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# Buffer capacity and Cu affinity of soil particulate organic matter (POM) size fractions

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## Summary

Particulate organic matter fractions (POM), defined as sand-sized organic separates in soils, are known to be labile organic components with a rapid turnover. Recently, POM fractions were identified to be metal-enriched in both metal-contaminated and uncontaminated soils. However, mechanisms for such metal-enrichment are poorly understood, because of the paucity of information on the chemical properties of POM. The aim of this study was to quantify the reactivity of POM towards Cu and to show a POM-size effect on this reactivity. POM was isolated from soils with different organic amendment managements: straw (S), conifer compost (CC), and non-amended (NA). Two POM size fractions were isolated by density-fractionation in water: 50–200  $\mu\text{m}$  and 200–2000  $\mu\text{m}$ . These fractions were studied for their metal contents, acid-base properties and affinity toward Cu. The buffer capacity and Cu affinity were modeled by FITEQL 4.0 software and compared between the two POM size fractions. Each POM size fraction provided a buffer capacity due to the presence of reactive sites, the greatest being for the 50–200  $\mu\text{m}$  POM fractions. A signature of organic inputs as seen by the buffer capacities was observed for the 50–200  $\mu\text{m}$  but not for the 200–2000  $\mu\text{m}$  POM fractions. But Cu affinity was comparable between the coarse and fine POM fractions and no significant differences were found between NA, S and CC samples. We checked the hypothesis that decreasing POM size due to degradation processes generates more reactive surface sites. Results confirmed that soil POM plays a key role as a metal sink, due to its chemical properties.

## Introduction

Particulate organic matter fractions (POM), as defined in our work, are the part of soil organic matter (SOM) that can be isolated in  $> 50\text{-}\mu\text{m}$  sand-sized fractions by physical separation in water (Balesdent *et al.*, 1991; Gregorich *et al.*, 2006). The material thus isolated is heterogeneous regarding its turnover, and includes animal and plant residues but also root fragments or fungal hyphae (Gregorich & Janzen, 1996; Chenu & Stotzky, 2000). In addition, Christensen (2001) defined POM as ‘uncomplexed’ organic matter in soil, neither present as readily recognizable litter components (i.e.  $> 2\text{ mm}$ ) nor incorporated into primary organo-mineral complexes. POM or ‘uncomplexed organic matter’ has been widely studied in order to establish relationships between POM characteristics and SOM dynamics and soil properties (Besnard *et al.*, 1996; Balesdent *et al.*, 1998; Whalen *et al.*, 2000). The quantity and quality of POM fractions have been shown to be strongly influenced by soil management, but the use of POM as an index of soil quality is a matter of discussion: there is no proof that POM fractions have a functional meaning (Wander, 2004).

An important POM characteristic related to SOM dynamics is the rapid turnover of C and N in POM fractions. This turnover generally decreases with decreasing POM size, indicating that smaller POM size fractions have a longer residence time in soil (Balesdent, 1996). In addition, several studies have shown that POM extracted from soils of uncontaminated, and moderately and strongly metal-contaminated soils was metal-enriched with respect to bulk soil (Ducaroir & Lamy, 1995; Flores-Vélez *et al.*, 1996; Balabane *et al.*, 1999; Besnard *et al.*, 2001; Balabane & van Oort, 2002). Enrichment factors between 1.5 and 3 were found for Pb and Zn, between 4 and 5 for Cd, but as high as 7 for Cu when comparing the metal contents of POM with those determined on bulk samples of surface horizons of agricultural soils from an area around a former zinc smelter (Lamy *et al.*, 2002). Two main hypotheses have been formulated to explain metals-enrichment in POM (Labanowski *et al.*, 2007): (i) absorption of even small amounts of metals by plants *via* roots and accumulation in shoots may lead to coarse plant debris locally enriched in metals before re-cycling to soils, and (ii) POM possess reactive surface sites involved in metal sorption. However, the first hypothesis seems unable to explain the *in situ* measured enrichment factors because metal contents determined on aerial parts of plants (except for metallophyte

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species) are generally small with respect to metal contents determined on coarse POM fragments. A recent paper on characterization of heavy metal adsorption on soil particulate organic matter (Guo *et al.*, 2006) seems to confirm the second hypothesis for Pb, Cu and Cd. After FTIR analysis of POM in the absence or presence of heavy metals, Guo *et al.* (2006) suggested that adsorption on POM was mainly due to complexes with carboxyl and hydroxyl groups. Moreover, several studies on samples collected from moderately and strongly metal polluted soils showed that metal contents of POM fractions increased with decreasing POM size (Balabane *et al.*, 1999; Balabane & van Oort, 2002; Lamy *et al.*, 2002). These authors suggested both an effect of abiotic processes (e.g. increasing reactivity for particles with decreasing sizes) as well as an influence of biodegradation leading to a relative enrichment of the most metal-contaminated POM parts. From laboratory incubations, Boucher *et al.* (2005a,b) showed that biodegradation of metal-contaminated leaves in an uncontaminated soil led to an increase in metal content of soil POM in the fraction 200–2000  $\mu\text{m}$ . Hence, POM appears as a metal sink in soils, but its role is poorly understood and the number of studies dealing with chemical properties of POM is small. In particular, metal reactivity towards POM is still poorly documented, contrasting with numerous studies on the reactivity of other fractions of soil organic matter towards metals, such as humic substances or bacteria (Milne *et al.*, 2003; Guiné *et al.*, 2006).

The objectives of the present paper were: (i) to show the chemical reactivity of POM, and (ii) to assess a size effect of this reactivity. For this, we used an original approach combining the extraction of two POM fractions, 200–2000 and 50–200  $\mu\text{m}$ , by physical fractionation in water and the potentiometric titrations of these POM fractions in the absence or presence of Cu. The POM fractions were extracted from soils amended either with straw or conifer compost and a non-amended soil. Data from potentiometric titrations were fitted with FITEQL 4.0 software in order to show and compare the various POM reactivities as a function of the POM-sizes and to discuss the role of POM towards sorption of heavy metals in soils.

## Materials and methods

### *Site description and sampling*

We used a 15-year-old field experiment located in the vineyard site of Mâcon Clessé (Burgundy, France, located at 46°25.087'N, 004°48.037'E). All soils were deep Eutric Cambisols (FAO-Unesco-ISRIC, 1988) with a fine clay-loam texture. The soil surface horizons displayed a pH between 6.5 and 7, and cation exchange capacity varying from about 17 to 25  $\text{cmol}_c \text{kg}^{-1}$  depending on organic amendments. The clay fraction, studied by X-ray diffraction, included mainly illite, kaolinite and interstratified 2:1 phyllosilicates, as well as quartz and feldspar. Two plots receiving different organic amendments at the soil surface were selected: (i) straw amendment (S) at a rate

of 10  $\text{t ha}^{-1}$  every 2 years, and (ii) conifer compost amendment (CC) at a rate of 100  $\text{m}^3 \text{ha}^{-1}$  every 3–4 years. A control sample was also taken from a non-amended (NA) plot with no organic inputs other than restitution of vine-shoot fragments and only chemical weeding of soil. Sampling was made at 0–5 cm depth in the surface horizon in the various plots. Four replicates of about 2 kg were sampled per plot. Samples were mixed and homogenized, air-dried, sieved to 2 mm and stored in plastic bags at room temperature.

### *Physical fractionation of the soil samples and POM analysis*

Mechanical dispersion of c. 50 g of soil samples (< 2 mm) was performed on a rotary shaker for 16 hours in polypropylene tubes with 125 ml of de-ionized water in the presence of 10 glass beads (6 mm). The suspension was then wet-sieved to 200 and 50  $\mu\text{m}$ , respectively. We separated the light fractions 200–2000 and 50–200  $\mu\text{m}$  by flotation in water as described by Balesdent *et al.* (1991). Thus POM corresponding to a light fraction mainly composed of floating organic debris was isolated from the dense mineral fractions. The two POM fractions isolated were oven-dried at 55°C. For the bulk soil samples and each size fraction, total N and C were determined by dry combustion in an autoanalyser (CE instruments NC 2500, Milan, Italy) and total Cu content by flame atomic absorption spectrometry (Varian SpectrAA 220, Mulgrave, Victoria) after  $\text{HNO}_3$  digestion for POM and  $\text{HF}/\text{HNO}_3$  digestion for bulk soil samples in a microwave system (CEM, MarsX, Matthews, NC). The purity of each isolated POM fraction was checked under a binocular microscope, and a petrographic polarized microscope was used to study general aspects of POM fractions.

### *Potentiometric titrations of POM*

We first studied the removal of indigenous POM Cu by shaking c. 0.3 g of POM in 50 ml of water at different pH (2, 3, 4, 5, 6) for 24 hours. At pH 2, total organic carbon (TOC) and Fe yields were greater than at pH 3.5 while Cu recovery was small (16% of the indigenous Cu content of POM). At pH 3.5, however, Cu removal was negligible, suggesting no POM modification. We thus decided not to acid-wash the POM before our experiments, and to begin titrations at around pH 3.5. At the end of titration, the TOC content in solution was monitored by high temperature catalytic combustion followed by  $\text{CO}_2$  measurement (Shimadzu TOC-VCSN, Kyoto, Japan). We found that around pH 8–9 less than 6% of total organic carbon initially introduced as solid matter in the titration cell was solubilized, indicating that our experimental conditions minimized modifications of POM during titration.

All titrations were thus conducted in a pH range between 3.5 and a maximum of 9 in order to minimize side-effects such as POM modifications (mineralization of POM at low pH or

solubilization of carbon at high pH). Copper was used here both for its relevance as a major environmental pollutant, especially in vineyard soils (Besnard *et al.*, 2001), but also as a probe of metal reactivity related to its well-known great affinity with soil organic constituents.

#### Acid-base properties of POM

The titrations were conducted using a pH meter (TIM 900, Radiometer, Copenhagen, Denmark) with a burette assembly (ABU901, Radiometer). A solution containing a known quantity of POM (1.6 g dm<sup>-3</sup> for POM 200–2000 or 0.8 g dm<sup>-3</sup> for POM 50–200 µm) and with an initial HClO<sub>4</sub> content of 10<sup>-3.5</sup> M and a constant ionic strength of 0.1 M NaClO<sub>4</sub> was titrated in a thermostated cell at 25°C. Titrations were conducted in the presence of a positive external pressure of N<sub>2</sub> applied above the solution to avoid solubilization of CO<sub>2</sub> during the experiments. Aliquots of NaOH used as titrant (0.05 M) previously standardized against potassium hydrogen phthalate were injected through a glass line. The pH was measured using a couple of electrodes: a reference electrode (XR 110, Radiometer) fitted with a salt-bridge junction filled with 0.1 M NaClO<sub>4</sub> (AL 120, Radiometer) and a glass electrode (XG 250, Radiometer). The titrator was programmed in a dynamic mode and successive fixed titrant additions of 10 µl were made every 5 minutes, a time lapse that preliminary experiments showed to be sufficient to attain stability.

For each point of the titration curve the charge balance equation for all ions in solution was used in accordance with Equation (1) to give the molarity of added protons in the system, expressed as  $Q$  (in eqH<sup>+</sup> g<sup>-1</sup> of POM):

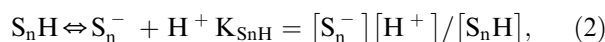
$$Q = \frac{[Ca]V_o / (V_o + V) - V[C_b] / (V_o + V) - [H^+] + [OH^-]}{[POM]}, \quad (1)$$

where  $[H^+] = 10^{-pH}$ ,  $[OH^-] = 10^{pH-pK_w}$  and  $K_w$  is the ionic product of water,  $[C_a]$  is the concentration of initial acidity (i.e. protons already dissociated at the beginning of the titration and the known initial concentration of added HClO<sub>4</sub>),  $V$  and  $[C_b]$  are respectively the volume (dm<sup>3</sup>) and the concentration (eq dm<sup>-3</sup>) of the base added;  $V_o$  is the initial volume and  $[POM]$  is the concentration of POM expressed in g dm<sup>-3</sup> introduced in the titration cell.

The potentiometric titration experiments were modelled using the FITEQL 4.0 optimization routine (Herbelin & Westall, 1999) to determine the intrinsic deprotonation constants, surface site densities and Cu binding affinities. Possible electrostatic behaviour of POM particles was not taken into account as an option in the model because of lack of data such as the specific surface area or capacitance of the Helmholtz layer.

Fitting of experimental titration curves of POM was tested with the hypothesis of either one or two acid-base exchange sites, based on the presence of carboxyl and/or phenol type groups

on particulate organic matter in soil (Guo *et al.*, 2006). Optimization of several parameters was made during the fitting procedure: site concentrations and apparent conditional constants. The apparent conditional dissociation constants were expressed as:

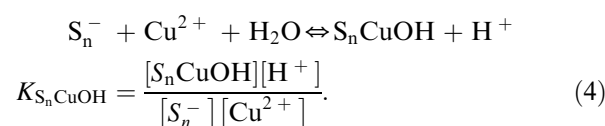
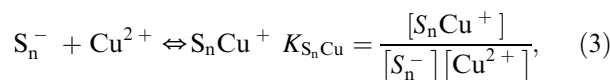


where  $S_n$  is a surface reactive functional group and  $n = 1$  or  $2$ .

#### Titration of POM in the presence of Cu

A solution containing a known quantity of POM and various amounts of a stock solution of Cu(ClO<sub>4</sub>)<sub>2</sub> (Strem Chemicals, Newburyport, MA) was titrated with 0.05 M NaOH at a constant ionic strength of 0.1 M NaClO<sub>4</sub>. The Cu concentrations in the cell were fixed in order to have a ratio: ligand content/Cu content = 8, where the ligand content was expressed as the total amount of reactive titratable functional group for a POM fraction (thus Cu = 7.4 × 10<sup>-5</sup> ± 0.7 M for POM 200–2000 µm or 5.8 × 10<sup>-5</sup> ± 1.2 M Cu for POM 50–200 µm). The free Cu concentration was measured during the titration with a Cu(II) ion-selective electrode (ISECu25, Radiometer) coupled to a reference electrode fitted with a salt-bridge junction (XR 110 and AL 120, Radiometer). Before the experiments, these electrodes were calibrated with a stock solution of Cu (10<sup>-5</sup>, 10<sup>-4</sup>, 10<sup>-3</sup> M) in 0.1 M NaClO<sub>4</sub>.

To determine the Cu binding affinity constants from the experimental curves in the presence of Cu, we considered that free Cu and hydrolysis products of Cu might form monodentate complexes with POM and we introduced acid-base properties previously determined (i.e. site concentrations and apparent conditional constants). The apparent stability constants of complex formation between POM and Cu were expressed as:



#### POM saturation with Cu

About 0.1 g of dry POM was mixed with 40 ml of 0.1 M NaClO<sub>4</sub>. Copper additions were made every 5 minutes from a stock solution (0.1 M or 0.001 M Cu). The pH of the solution was maintained constant at six with small additions of NaOH (0.1 M) or HClO<sub>4</sub> (0.1 M). The free Cu concentration was measured during the titration with a Cu(II) ion-selective electrode (ISECu25, Radiometer) coupled to a reference electrode fitted

with a salt-bridge junction (XR 110 and AL 120, Radiometer) calibrated as above.

The quantity of sorbed Cu was determined by difference between total Cu in solution and free Cu. In order to neglect Cu hydrolysis, we stopped the Cu adsorption experiments when the free Cu concentration in solution was  $6 \times 10^{-4}$  M as determined by the Cu-selective electrode. For such a free Cu concentration at pH 6, thermodynamic calculations indicated that 97.5% of Cu in solution is in the free form. The Cu saturation experiments were made in triplicate for POM size fractions 200–2000  $\mu\text{m}$  of the three samples NA, S and CC.

### Statistical analysis

Significant differences ( $P < 0.05$ ) between data were determined with XLstat (Microsoft) with Student's *t*-test on the means of two or three replicates.

## Results and discussion

### POM characteristics

Morphological aspects of the POM fractions studied by optical microscopy are presented in Figure 1 and chemical aspects are given in Table 1. For both POM size fractions, continuous size heterogeneity was observed due to biodegradation processes in soils. The 200–2000- $\mu\text{m}$  size fractions still showed recognizable plant structures ascribed to fragments of vine-shoot (Figure 1a), straw (Figure 1c), and very dark coloured conifer compost residues (Figure 1e). But the general aspect of 50–200- $\mu\text{m}$  size POM fragments in the fractions of the three soils was similar (Figure 1b, d, f). Plant cell structures were still visible but the origin of the organic input was difficult to establish at this scale.

Total POM fractions 50–2000  $\mu\text{m}$  represented a small part of the mass of soil, 1.3% for the NA soil, 1.8% for S, but 6.1% for CC (Table 1). However, total POM fractions represent a large reservoir of organic carbon in soil: about 25% of total organic carbon content in soil for NA and S, and 47% for CC. Similar findings were noted by Besnard *et al.* (2001) in calcareous vineyard soils with various organic amendments. These authors observed that total POM accounted for 20–55% of total organic carbon in soils. Furthermore, Christensen (2001) reported that uncomplexed organic matter in soil can account for 10–40% of organic carbon in surface horizons depending on several factors such as land use, vegetation type, climate, soil type or faunal activity.

Organic carbon contents of POM and C/N values decreased with the decreasing POM size, in agreement with findings of Balabane & van Oort (2002) and Besnard *et al.* (2001), thus indicating that the finest POM fractions were more biodegraded. The 200–2000- $\mu\text{m}$  POM size fractions of the three samples showed comparable organic carbon contents and C/N values ( $P > 0.05$ ). Thus, although the morphology of 200–2000- $\mu\text{m}$  POM fractions was different (Figure 1), no bio-

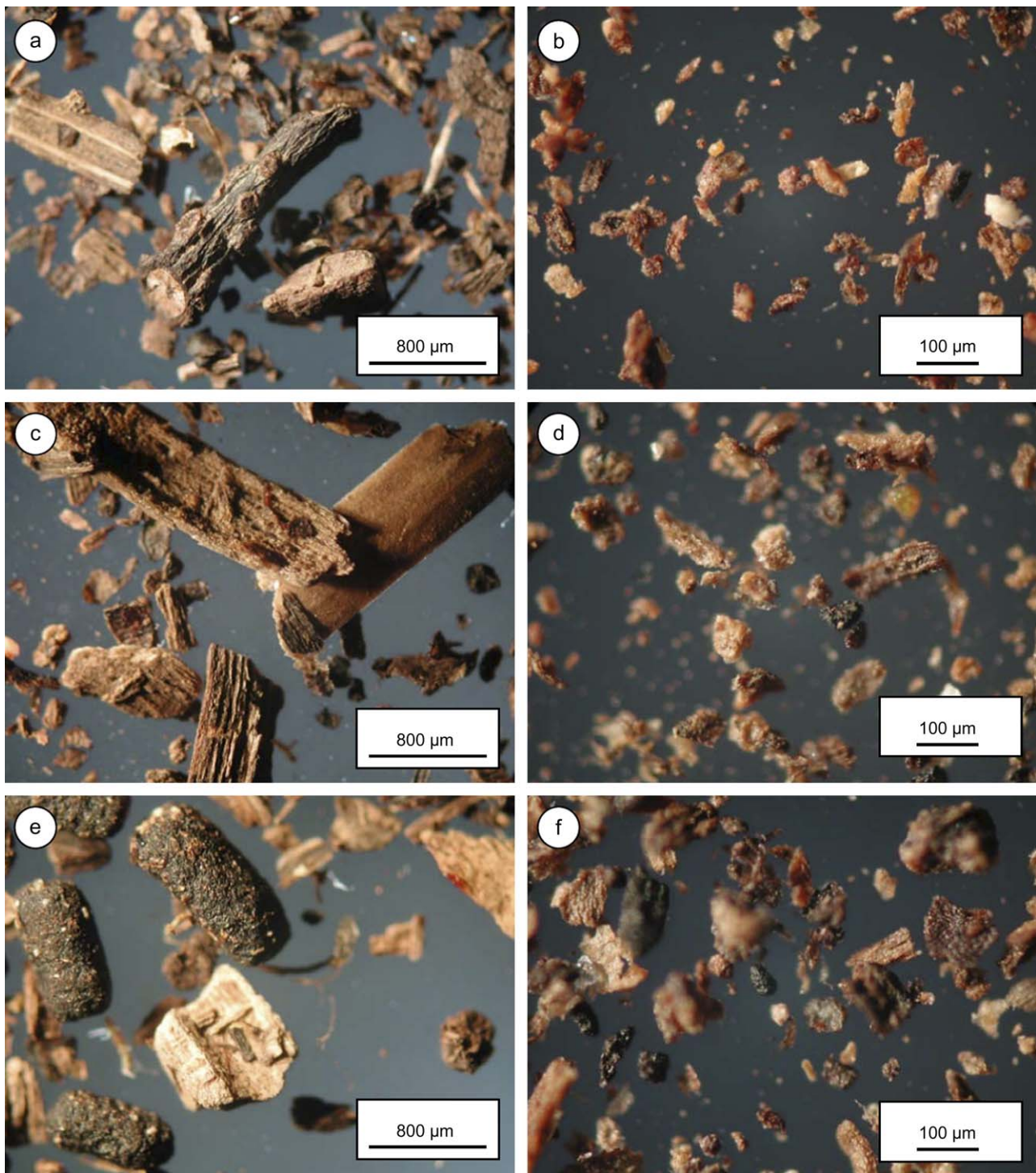
geochemical signature of the exogenous organic inputs was observed for these fractions. For the 50–200- $\mu\text{m}$  POM size fractions, the organic carbon contents and C/N values measured for CC differed significantly from those of NA and S, but little difference in the morphological aspects of these fine POM particles was observed (Figure 1). Similarly, Fortuna *et al.* (2003) found that addition of compost to a cultivated sandy loam soil significantly increased POM-C contents.

All three soil samples had comparable Cu contents of about 60 mg  $\text{kg}^{-1}$  soil (Table 1), but Cu contents in the extracted POM fractions were found two- to eightfold greater than the bulk samples. For all three soils, a greater Cu content was observed in the finest POM fraction (Table 1). Accumulation and increasing metal enrichment with decreasing POM size have also been observed for other metals and for different types of pollution (Florez-Vélez *et al.*, 1996; Balabane *et al.*, 1999; Besnard *et al.*, 2001; Balabane & van Oort, 2002; Lamy *et al.*, 2002). In our samples, the smallest Cu contents in POM were observed for the S and CC soils. However, in these soils the contribution of total POM pool to the total Cu content of bulk soil was 9.9% for S and 16.1% for CC, greater than for the NA soil (8.8%) (Table 1). Thus, introduction of exogenous organic matter into these soils induced a change in the initial Cu distribution towards the freshly introduced organic debris. This change was larger for compost than for straw amendment, and clearly visible in the finest POM fractions. These results suggest that incorporation of exogenous organic material into various particle-size fractions modifies Cu speciation. In the amended soils, the smaller Cu content measured in POM may be due to a dilution effect. Besnard *et al.* (2001) also observed a small Cu content in POM extracted from a soil amended with oak bark. They concluded either a poor reactivity of the oak-bark towards Cu or to a dilution effect due to the great mass of the POM fraction in this soil.

### Buffer capacity of POM

Examples of typical titration curves for the 200–2000 and the 50–200- $\mu\text{m}$  POM size fractions are given in Figure 2a, showing the reproducibility of titrations. An example of calculated curve is given in Figure 2b. The number of mole of protons added below pH 4 should tend to 0, but as seen in Figure 2b, the numerical value of  $Q$  increases below pH 4. Such behaviour could be due to uncertainties in the response of pH electrodes classically observed at low pH (Guiné *et al.*, 2007). At pH  $> 8$ ,  $Q$  decreases rapidly (Figure 2b) and this appears unusual. This may be due to proton release related to solubilization of the organic carbon of POM. Therefore, modelling of titration curves was restricted to the pH range 4–8.

Figure 3 gives  $Q$  curves versus pH calculated from potentiometric data of each POM sample, and shows that each POM fraction contributed an additional buffer capacity to the solution between pH 4 and 8 compared with a solution without POM. Titration curves were rather similar in shape between



**Figure 1** Microscopic observations of extracted POM: 200–2000- $\mu\text{m}$  POM size fractions (left) extracted from NA (a), S (c), CC (e) and 50–200- $\mu\text{m}$  POM size fractions (right) extracted from NA (b), S (d), CC (f) samples. (NA = non-amended soil, S = soil amended with straw and CC = soil amended with conifer compost). Figure in colour online.

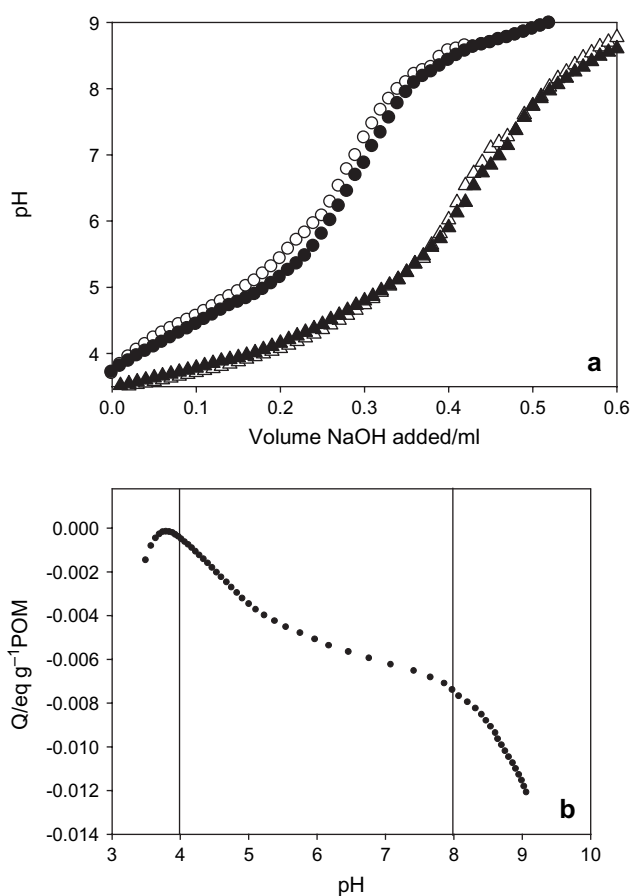
size-fractions but buffer capacities were found to be greater for the finest POM fractions. Furthermore, when normalized to the grams of titrated POM, the coarser POM fractions from

the three soils showed comparable buffer capacities. In contrast, the finest POM fractions exhibited significantly different buffer capacities, the greatest being observed for the fine POM



**Table 1** Chemical characteristics of POM extracted from the three soil samples (NA = non-amended soil, S = soil amended with straw and CC = soil amended with conifer compost) (mean  $\pm$  standard error)

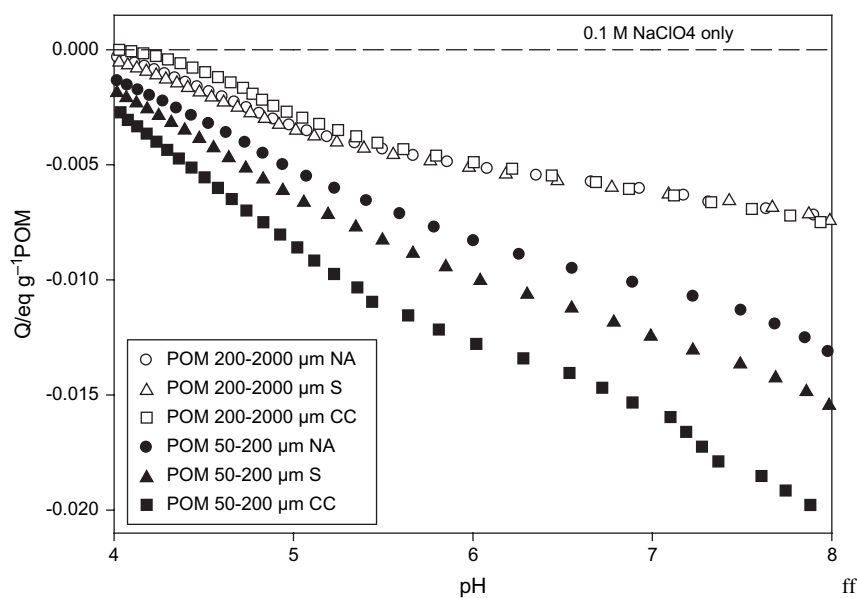
	Content /g kg <sup>-1</sup> soil	Organic C		C/N	Cu	
		/g kg <sup>-1</sup> POM	/g kg <sup>-1</sup> soil		/mg kg <sup>-1</sup> POM	/mg kg <sup>-1</sup> soil
NA						
POM 200–2000 $\mu$ m	4.5 $\pm$ 0.3	358.0 $\pm$ 7.7	1.6 $\pm$ 0.1	24.0 $\pm$ 0.8	395.1 $\pm$ 13.5	1.8 $\pm$ 0.1
POM 50–200 $\mu$ m	7.5 $\pm$ 0.3	189.9 $\pm$ 8.2	1.5 $\pm$ 0.1	17.7 $\pm$ 0.5	486.7 $\pm$ 9.6	3.7 $\pm$ 0.2
Bulk soil			12.3 $\pm$ 0.3	11.7 $\pm$ 0.3		62.4 $\pm$ 0.4
S						
POM 200–2000 $\mu$ m	5.8 $\pm$ 1.1	341.0 $\pm$ 12.5	2.0 $\pm$ 0.4	24.4 $\pm$ 2.1	281.3 $\pm$ 26.3	1.6 $\pm$ 0.2
POM 50–200 $\mu$ m	12.6 $\pm$ 0.7	204.0 $\pm$ 3.9	2.5 $\pm$ 0.2	17.5 $\pm$ 0.2	369.0 $\pm$ 10.7	4.5 $\pm$ 0.1
Bulk soil			17.5 $\pm$ 1.50	13.1 $\pm$ 0.2		61.1 $\pm$ 0.6
CC						
POM 200–2000 $\mu$ m	31.8 $\pm$ 6.1	360.3 $\pm$ 5.7	11.5 $\pm$ 1.6	25.5 $\pm$ 0.6	114.8 $\pm$ 7.3	3.6 $\pm$ 0.4
POM 50–200 $\mu$ m	29.1 $\pm$ 2.0	296.7 $\pm$ 8.5	8.7 $\pm$ 0.7	20.4 $\pm$ 0.3	203.4 $\pm$ 4.4	5.9 $\pm$ 0.4
Bulk soil			43.0 $\pm$ 3.1	17.5 $\pm$ 0.5		58.8 $\pm$ 0.1

**Figure 2** (a) Potentiometric pH versus V titration curves of 200–2000  $\mu$ m ( $\circ$  repetition 1,  $\bullet$  repetition 2) and 50–200  $\mu$ m ( $\Delta$  repetition 1,  $\blacktriangle$  repetition 2) sized POM extracted from soil CC, amended with conifer compost. (b) Example of calculated Q curves from potentiometric data of POM 200–2000  $\mu$ m extracted from soil S, amended with straw.

fraction from CC (Figure 3). Within our experimental conditions, we fitted the potentiometric data using either a one-site or a two-site model. An example of such calculations is given in Figure 4. Whatever the POM fraction, the one-site model did not account for the entire buffer capacity titrated between pH 4 and 8: for pH > 5.8, nearly all sites were deprotonated and no further buffering was possible. The two-site model, however, provided a good fit to the experimental data, indicating that at least two types of reactive functional groups were present and active on POM surfaces between pH 4 and 8.

The deprotonation constants and surface-site densities calculated after fitting titration data between pH 4 and 8 are given in Table 2. The buffer capacity can be linked to the presence of carboxyl and phenolic groups, as highlighted by Guo *et al.* (2006), by infra red spectrometry of POM, such sites being created after degradation of soil organic residues (Stevenson, 1982; Kelleher *et al.*, 2006). But  $pK_{S_{nH}}$  values in this study are also consistent with the presence of microbial adhesion on POM surface (i.e. bacteria and fungi) shown by Chenu & Stotzky (2000), such micro organisms exhibiting acid-base properties with carboxyl, phosphate and phenolic groups (Fein *et al.*, 1997; Guiné *et al.*, 2006).

For a given POM size fraction,  $pK_{S_{1H}}$  or  $pK_{S_{2H}}$  values did not differ significantly between NA, S and CC (Table 2). These results indicate that organic inputs had only little influence on deprotonation constants for a given POM size fraction. But when comparing POM size fractions within a soil sample, values of deprotonation constant ( $pK_{S_{1H}}$  and/or  $pK_{S_{2H}}$ ) were found to be significantly different between the 50–200- $\mu$ m and 200–2000- $\mu$ m POM size fractions for the two amended soils, whereas no significant difference was observed for the NA soil. This absence of size effect on the buffer capacity for the NA soil suggested that similar kinds of functional reactive groups were present both in the 50–200 and 200–2000  $\mu$ m POM fractions.



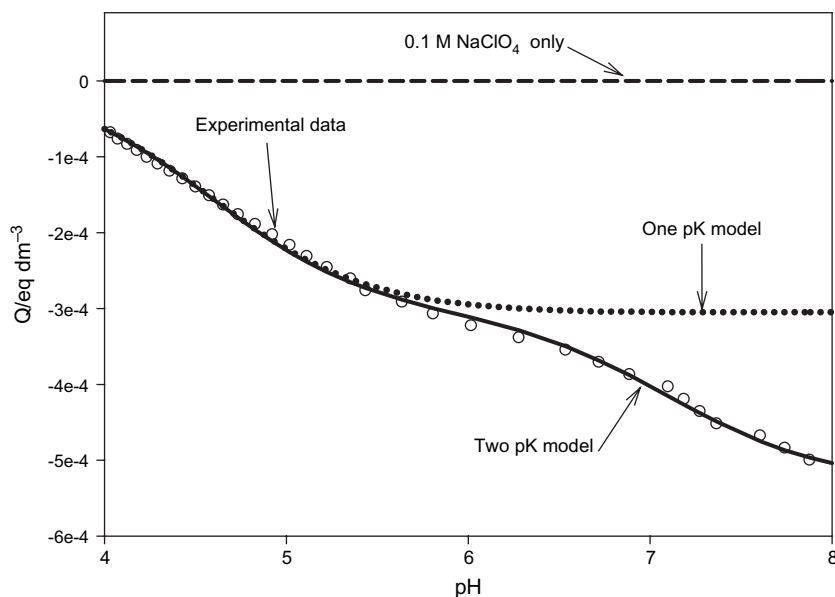
**Figure 3** Calculated  $Q$  curves from potentiometric titration of 200–2000  $\mu\text{m}$  ( $\circ$ ,  $\square$ ,  $\triangle$ ) and 50–200  $\mu\text{m}$  ( $\bullet$ ,  $\blacksquare$ ,  $\blacktriangle$ ) sized POM extracted from the three soil samples. NA = non-amended, S = amended with straw and CC = amended with conifer compost.

However, after the introduction of new organic residues, a size effect on the buffer capacity becomes visible. Given the rapid turnover rates mentioned for coarser organic residues (Balesdent, 1996), the chemical signature of the finer POM fractions (e.g.  $\text{pK}_{\text{S}_{\text{NH}}}$ ) of the different organic amendments will become more marked with time. No significant differences were observed between NA, S and CC for the surface site densities of coarse and fine POM fractions (Table 2). But within each soil, the site density was always larger for the 50–200- $\mu\text{m}$  than for the 200–2000- $\mu\text{m}$  POM size fraction. This is particularly clear when expressing the site density values ( $\Sigma[S_1] + [S_2]$ ) in  $\text{eq g}^{-1}$  of organic carbon of the POM fraction, that is  $0.63 \pm 0.07 \text{ meq g}^{-1}$  Corg for the three NA, S and CC POM 200–2000  $\mu\text{m}$  frac-

tions, and  $2.20 \pm 0.11 \text{ meq g}^{-1}$  Corg for the three POM 50–200  $\mu\text{m}$  fractions. This last result corroborates the idea that decreasing POM size generates functional reactive sites.

#### POM-Cu interaction

Cu saturation curves at a constant pH of 6 for POM 200–2000  $\mu\text{m}$  are given in Figure 5a. Even for adsorbed Cu greater than  $10\,000 \text{ mg kg}^{-1}$  of POM, saturation was not attained. This Cu content was 25–90 times larger than the initial Cu content of POM and showed that POM can adsorb large quantities of Cu. The quantity of Cu adsorbed was not significantly different between NA, S and CC, suggesting that the



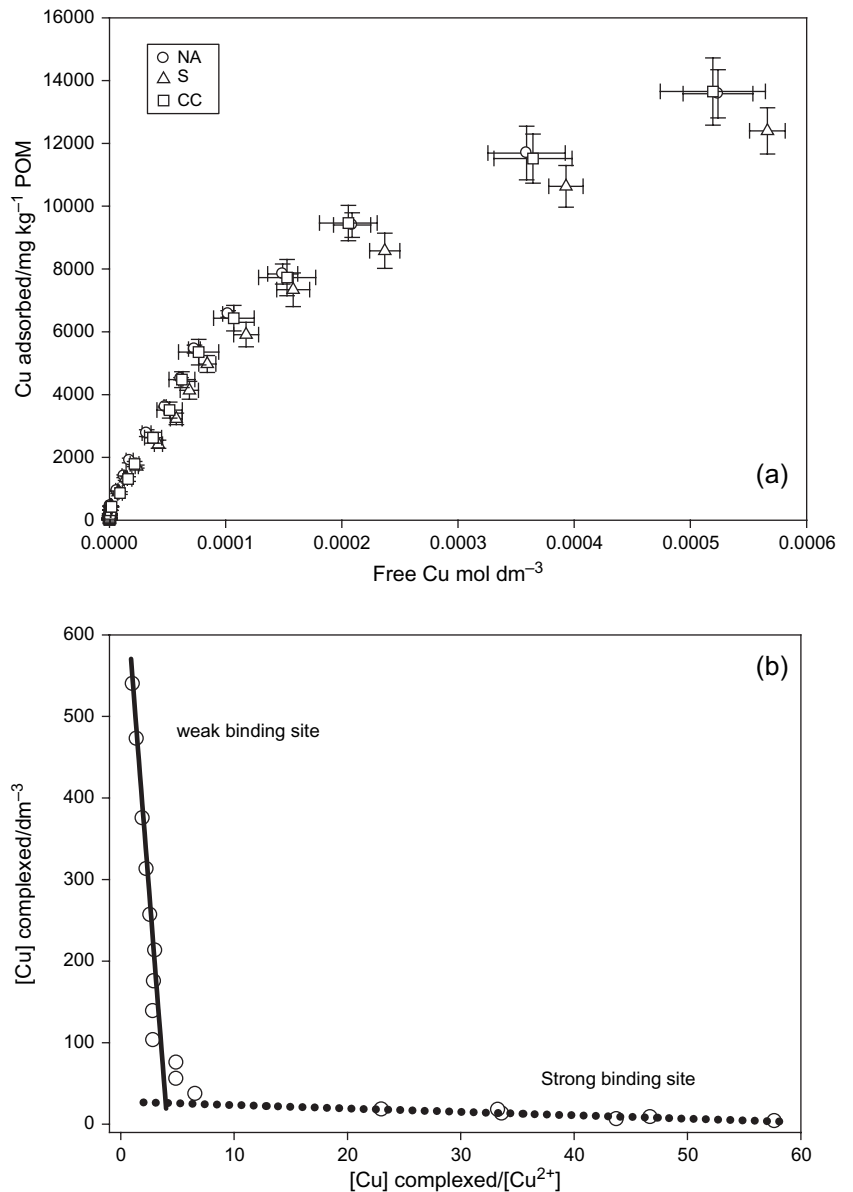
**Figure 4** Results from FITEQL 4.0 modelling of potentiometric titration data for one titration performed using POM 50–200  $\mu\text{m}$  extracted from soil amended with conifer compost. Also shown is the titration curve for the electrolyte in the absence of POM. Results of modelling are depicted for the best fit with the hypothesis of the presence of one or two-sites.



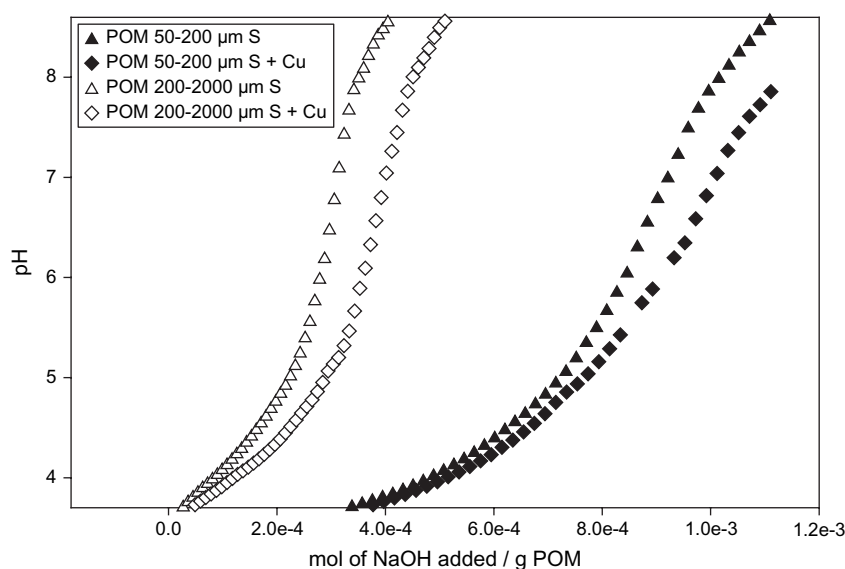
**Table 2** Deprotonation constants and site densities as calculated by FITEQL (mean  $\pm$  standard error) (POM-NA = POM extracted from non-amended soil; POM-Straw = POM extracted from soil amended with straw and POM-CC = POM extracted from soil amended with conifer compost)

	$pK_{S1H}$	$pK_{S2H}$	$\frac{[S_1]}{10^{-4} \text{ eq g}^{-1} \text{ POM}}$	$[S_2]$
<b>POM-NA</b>				
200–2000 $\mu\text{m}$	$4.86 \pm 0.18^a$	$7.28 \pm 0.18a$	$1.37 \pm 0.28a$	$0.69 \pm 0.14a$
50–200 $\mu\text{m}$	$4.68 \pm 0.23a$	$6.90 \pm 0.25a$	$2.48 \pm 0.18b$	$1.56 \pm 0.13b$
<b>POM-Straw</b>				
200–2000 $\mu\text{m}$	$4.81 \pm 0.13a$	$7.46 \pm 0.24a$	$1.69 \pm 0.28a$	$0.76 \pm 0.13a$
50–200 $\mu\text{m}$	$4.71 \pm 0.12a$	$6.99 \pm 0.18b$	$2.85 \pm 0.12b$	$1.95 \pm 0.15b$
<b>POM-CC</b>				
200–2000 $\mu\text{m}$	$5.00 \pm 0.14a$	$7.38 \pm 0.15a$	$1.43 \pm 0.25a$	$0.76 \pm 0.12a$
50–200 $\mu\text{m}$	$4.57 \pm 0.16b$	$7.02 \pm 0.08b$	$3.83 \pm 0.31b$	$2.44 \pm 0.32b$

<sup>a</sup>Different letters within columns and for one POM sample indicate significant difference by Student test at  $P < 0.05$ .



**Figure 5** (a) Cu saturation curves of POM 200–2000  $\mu\text{m}$  extracted from NA, S and CC (NA = non-amended soil, S = soil amended with straw and CC = soil amended with conifer compost). (b) Example of calculated curve using the Langmuir double surface equation from Cu saturation data for POM 200–2000  $\mu\text{m}$  extracted from soil S.



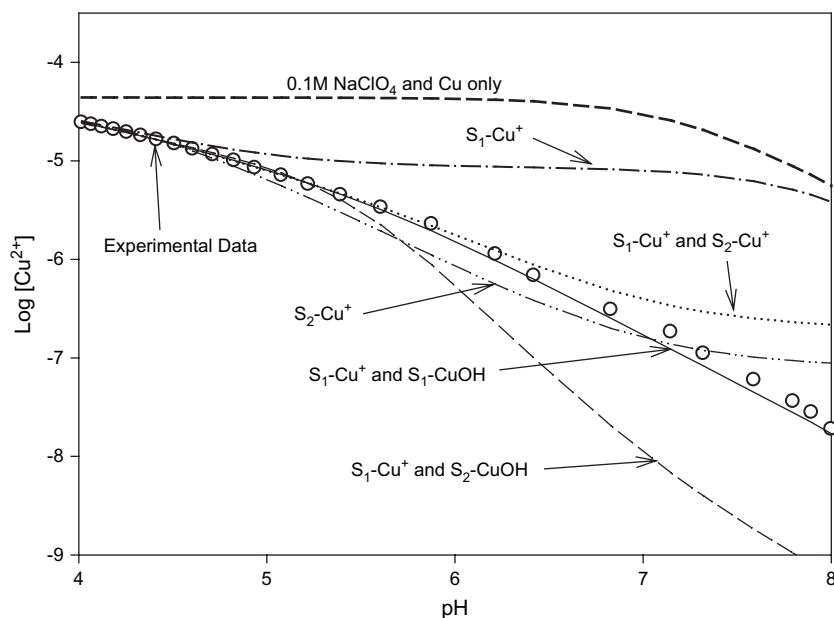
**Figure 6** Potentiometric titration data in the absence and presence of Cu (ratio R : ligand/Cu = 8) of 50–200  $\mu\text{m}$  ( $\Delta$ ,  $\diamond$ ) and 200–2000  $\mu\text{m}$  ( $\blacktriangle$ ,  $\blacklozenge$ ) sized POM extracted from soil S.

type of organic inputs had no influence on Cu sorption onto coarse POM. From these data, an example of a calculated curve using the Langmuir double surface equation (Fitch & Stevenson, 1984) is given in Figure 5b and suggests the presence of two copper-reactive sites in solution, that is strong and weak binding sites.

Titration curves of 200–2000- $\mu\text{m}$  or 50–200- $\mu\text{m}$  POM size fractions in the absence and presence of Cu are shown in Figure 6 for the S soil. The other POM samples showed a similar behaviour. The ability of the POM size fractions to complex Cu is expressed as soon as pH 4 by the proton release due to Cu-complexation. The interactions of Cu with POM during the alkali titration correspond to a competition between the metal ion and protons for complexing sites on the ligand.

The metal distribution on POM fragments has also been analysed using micro-X-ray fluorescence on impregnated undisturbed soil thin sections, in different metal contaminated arable soils (Lamy *et al.*, 2005; Labanowski *et al.*, 2007) or vineyard soils (Hinsinger *et al.*, 2005). These chemical mappings of heavy metal distribution on organic debris revealed rather heterogeneous distribution patterns for Zn and Cu on POM. These results point to surface fixation of metals and are in accordance with the findings of our work.

Results from FITEQL calculations from potentiometric data with different hypotheses of complex formation are presented in Figure 7 for NA. Fits for CC and S were similar. The observed decrease of free Cu contents between pH 4 and 8 was enhanced by the presence of POM, indicating increased adsorption of Cu



**Figure 7** Results from FITEQL 4.0 modelling of potentiometric titration data in the presence of copper ( $R = 8$ ) for one titration performed using POM 50–200  $\mu\text{m}$  extracted from NA, the non-amended soil. Results of modelling are depicted for different complexation hypotheses.

on POM surfaces with increasing pH. For all titrations between pH 4 and 8 with POM in the presence of Cu, the best fit was obtained with the hypothesis of the formation of two complexes involving the first type of site:  $S_1$ -Cu<sup>+</sup> and  $S_1$ -CuOH. Under our experimental conditions, the hypothesis that Cu complexes with the other type of site,  $S_2$ , did not produce the best fit (Figure 7). Nevertheless, Cu-complexes with  $S_2$  cannot be excluded under different experimental conditions. Indeed, in our study, only a small amount of Cu was added, thus favouring complexation by  $S_1$  type sites, the stronger sites as suggested in Figure 5b, while other ligand/Cu ratios could lead to formation of other types of complexes. Table 3 shows the apparent stability constants for Cu-complexes with the two POM size fractions, as computed by FITEQL. Whatever the size of POM or organic amendments, no significant differences were observed between the stability constants. Hence, reactivity of soil POM towards Cu seemed to be unaffected by the nature of the organic inputs and no size effect could be assessed. This result suggested that whatever the size of the POM fraction, the same kind of functional groups were metal-reactive. Consequently, the recurrently observed larger Cu content of the 50–200- $\mu$ m POM size fractions with respect to the 200–2000  $\mu$ m fraction (Table 1) is explained by the larger surface site content of the 50–200  $\mu$ m fraction (Table 2) rather than by a different Cu affinity (Table 3). To our knowledge, these results on the quantification of complexing capacities of soil POM fractions are new. When comparing the values obtained with literature data for other reactive organic fractions with well-known large Cu-affinities (e.g. for humic substances), they were found to be in the same range (Lamy *et al.*, 1988; Brown *et al.*, 1999). POM can thus be considered as playing an important role as a metal sink in soils, mainly due to its chemical acido-basic and complexing properties. One of the remaining questions, the object of future work, is the role of POM as a source for metals in soils.

## Conclusions

In this work, we showed that POM fractions extracted from soils exhibited a buffer capacity and a copper affinity that were both

**Table 3** Apparent stability constants of Cu-POM complexes computed by FITEQL (mean  $\pm$  standard error,  $n = 3$ )

	POM 50–200 $\mu$ m		POM 200–2000 $\mu$ m	
	Log $K$			
	$S_1$ -Cu <sup>+</sup>	$S_1$ -CuOH	$S_1$ -Cu <sup>+</sup>	$S_1$ -CuOH
Non-amended	4.45 $\pm$ 0.22a <sup>a</sup>	6.63 $\pm$ 0.33a	4.61 $\pm$ 0.18a	6.71 $\pm$ 0.28a
Straw	4.35 $\pm$ 0.19a	6.79 $\pm$ 0.23a	4.49 $\pm$ 0.19a	6.42 $\pm$ 0.30a
Conifer compost	4.29 $\pm$ 0.21a	6.51 $\pm$ 0.29a	4.61 $\pm$ 0.15a	6.34 $\pm$ 0.25a

<sup>a</sup>Different letters within columns indicate significant difference at  $P < 0.05$ .

quantified by potentiometric titrations. In addition, we found that:

- 1 Decreasing POM size generates functional reactive sites resulting in a larger surface site content of the 50–200  $\mu$ m POM fractions compared with the 200–2000  $\mu$ m POM fractions.
- 2 Under our experimental conditions, the buffer capacities can be linked to the presence of at least two classes of reactive sites, consistent with the presence of carboxyl, phosphate and/or phenolic groups.
- 3 The chemical signature of introducing new organic residues in the soils is expressed by changes in the buffer  $pK_{SnH}$  values only in the fine POM fractions.
- 4 Finally, copper affinities of POM fractions were independent of the size of the fractions, as well as independent of organic inputs. The values obtained were in the range of those reported for reactive soil organic fractions such as humic substances. This last result underlines the role of the metal sink function of POM in soils.

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