationship was not apparent for the High C treatment where the Eh reached a minimum after 10 days flooding (0 mV) and increased thereafter (Fig. 1) whilst Fe_E gradually increased over time (Fig. 5). This suggests that at 10 days in the High C treatment, the MnO_2/Mn^{2+} or Fe_2O_3/Fe^{2+} systems are not operating yet and the system is being controlled the H_2/H^+ redox system (Glinski et al., 1996). Evidence that later the MnO_2/Mn^{2+} or Fe_2O_3/Fe^{2+} systems take control can be seen in the relationship between the High C anaerobic samples at 21 and 42 days and the High C re-oxygenated samples. Thus, as a result of the pH increasing as Eh decreases in the Low C treatment, Fe_E and $[Fe^{2+}]$ increase with pH in these conditions. The relationship between pH and $[Fe^{2+}]$ is not so evident for the High C treatment.

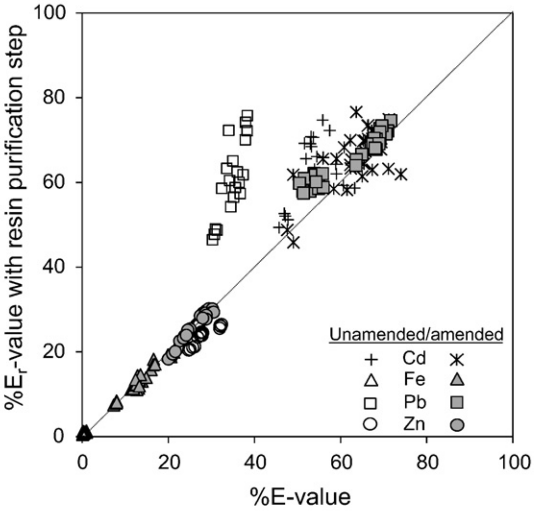

Fig. 3. Comparison between the isotopic exchangeability of metals determined without (E) and with (E_r) an ion exchange resin purification for samples taken at 0, 10, 21 and 42 days of submergence. Values expressed as % E-value over the total pool of metal in soil.

The reductive dissolution of Fe oxy-hydroxides and the associated increase in $[Fe^{2+}{}_{Sol}]$ has implications for the solubility of the other elements. Firstly, Roth et al. (1969) suggested that negatively charged exchange sites on clay surfaces can be blocked by positively charged Fe oxy-hydroxide coatings. Under anaerobic conditions the removal of these coatings by biological or chemical reductive dissolution can increase the cation exchange capacity (CEC) of the soil (Favre et al., 2002) and create new sorption surfaces for trace metals. Secondly, an increase in $[Fe^{2+}{}_{Sol}]$ creates competition with other trace metals for sorption sites on the CEC, and this can increase $[M_{Sol}]$ of other trace metals. Thirdly, trace metals associated with the Fe oxy-hydroxide coatings may also be released. In contrast to $[Fe^{2+}{}_{Sol}]$, further incubation time did not result in increases in $[Mn_{Sol}]$, suggesting that most of the reductive dissolution of Mn oxides took place during the early stages of submergence. As with $[Fe^{2+}{}_{Sol}]$, increases in $[Mn_{Sol}]$ potentially increase competition for sorption sites for trace metals.

3.4.3. Zinc

For the untreated and anaerobically incubated soils, Zn_E remained largely unchanged (%E variations were <5%) during the incubation period, reflecting the minimal changes in pH and redox status. There were no major changes in $[Zn_{Sol}]$ in soils incubated in aerobic conditions for 42 days but a very small steady increase was found in $[Zn_{Sol}]$ for the anaerobic untreated soils, rising from 100 to 150 μg L^{-1} (Fig. 1). This does not appear to be related to the release of metals via reduction of Fe and Mn oxides, but may reflect the small initial decrease of 0.3 pH units observed. With the onset of reducing conditions, Zn_E in the High C amended soils remained invariably around 30% due to (i) the lower soil pH after 10 days and (ii) the subsequent small recovery that pH showed throughout the incubation time (Fig. 1). In the Low C amended soils Zn_E slowly declined from 25 to 21% with increasing submergence time. Several mechanisms may cause the decrease in Zn_E found in the Low C treatment. Mineral precipitation has often been cited as a reason for decreasing Zn availability in flooded soils, including the formation of $ZnFe_2O_4$ or similar franklinite-like minerals (Sajwan and Lindsay, 1986), Zn occurring in layered double hydroxides and phyllosilicates, which are present at a range of pH values (Khaokaew et al., 2012) or the

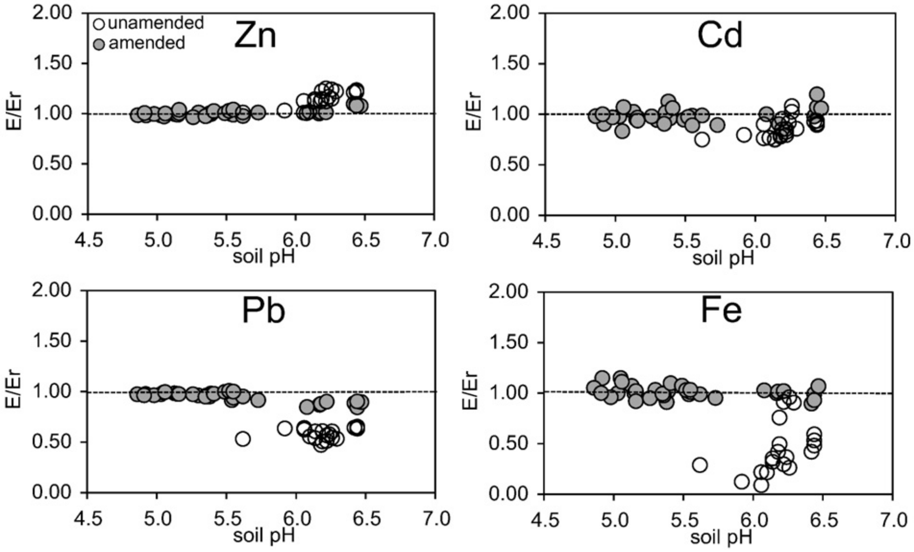

Fig. 4. Relationship between E/Er and pH for Zn, Fe, Pb and Cd for samples taken at 0, 10, 21 and 42 days of submergence.

formation of $ZnCO_3$ and ZnS Bostick et al. (2001). The formation of sulphide minerals is unlikely in our study as the declining trend in Zn_E was reported at redox potentials above the onset of sulphate reduction. High pH and the presence of bicarbonate are known to depress Zn availability in flooded paddy fields (Breemen et al., 1980) with high organic matter concentrations strongly enhancing this process. However, our analysis suggests that with no carbonate phases being present in the soils the precipitation of $ZnCO_3$ is unlikely to have occurred. Thus the concentration of Zn_E is considered essentially pH determined (Fig. 7). A strong negative correlation ($r = -0.84$, $p < 0.001$) between Zn_E and soil pH was found for treatments where Eh < 300 mV (Fig. 6). Despite relatively small changes in Zn_E, a > 10-fold increase in $[Zn_{Sol}]$ (from 0.1 to 2 mg L^{-1} for Low C treated soils and 6 mg L^{-1} for High C treated soils) was observed at 10 days (Fig. 1), followed by a decline with time in response to the rising pH. In a similar manner to Zn_E, the results suggest that $[Zn_{Sol}]$ is primarily controlled by pH. A strong negative correlation between pH and $[Zn_{Sol}]$ was found for amended soils ($r = -0.94$, $p < 0.001$, Fig. 7). However, a smaller competition effect with Fe^{2+} may be identified after 42 days for the Low C amendment, where despite the Low C pH being higher than the control, and with a lower %

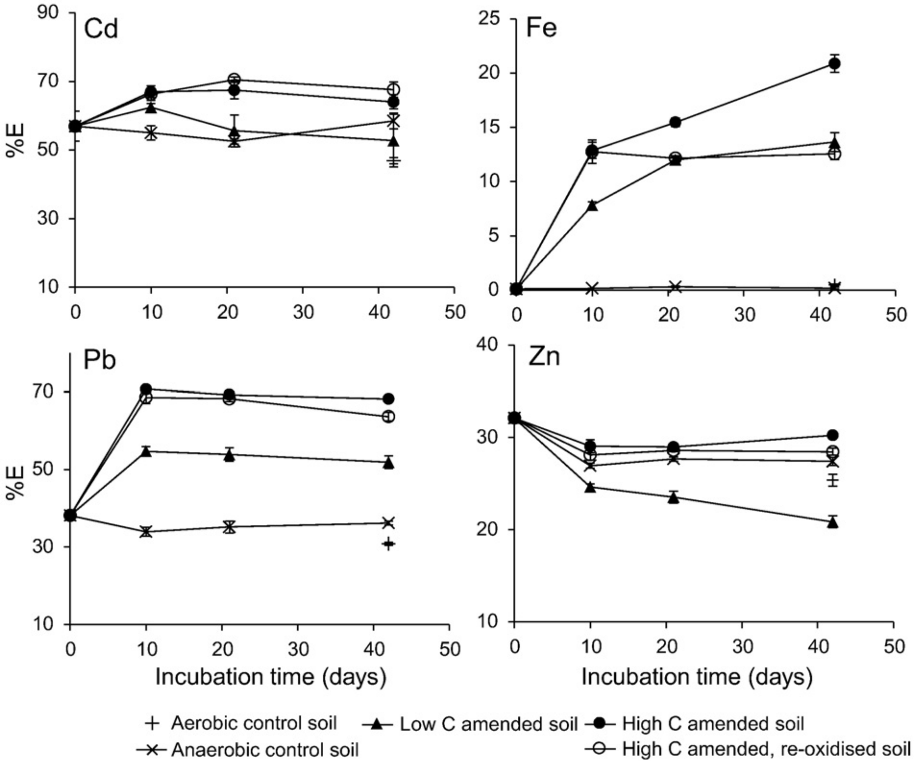

Fig. 5. Changes in % E-values of Cd, Fe, Pb and Zn during 42 days incubation. Error bars denote ±1 SD.

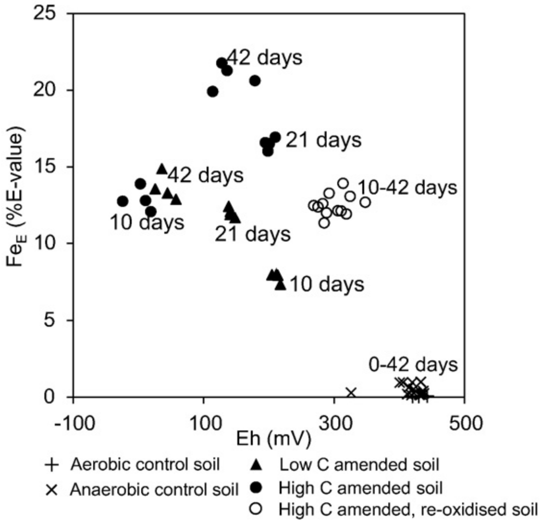

Fig. 6. Comparison between Fe E-values and redox potential for samples taken at 0, 10, 21 and 42 days of submergence.

Zn_E value, the concentration of Zn_{Sol} was higher. Van Laer et al. (2010) found a similar response.

3.4.4. Lead

In aerobically and anaerobically incubated unamended soils, variations in Pb_E throughout the experiment were small (<7%) and $[Pb_{Sol}]$ remained at low concentrations (<6 μg L^{-1}). Where reductive dissolution of Mn and Fe oxides occurred, large changes in Pb_E and $[Pb_{Sol}]$ were observed and followed a reverse trend with increasing pH (Fig. 7) across the dataset. A steep increase in Pb_E from 38% to 55% was found in Low C soils after 10 days, reaching 71% in High C soils. Whilst a decrease in pH will be a major factor increasing Pb_E (Izquierdo et al., 2013), it is likely that the reductive dissolution of oxides will also increase Pb_E further by allowing isotopic exchange with previously non-labile Pb. The affinity of Pb to bind to Fe and Mn oxyhydroxides is well recognised (McKenzie, 1980; Sauvé et al., 2000; Izquierdo et al., 2013). Evidence of the interactions between the previously described increase in Fe_E and Pb_E is shown in Fig. 8. Such a relationship was not found for the other studied metals. However, when $[Fe_E]$ was >15% it did not result in further increases in Pb_E. This may be a result of Pb_E being limited by Mn and Fe oxide surfaces that are dissolved early on in the development of anaerobic conditions. Other possible additional sources of Pb that may contribute to Pb_E may arise from the dissolution of mineral phases through pH changes or increases in DOC (e.g. Sauvé et al., 1998). After the increase of Pb_E in both the C amended treatments peaked at 10 days, it subsequently declined slowly at a rate of 1–2% at each sampling (Fig. 3), coinciding with the recovery of soil pH.

Concomitant with the changes in Pb_E, $[Pb_{Sol}]$ rose to 40 μg L^{-1} (Low C) and 250 μg L^{-1} (High C) after 10 days incubation and subsequently stabilised (Fig. 1). The very narrow fluctuations of $[Pb_{Sol}]$ and Pb_E after 10 days (Figs. 1 and 3) probably result from the balance between (i) slowly rising pH enhancing re-absorption, (ii) continued reductive dissolution of Fe oxides releasing previously occluded Pb and (iii) competitive sorption with Zn^{2+}, Mn^{2+} and Fe^{2+}. However, it should be noted that $[Pb_{Sol}]$ was 10-fold lower than $[Zn_{Sol}]$ (Fig. 1), despite the fact that both metals have similar total and labile concentrations. This may be attributed to Pb being typically more strongly retained on oxides than Cd and Zn.

3.4.5. Cadmium

Cadmium was the most labile of the trace elements studied with Cd_E ranging from 50 to 70% of the Cd_{Total} across treatments. This is in line with the sequential extraction results which suggested that 30% of Cd_{Total} in the aerobic soil and 70% of the Cd_{Total} in the reduced soil are readily available i.e. soluble (F0) and exchangeable (F1) (Fig. 2). In the absence of additional organic C source, Cd_E (57%) and $[Cd_{Sol}]$ solubility (4 μg L^{-1}) remained largely unchanged over the incubation period regardless of whether this was in an aerobic or anaerobic environment (Figs. 1 and 5). In anaerobic soils Cd_E increased 6–10% after 10 days. For the Low C soils, this was followed by a slow declining trend in response to increasing pH. For the High C soils where a weaker recovery in pH was noted, Cd_E declined very slowly over the remaining incubation period (Fig. 5). Thus, the size of Cd_E appeared to be essentially related to soil pH. This is demonstrated by a negative relationship ($r = -0.79$, $p < 0.001$) between % Cd_E and soil pH across all the

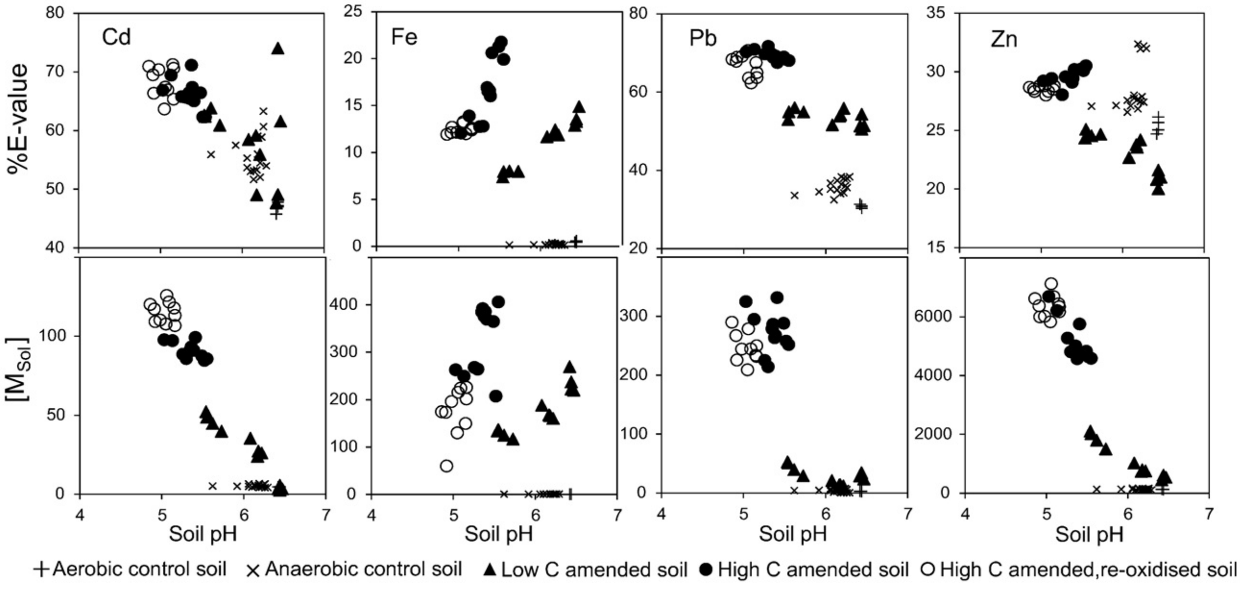

Fig. 7. Isotopically exchangeable (E-value) and soluble pool of metals vs soil pH. Metal solubility values $[M_{Sol}]$ in mg L^{-1} for Fe and μg L^{-1} for Cd, Pb and Zn.

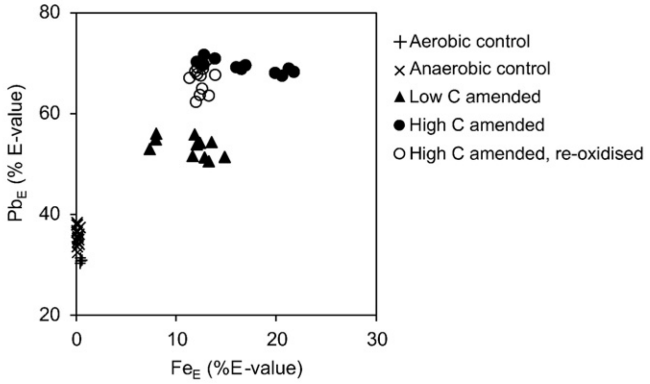

Fig. 8. Relationship between Pb and Fe E-values during 42 days incubation.

treatments (Fig. 7). For both Cd and Zn, decreasing lability with increasing pH is consistent with the literature on aerobic soils (Degryse et al., 2004; Izquierdo et al., 2013; Sterckeman et al., 2009; Tye et al., 2003). Similarly [Cd_{Sol}] rose after 10 days (Fig. 1) for the Low and High C amendments, probably as a result of increased concentrations of Cd_E and the lower pH. However, unlike for [Zn_{Sol}] in the Low C amendment, there was no evidence that competition from Mn^{2+} and Fe^{2+} ions produced [Cd_{Sol}] concentrations higher than the unamended treatment. The above observations suggest that changes in Cd_E and [Cd_{Sol}] are mainly linked to soil pH rather than redox status. There was a strong correlation between [Cd_{Sol}] and pH ($r = -0.95$, $p < 0.001$) across the dataset.

3.5. Changes in metal isotopic exchangeability and solubility with re-oxidation

After 5 days re-oxygenation, the redox potential returned to ~+300 mV after each sampling, although with a declining trend over time (Fig. 1). Reactions involving Fe^{3+} reduction consume protons and the reverse occurs when reduced species of Fe^{2+} are re-oxidised and precipitate as Fe^{3+} oxy-hydroxides. It is therefore not surprising that the lowest pH values (pH ~ 5.0) recorded in the current experiment were from these re-oxidising soils. Thus soil acidification in the anaerobic-aerobic transition may have a major impact on metal lability and solubility. Re-oxygenation decreased Fe_E and solubility by 50% (Figs. 1 and 5), providing evidence of partial Fe^{2+} oxidation and re-precipitation of Fe oxy-hydroxides. The concentrations did not return within the 5 day re-equilibration with O_2 to those measured on aerobically incubated soils but [$Fe^{2+}{}_{Sol}$] remained > 100 mg L^{-1} and $Fe_E > 10\%$, and no changes on NPOC levels or any re-precipitation of [Mn_{Sol}] as oxides was reported. Although the measured redox potential was typical of a mildly reduced soil, with mV values higher than those characteristic of Fe^{3+} reduction, the solution chemistry was still more representative of a well reduced system with respect to [$Fe^{2+}{}_{Sol}$] and [Mn_{Sol}] concentrations. This suggests a hysteresis effect such that changes in the soil solution chemistry may lag behind Eh adjustments during periods of the anaerobic-aerobic transition.

Iron (Fe^{2+}) oxidation and the re-precipitation of oxides prompted by the shift towards aerobic conditions results in (i) the creation of new sorption surfaces and (ii) simultaneously decreases competitive sorption with other metals for the remaining sorption sites (e.g. humus, oxides). Re-precipitation of Fe oxides appeared to decrease [Pb_{Sol}] and Pb_E, despite the lower pH, demonstrating the strong affinity of oxides for Pb (Ainsworth et al., 1994). Increases in [Cd_{Sol}] and [Zn_{Sol}] during the anaerobic-aerobic transition were most likely associated with the decrease in pH (Fig. 1). Gambrell (1994) found a similar response when marsh sediments underwent oxidation. In our study, only small changes in the Cd_E and Zn_E were found, suggesting that the H^+ produced during oxidation is competing with Zn and Cd held on cation exchangeable sites. However, it is apparent that after 5 days re-oxidation the impact on the soluble pools of Cd and Zn was not reflected in changes in M_E (Fig. 1). In a similar way in which oxygen depletion and subsequent electrochemical changes (including reductive dissolution of Fe oxides present in soil) do not immediately take place after submergence, exposure to oxygen for 5 days did not fully reverse the changes that soils underwent during the flooding period.

3.6. Comparison between E-values and metal fractionation

Both E-values and sequential extractions have been used to characterise metals in the solid phase of soils. However there is no reason to pre-suppose agreement as sequential extractions are based on the dissolution of selected solid phases (or more correctly phases with similar physico-chemical characteristics) and therefore do not discriminate between isotopically exchangeable (labile) and non-labile metal. However, within this work a basis for comparison exists because we have demonstrated that no carbonate phase appears to be present in the F2 fraction. This means that the F2 fraction should largely consist of specifically adsorbed metal which should largely be isotopically exchangeable and demonstrate pH dependence (Forbes et al., 1976). Thus the more mobile fractions of sequential extraction i.e. F0–F2 (the solution phase, exchangeable phase and the chemisorbed phase) should all potentially be included within the ID measurement, providing an effective check on the E-value measurements.

Good agreement was found between Zn_E and the combined soluble, exchangeable specifically adsorbed fractions (F0–F2) of the sequential extraction (Fig. 9). Where anaerobic conditions did not develop, the Zn_E slightly exceeded the combined F0 + F1 + F2 fractions, possibly due to some metal bound to oxides and yet remaining isotopically exchangeable. Young et al. (2005) also reported E-values between the combined Tessier F1–F2 and Tessier F1–F3 fractions for aerobic urban soils for Zn. Similarly, the combined F0–F2 proportion provided a reasonable estimate of Pb_E, although the E-value was found to be slightly higher across the 4 treatments. Again this suggests that some isotopically exchangeable Pb also occurs in the oxides and organic matter fractions. For Cd, the combined F0–F2 fraction was also in good agreement with Cd_E, although slightly lower. Iron in aerobic soil is virtually non-labile which is consistent with most Fe hosted in the residual phase (F5) with smaller proportions being found in the oxide (F3) and organically bound (F4) fractions. Reductive dissolution of Fe oxides resulted

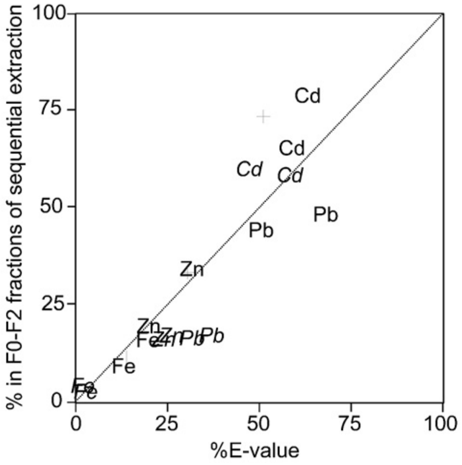

Fig. 9. Comparison between Cd, Fe, Pb and Zn E-values and the F0 + F1 + F2 combined fractions of sequential extraction procedure. Data reported are mean values for each treatment after 42 days incubation (n = 4). Labels in italics denote soils where reducing conditions did not develop i.e. aerobically and anaerobically incubated unamended control soils.

in an increase in Fe_E and a transfer to F0–F2 fractions (Fig. 9). Thus, the Fe_E in reduced soils is approximately equivalent to the sum of the F0 + F1 + F2 fractions, with much of the Fe_E in solution (F0) as $[Fe^{2+}{}_{Sol}]$. Results therefore suggest that there is reasonable agreement between the F0–F2 fractions and the ID assays. In particular this demonstrates that the solution phase complexes are labile confirming a key fundamental condition for ID assays.

3.7. General discussion

In this work the applicability of ID assays in submerged soil environments where VFA's in particular, may be a significant complex for trace metals in the soil solution was examined. These conditions (aerobic to anaerobic transition) may occur during and after the fermentation stages of soil reduction, which is one of the key stages in the reduction process for trace elements because of the potential for rapid pH and Eh change. Comparisons of ID measurements with and without a resin purification step revealed good agreement between M_E and M_{Er} values, with the exception of the unamended Pb_E samples due to methodological problems (Section 3.4). This suggests that the by-products of fermentation, mainly acetate, do not produce non-labile metal colloids in solution over a pH range of 4.5–7, therefore not violating one of the fundamental conditions of the ID assay. This is despite acetate-metal complexes generally have higher Log_{10} K values than those for humic and fulvic acids metal complexes. For example, Log_{10} K values from the Minteq 4 database for acetate-metal complexes are 2.64, 2.86, 2.68 and 1.4 for Zn, Cd, Pb and Fe respectively. In the WHAM 7 (Lofts and Tipping, 2011) database Log_{10} K values for humic acids are 1.87, 1.67, 2.37 and 1.76 for Zn, Cd, Pb and Fe respectively and 1.68, 1.51, 2.15 and 1.46 for fulvic acids. Whilst we have demonstrated solution acetate-metal complexes do not present methodological problems for ID assays, testing the applicability of the ID assays for individual elements and at the extent of the anaerobic conditions expected would still be advisable as the behaviour of the metal and isotope within the system needs to be understood. For example, Izquierdo et al. (2016) demonstrated that ^{67}Zn used in ID assays of rice paddy soils underwent fixation. A further example is that in very reduced systems (−400 mv) non-labile metal sulphide colloids (e.g. PbS and CdS) may be produced (Weber et al., 2009). These would be expected to be non-labile and would require the resin purification step within the ID assay.

Accepting that the addition of sucrose in the quantities used in these experiments do not represent entirely realistic field conditions, the results have demonstrated some of the recognised mechanisms that determine trace element speciation and transport in submerged soils, for which ID assays can develop a deeper understanding of the interactions between elements. Importantly, the use of ID, unlike common chemical extraction schemes (e.g. 0.05 M EDTA), provides a mechanistic basis for describing these interactions. Firstly, it was evident that under these conditions, pH was the dominant major factor determining M_E and $[M_{Sol}]$ for Zn and Cd. It is recognised that solubility is pH dependent. Whilst for Zn and Cd the changes in pH created relatively small changes in E-values, the effect of submergence on Pb_E was much larger and appeared to be a two stage process: a sharp initial increase in Pb_E caused by the reductive dissolution of Fe and Mn oxy-hydroxides or other mineral phases, followed by a second phase when solubility was controlled by pH. In particular, after C amendment there was a substantial redistribution of Pb from the oxide mineral phases to the specifically adsorbed phase as demonstrated by the sequential extractions. Several authors have indicated that the dissolution of Fe and Mn oxy-hydroxides can lead to new surfaces being created, that can then adsorb metals. For example, Iu et al. (1981) suggested that the dissolution of Fe and Mn oxides in submerged soils may have an antagonistic effect on Zn bioavailability because their dissolution can create surfaces with high adsorptive capacity for Zn. However, in the current study, competition from $[Fe^{2+}{}_{Sol}]$ and $[Mn_{Sol}]$ was identified and this also had an effect on $[Zn_{Sol}]$ in the Low C amendment at 42 days. For the other metals this competition effect was not identified, probably because concentrations of $[Cd_{Sol}]$ were much lower and the solubility of $[Pb_{Sol}]$ was associated with the large increase in E-values caused by the dissolution of oxide phases and the role of pH.

Within the broader pH-dependency found for Zn, Cd and Pb in our model system, the work has identified two key stages in the reduction-oxidation cycle where the use of E-values would develop greater understanding in more realistic field conditions. The first key area is to help provide further insight into the mechanisms underpinning metal availability when soils undergo re-oxidisation, and when there is potential of (M^{2+}) exposure to plants and biota through the accompanying acidification. This process has been identified as the cause of Cd enrichment in rice, which occurs primarily during the grain filling stage that coincides with soil oxidation when flooded paddy soils are drained in preparation for the harvest and pH decreases (de Livera et al., 2011; Simmons et al., 2008). A further key factor in submerged soils that was not examined in this work due to predetermined sampling times were the rapid changes in lability and solubility caused by the initial drop in pH, which is often found within the first 10 day period of submergence as a result of fermentation, when the most readily available C present in the soil is utilised (Bell, 1969). This initial fermentation period and the subsequent effects on metal speciation are worth investigating further, particularly in paddy soils where organic material is used as fertiliser but also in countries such as the UK where small flooding events predominantly occur within a 0–10 day cycle (Cannell et al., 1980).

4. Conclusions

Given the complexity of the competing interactions affecting trace element solubility in anaerobic soils, an approach where E-values are combined with mineralogical analysis, in this instance sequential extraction assays, was used to identify the causes of changes in E-values and solubility, and provide greater understanding of the behaviour of trace element cycles in submerged soils. Results from our model system demonstrated that without an extra source of C, initiation of changes in redox status were only just occurring after 42 days of submergence. However the presence of a carbon source, produced significant responses, dependent on the quantity of C available. These responses stemmed from changes in pH and the reductive dissolution of Mn/Fe oxy-hydroxides and had an impact on the mobility and solubility of Cd, Pb and Zn. Our findings using exchange resins indicated that the presence of VFA's as a product of fermentation did not invalidate the suitability of isotopic dilution techniques in environments undergoing fermentation. This is also supported by the good agreement between E-values and the combined soluble, exchangeable and adsorbed phases of the sequential extraction.

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