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Turnover of intra- and extra-aggregate organic matter at the silt-size scale

I. Virto [a,1], C. Moni [a], C. Swanston [b], C. Chenu [a,*]

[a] UMR BIOEMCO, AgroParisTech, Campus Grignon, Bâtiment EGER. 78850 Thiverval-Grignon, France
[b] Northern Research Station, 11 campus Blvd, Newton Square, PA 19073, USA

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ABSTRACT

Temperate silty soils are especially sensitive to organic matter losses associated to some agricultural management systems. Long-term preservation of organic C in these soils has been demonstrated to occur mainly in the silt- and clay-size fractions, although our knowledge about the mechanisms through which it happens remains unclear. Although organic matter in such fractions is usually assumed to be present in the form of organo–mineral complexes, a large percentage of this fraction appears to be micro-aggregated. In this study we hypothesized that slaking-resistant silt-size aggregates should be preferential sites of long-term organic matter preservation in soils, by physical entrapment that would allow for stabilization by interaction with mineral surfaces. To verify this, we studied the organic C stocks and turnover within and outside slaking-resistant aggregates in the silt-size fraction (2–50 μm) after complete dispersion of larger aggregates (>50 μm) in a wheat–maize (C3–C4) chronosequence of a silty soil in Northern France. Changes in the natural abundance of ^{13}C from C3 to C4 plants were used to assess the turnover time of organic C in the different isolated fractions. Slaking-resistant silt-size aggregates stored almost half of the soil organic C, both as entrapped silt-size light (<1.6 g cm^{-3}) organic particles (μLF) and as organic C in the mineral fraction. We found that overall, organic C stored in the silt-size fraction had longer turnover times than sand-size particulate organic matter. However, we found similar turnover rates within and outside slaking-resistant aggregates of the silt-size fraction, both for μLF and for the organic C recovered with minerals. Moreover, the apparent turnover rates of μLF (167–330 years) were slower than that of C in the clay-size fraction (100 years). We postulate that a rapid turnover rate of silt-size aggregates is needed to explain preservation of this μLF by physical entrapment. Our results also demonstrated the heterogeneity of organic matter kinetics in the silt- and clay-size fraction, which should be accounted for when modelling organic matter dynamics in silty temperate soils.

1. Introduction

Long-term storage of organic C in soils is at present a key issue in environmental research. Agricultural management often results in soil organic C losses, and silty soils in temperate areas where organic matter (OM) is the major agent of aggregate formation and stabilization are especially sensitive to this (Balesdent et al., 2000).

Three mechanisms have been described through which OM can be preserved in soils: chemical recalcitrance, physical inaccessibility to microorganisms and their enzymes, and/or interaction with the mineral surfaces (Lützow et al., 2006). The two latter mechanisms are dependent on the presence of multivalent cations, the presence of mineral surfaces capable of adsorbing organic materials and the architecture of the soil matrix (Baldock and Skjemstad, 2000). The role of soil architecture (structure) in this process has been defined by several authors (Tisdall and Oades, 1982; Beare et al., 1994; Golchin et al., 1994; Angers and Chenu, 1997; Six et al., 1999, 2004). In temperate soils, where OM is the major agent of aggregate formation and stabilization, it is presently understood as a dynamic cycle in which aggregates protect OM from degradation. At the same time, OM contributes to the stabilization of soil aggregates (Balesdent et al., 2000). Decomposition proceeds at a slower pace within aggregates (Puget et al., 2000), due to physical protection, and this would allow for adsorption of a part of the metabolites from OM decomposition to the smallest mineral particles. Decomposition within aggregates eventually leads to a decrease of the size of organic particles, and to the concentration of the more resistant plant structural materials in the oldest occluded organic particles in the soil (Golchin et al., 1994; Baldock et al., 1997). According to Six et al. (2004), very stable microaggregates and clay- and silt-size organo–mineral complexes are finally released from larger aggregates and incorporated into newly formed macro-aggregates, closing the cycle. According to this model, stable silt and clay-size aggregates would contain the oldest and most processed organic particles. Also, mineral particles within them would be especially enriched in old organic matter.

Indirect evidence of highly stable aggregates within the silt-size fraction of different soils has been provided by different authors using

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[*] Corresponding author. Tel.: +33 1 30 81 52 16; fax: +33 1 30 81 54 97.
E-mail address: Claire.Chenu@grignon.inra.fr (C. Chenu).

[1] Present address: Departamento Ciencias Medio Natural, ETSIA, Universidad Pública de Navarra, Campus Arrosadia, 31006 Pamplona, Spain.

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incomplete dispersion of soil samples. Skjemstad et al. (1993) demonstrated that a considerable proportion of the OM in clay and silt fractions of some Australian soils was resistant to photo-oxidation and was thus likely to be protected within highly stable microaggregates. Shang and Tiessen (1998) observed that an important amount of uncomplexed OM (*sensu* Gregorich et al., 2006) was liberated when the silt-size fraction of intermediate density (2.0 to 2.4 g cm^{-3}) obtained after mild dispersion of two tropical soils was further dispersed. Roscoe et al. (2000) observed that progressive ultrasonic dispersion of a Latosol persistently rendered large amounts of organic C in the highly stable silt-sized fraction. They suggested that this fraction must be composed of microaggregates strongly stabilized by OM. Balabane and Plante (2004) used also progressive dispersion of soil samples to demonstrate that an amount of silt-sized aggregates should exist within the silt-size fraction obtained after weak and moderate dispersion. This is in line with Gerzabek et al. (2001), who deduced that the silt-size fraction isolated after gentle fractionation of an agricultural soil receiving organic amendments was likely to contain microaggregates. This fraction was also observed to contain most of the recently added organic C. Finally, Plante et al. (2006) also observed that silt-size fractions isolated after mild dispersion were the most responsive ones to changes in organic C inputs in a long-term experiment in Alberta (Canada). They concluded that this fraction is important for the long-term stabilization of OM through both physical occlusion in microaggregates and protection by interaction with minerals.

Highly stable silt-size aggregates seem then to be important sites for OM stabilization in soils. However, very few studies have been specifically devoted to examine OM stabilization at the silt-size scale or within silt-size aggregates in the mid (decadal) term. In a recent study, Virto et al. (2008) proposed a new methodology which allowed for an optimal recovery of slaking-resistant silt-size aggregates in a cultivated silty temperate soil. They found that silt-size aggregates recovered after complete dispersion of the fraction >50 μm stored half of the total soil organic C. As previously found by Chenu and Plante (2006) for clay-size aggregates, OM within silt-size aggregates was found to exist both in the form of uncomplexed light particulate OM and OM associated to the mineral fractions.

In this work we hypothesized that OM within slaking-resistant silt-size (2–50 μm) aggregates of silty temperate soils should have a longer turnover time than OM stored elsewhere in the soil. Our objective was to gain knowledge on the protection of OM at the silt-size scale in a silty soil after nine years of conversion from C3 to C4 crops, by (i) comparing the mean residence time of organic C in slaking-resistant silt-size aggregates to that of organic C in other soil fractions, and (ii) determining whether the OM stored within such aggregates has different renewal kinetics depending on its location (i.e. in the clay-size fraction, in entrapped particulate OM or entrapped silt particles). That for, we applied the new methodology for slaking-resistant silt-size aggregates isolation to samples from a wheat/maize chronosequence, and used ^{13}C natural abundance to estimate the turnover and mean residence time of C in the different soil fractions.

2. Materials and methods

2.1. Soil and sampling

We studied the soil from the Closeaux Field Experiment, at the INRA Experimental Station in Versailles, Île de France, France. The field has been under C3-type crops since about 1930. A wheat (C3) monoculture was established in the late 1980s and in 1994 a chronosequence was created, by converting several plots to maize (C4 plant) each year until 2001. This allows for *in situ* ^{13}C labeling of organic C inputs since the only difference between plots is the origin of the plant-derived organic C entering the soil (Rasse et al., 2006). The soil is conventionally tilled (moldboard plough down to 30 cm) annually in all plots. We sampled the topsoil (0–25 cm) of the control plots under wheat, and the plots with 3 and 9 years of continuous maize cropping in April 2002. For each treatment (wheat, 3 years maize and 9 years maize), three replicate plots were sampled. Three sub-samples were collected per plot (wheat or maize), and pooled to produce a composite sample. Clods from field-moist samples were broken apart, and gently crushed to pass a 5 mm-openings mesh, allowed to air-dry and stored in a dry place at room temperature.

The soil has been described as an Eutric Cambisol in the FAO Reference Base (Rasse et al., 2006), which corresponds to a Eutrochrept in Soil Taxonomy (Soil Survey Staff (S.S.S), 2003) or as a neo Luvisol (FAO), (F.van Oort, oral communication). The upper horizon (Ap) contains 1.3% of organic C in average, and has a pH of 6.8. It contains 32% sand, 51% silts and 17% clay (Balabane and Plante, 2004).

2.2. Soil fractionation

We used the combined physical and density fractionation protocol described in detail in Virto et al. (2008). The summarized flow-chart for this fractionation is shown in Fig. 1. It comprises three consecutive steps: (1) dispersion of the whole soil sample and recovery of the completely dispersed sand-size fraction, non-aggregated clays and the silt-size fraction containing slaking-resistant silt-size aggregates, (2) density fractionation of the silt-size fraction to recover slaking-resistant aggregates, and (3) dispersion of such aggregates and separation of their constituents. For homogeneity in the terminology and for analogy with Virto et al. (2008) we denote as *occluded* the fractions obtained from the dispersion of silt-size aggregates (clay-size particles, light OM and silt-size mineral particles), and as *non-occluded* those not forming part of such aggregates.

Briefly, in the first step fifteen g of soil (see sampling section) were placed in a 30-mL plastic flask with five glass beads (Ø = 5 mm), and the flask was stopped with a 50-micron-opening nylon cloth (Gilson Co. Inc., Columbus, OH). This flask was introduced into a 250-mL bottle to which 200 mL of deionized water were added. The whole was shaken for 4 h in a vertical orbital shaker so that all particles <50 μm were allowed to pass through the nylon cloth as they were released from aggregates. Slaking-resistant silt-size aggregates were thus no longer subjected to the dispersing effect of glass beads and abrasive sand grains once out from the flask. The sand-size fraction (>50 μm) remaining in the flask, containing sand minerals and particulate OM>50 μm (POM) was recovered, oven-dried (105 ºC), weighed and ground to powder, and the fraction <50 μm was further separated into non-occluded clay-size (<2 μm), fine silt (2–20 μm) and coarse silt (20–50 μm) fractions by sedimentation and sieving. Non-occluded clay-size particles were freeze-dried and ground to powder. Aliquots of the fine and coarse silt-size fractions were oven-dried (105 ºC) and ground to powder for chemical analysis of the whole fractions before further fractionation.

In step 2, the silt-size fractions (2–20 and 20–50 μm) were density fractionated, in a sequential protocol with density limits set at 1.6 g cm^{-3} and 2.5 g cm^{-3}, which rendered three differentiated fractions: a light fraction (ρ < 1.6 g cm^{-3}) consisting mainly of non-occluded uncomplexed light organic particles (denoted as non-occluded μLF), a heavy fraction (ρ > 2.5 g cm^{-3}) containing mostly non-occluded silt-size minerals, and a fraction of intermediate density (1.6<ρ<2.5 g cm^{-3}) that has been observed to be composed by slaking-resistant aggregates (Virto et al., 2008). Density limits were set assuming that the proportion of organic vs. mineral components of the separates decreases with increasing density (Chenu and Plante, 2006). Density fractionation was accomplished using solutions of sodium polytungstate (SPT, $Na_6(H_2O_{40}W_{12})$) at different concentrations. Silt-size aggregates and non-occluded silts were oven-dried (105 °C) and weighed. The non-occluded μLF was rinsed on a 0.1-μm pore-size nitrocellulose filter using ~800 mL of deionized water under vacuum suction, freeze-dried and weighed.

In step 3, slaking-resistant aggregates of the fine (2–20 μm) and coarse (20–50 μm) silt-size fractions from the control (wheat) and final

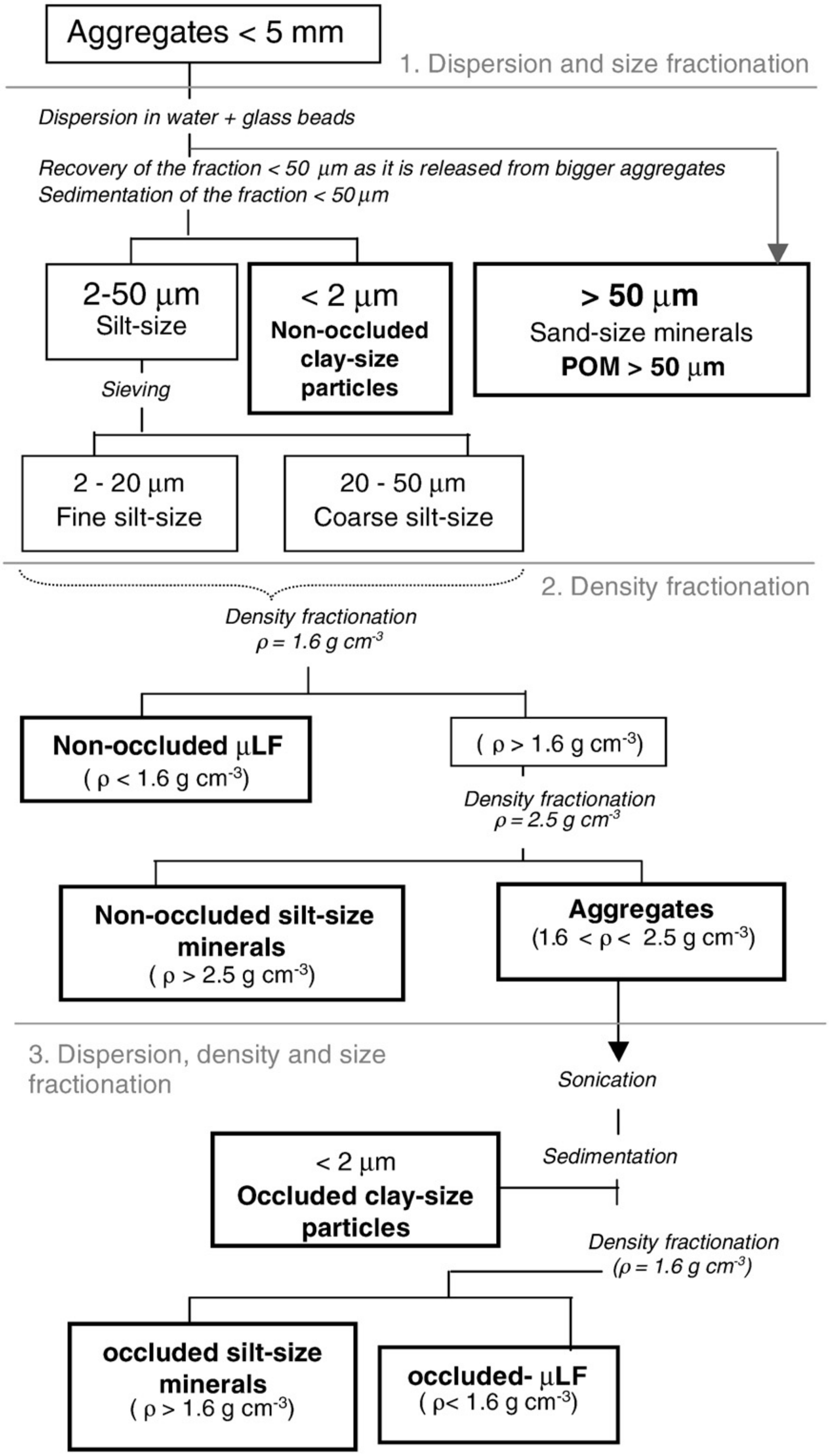

Fig. 1. Flow-chart of the fractionation protocol.

(9 years under maize) plots were further dispersed using ultrasonication. One-g of coarse and fine silt-size slaking-resistant aggregates were re-moistened using 20 mL of deionized water, vigorously shaken (vortex-type shaker, 3 min) and transferred to a glass beaker into which 30 mL of deionized water were added. Samples were then dispersed by means of an ultrasonic probe (Vibra-cell^{TM} 75041. Bioblock Scientific. Illkirch, France) set at 100% of 750 W and submerged in the solution for 10 min, equivalent to an input of 950 J mL^{-1}.An ice bath was used to avoid temperature rising and to avoid reduction in cavitation during sonication (Roscoe et al., 2000). The suspension was then poured into 100 mL cylinders, shaken to achieve homogeneous distribution of the dispersed material, and the clay-size (<2 µm) fraction obtained from silt-size aggregates (occluded clays) was separated through sedimentation, flocculated in $SrCl_2$ 10^{-3} M, recovered after centrifugation, freeze-dried, weighed and ground to powder. We assumed the organic material present in this fraction as mainly composed by organic C adsorbed onto clay-size minerals. Some very fine organic particles and charcoal particles might also have been recovered in this fraction.

The material retained in the cylinders was divided into 2–20 µm and 20–50 µm fractions by sieving through a 20-µm-openings steel sieve and both fractions were subjected to density fractionation (ρ 1.6 g cm^{-3}) in order to separate the light organic particles occluded within silt-size aggregates (occluded µLF) from the silt-size mineral phase within such aggregates (occluded silt-size minerals). Finally, both the µLF and the occluded silt-size minerals were freeze-dried, weighed and ground to powder.

2.3. Organic C content, C isotope analysis and turnover

Total C and N in whole soil samples and in each particle-size and density fraction were determined by dry combustion on an elemental autoanalyzer (NA 1500-NCS, Carlo Erba, Milano, Italy) coupled in continuous flux to an isotope ratio mass spectrometer for isotopic analysis. Results of the isotopic analyses are expressed in δ^{13}C against the PBD international standard, and were calculated as in Dignac et al. (2005). The small recovered amounts of non-occluded 2–20 µm µLF did not allow for C and N analyses.

The equation proposed by Balesdent and Mariotti (1996) was used for the conversion of the δ^{13}C values into proportions (f) of maize-derived soil organic C in the different studied fractions:

$$f = \frac{\delta_{\text{sample}} - \delta_{\text{control}}}{\Delta}, \qquad (1)$$

where δ_{sample} is the δ^{13}C of the studied soil sample or fraction, δ_{control} is the δ^{13}C of the corresponding fraction in the control plots (under wheat, C3 plant), and Δ is the difference in the δ^{13}C between maize plant material and wheat plant material. This equation is unbiased even if some ^{13}C enrichment occurs along with OM decomposition, if the two compared soils or plots have similar C dynamics (Balesdent, 1996).

To estimate de value of Δ, δ^{13}C values of maize and wheat tissues given by Dignac et al. (2005) for the same field trial were used. These values were −12.8‰, −12.5‰ and −11.8‰ in maize leaves, stems and roots, respectively, and −28.5‰, −27.6‰ and −28.4‰ in the respective parts of wheat plants. A weighed average was calculated according to the different contribution of different plant organs to the total plant biomass returned to the soil in the Ap horizon of the same experimental field, as given by Bahri et al. (2006).

The three analyzed field replicates at 3 and 9 years after maize cropping showed some heterogeneity in their natural ^{13}C abundance in the studied fractions. Values of f were calculated for each replicate independently in relation to the average value of δ^{13}C in the control plots (under wheat) for each fraction (Rasse et al., 2006).

We verified that the organic C content in the studied fractions was at steady-state (Fig. 2), which means that the rate of decay of old C equals the rate of incorporation of new C in all plots. We assumed single exponential kinetics for the incorporation of maize-derived C into the different isolated fractions to estimate a time constant, k, as described by Balesdent (1996). He stated that the incorporation of maize-derived C into the 0–50 µm size fraction in similar Cambisols in Île de France can be fitted to a linear regression curve for modeling purposes. In our case, using a linear or exponential regression did not affect the values of k in the silt-size and clay-size fractions, due to the slow incorporation of new C into these fractions (Balesdent, 1996). Since comparisons were to be made also with larger size fractions, where the incorporation kinetics of organic C have been seen to be exponential, we calculated the values of $1-f$ (remaining wheat-derived C) obtained for the three replicates of the different soil fractions after 3 and/or 9 years of maize cultivation and fitted them to an exponential decay equation for all of our fractions:

$$(1-f) = e^{-kt}, \qquad (2)$$

where t is time under maize cultivation. The mean apparent residence time of C in each fraction was then estimated using $T = 1/k$.

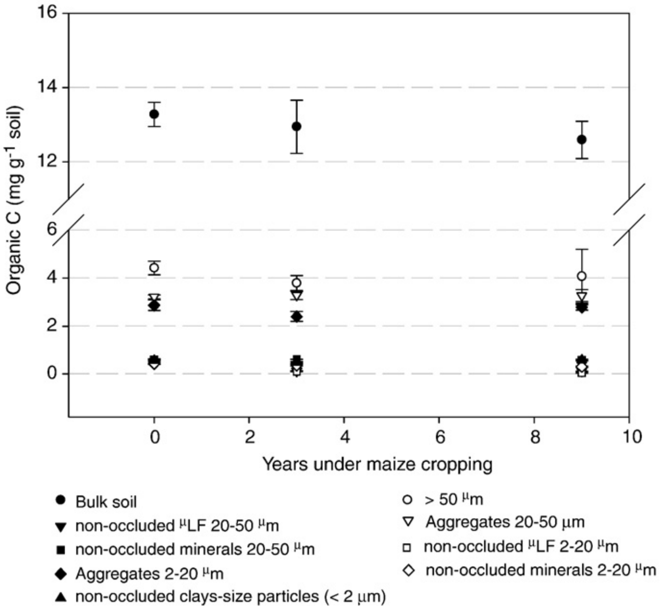

Fig. 2. Variation of organic C content in bulk soil and size and density fractions among the plots of the experimental field. Mean ± standard errors ($n = 3$ for years 0, 3 and 9 in the wheat/maize chronosequence). >50 µm: fraction >50 µm (completely dispersed).

2.4. Statistical analysis

Data are presented as mean values with standard errors. Analysis of variance (ANOVA) was used to determine differences between fractions using the univariate linear model of SPSS 14.0 (SPSS Inc., 2005. Chicago, IL, USA). Duncan's test was used to identify homogeneous groups. The 2-tailed T-test command of the same package for paired samples was used to determine differences in the values of f in time for each fraction. f data after 3 and 9 years of maize cropping were fitted to the exponential Eq. (2) by the Marquardt–Levenberg algorithm using the SigmaPlot 8.0 software package (Systat Software, 2002. Point Richmond, CA, USA). A level of significance of $P<0.05$ was considered.

3. Results

3.1. Fractionation and organic matter distribution and losses

The duration of maize cropping after wheat did not modify the total C content of the studied soil layer (0–25 cm) or that of the studied fractions (Fig. 2). This means that the change in crop did not cause major changes in the C content of any of the studied fractions in the arable layer of this conventionally-tilled soil.

The fractionation and dispersion protocol allowed for the quantification of the mass of slaking-resistant silt-size aggregates and the amount of organic C stored in the form of POM, µLF, and in the silt-size minerals and clay-size fraction (Tables 1 and 2).

The mass recovery after steps 1 and 2 was satisfactory (97.5 ± 0.6%, Table 1), while 9.60 ± 2.8% of the initial C content was lost during these two steps (Table 1). Some amounts of the slaking-resistant silt-size aggregates mass and organic C were also lost after aggregate dispersion and density fractionation (step 3, Table 2). Carbon losses are usual when concentrated solutions of SPT are used due, in part, to solubilization of Na-saturated humic substances (Sollins et al., 2006; Chenu and Plante, 2006). The dissolved fraction does not likely represent a proportional part of all the studied fractions. Crow et al. (2007) observed that a light isotope fraction was preferentially mobilized in the SPT solutions during density fractionation of two forest soils. We used the isotopic values of the fraction 20–50 µm before density separation and those recalculated

Table 1
Mass and C balance after fractionation steps 1 and 2 (see Fig. 1).

Size class (μm)	Non-occluded μLF	Slaking-resistant silt-size aggregates	Non-occluded minerals	Total	
	$\rho<1.6$ g cm^{-3}	$1.6<\rho<2.5$ g cm^{-3}	$\rho>2.5$ g cm^{-3}		
	Mass of fractions (g 100 g^{-1} soil)				
					PSD[a]
>50				33.7 ± 1.3	31.85
20–50	0.08 ± 0.02	8.93 ± 0.53	32.4 ± 0.6	41.5 ± 0.9	27.4 ± 1.8
2–20	–	7.21 ± 0.36	13.4 ± 0.6	20.7 ± 0.7	22.2 ± 0.7
<2 (non-occluded)				1.66 ± 0.08	16.9 ± 0.7
Total, calculated				97.5 ± 0.6	97.5 ± 0.07
	C in fractions (mg C g^{-1} and % of total SOC (*in brackets*))				
Bulk soil				12.9 ± 0.3	
>50 (POM)				12.3 ± 1.3 (*31.6 ± 2.8*)	
20–50	171.1 ± 46.4 (*0.72 ± 0.11*)	36.8 ± 1.9 (*25.1 ± 0.6*)	1.82 ± 0.02 (*4.60 ± 0.15*)	9.96 ± 0.18 (*30.3 ± 0.8*)	
2–20	–	37.9 ± 1.3 (*20.6 ± 0.9*)	2.79 ± 0.11 (*2.80 ± 0.19*)	16.6 ± 0.6 (*24.3 ± 0.8*)	
<2 (non-occluded)				32.5 ± 0.6 (*4.15 ± 0.18*)	
Total, calculated				11.7 ± 0.4 (*90.4 ± 2.8*)	
	C-to-N ratio in fractions				
Bulk soil				10.7 ± 0.2	
>50				19.1 ± 1.5	
20–50	14.8 ± 2.3	9.77 ± 0.11	7.49 ± 0.15	8.97 ± 0.42	
2–20	–	8.95 ± 0.10	7.24 ± 0.15	8.80 ± 0.09	
<2 (non-occluded)				7.32 ± 0.04	

Average ± standard error ($n=9$).

[a] Particle-size distribution after complete dispersion as in Balabane and Plante, 2004.

from the density separates to evaluate this effect. We did not observe significant differences between the calculated and the actual isotopic balance in this fraction (data not shown).

Detachment of organic materials from organo–mineral complexes as a result of sonication has also been pointed out as a possible cause for C losses in fractionation protocols involving sonication (Schmidt et al., 1999). Part of the C losses after slaking-resistant silt-size aggregates dispersion might have this origin, although recent research indicates that disrupting energies of up to 1500 J mL^{-1} have only minor effects on the SOC content of the clay fraction (Yang et al., 2009).

The silt-size fraction as a whole represented 62.2 ± 1.1% of the soil mass and 54.6 ± 1.12% of the total soil C (Table 1). Within this fraction, slaking-resistant aggregates represented 16.1 ± 0.8% of the total soil mass, and stored 45.7 ± 1.1% of the total soil organic C (Table 1). This C was mainly associated to the occluded clay-size particles (Table 2), which contained 59% and 69% of the total organic C in coarse and fine silt-size slaking-resistant aggregates, respectively. Occluded clay-size particles were also richer in C than non-occluded clay-size particles (Tables 1 and 2). Also a significant amount of C was found in the occluded μLF (23% and 13% in coarse and fine silt-size slaking-resistant aggregates, respectively), and associated to occluded minerals 2–20 μm in size (18% and 17%, respectively, Table 2). Organic C stored in the occluded μLF was 5 and 7.5 times that in the non-occluded μLF in the fine silt and coarse silt-size fractions, respectively.

Non-occluded clay-size particles (1.66 ± 0.08% of the soil mass) stored 4.15 ± 0.18% of the soil C (Table 1).

The C-to-N ratio of the bulk soil sample was of 10.7 ± 0.2 (Table 1). C-to-N ratios of the separates decreased in the order POM (>50 μm) > μLF > silt-size aggregates ≈ occluded clay-size particles > non-occluded silt-size minerals ≈ non-occluded clay-size particles. No differences were observed between the occluded μLFs and their non-occluded counterparts (Tables 1 and 2).

3.2. Incorporation of maize-derived C in the separates

Fig. 3 shows the concentration of maize-derived C in the separates of steps 1 and 2 of the fractionation protocol. After 9 years of

Table 2
Mass distribution, C concentrations, C balance and C-to-N ratio of clays, μLF and silt-size minerals occluded within fine (2–20 μm) and coarse (20–50 μm) slaking-resistant silt-size aggregates.

Size class (μm)		Slaking-resistant aggregates 2–20 μm				Slaking-resistant aggregates 20–50 μm			
		mg g^{-1} aggregate	mg C g^{-1}	mg C g^{-1} aggregate	C/N	mg g^{-1} aggregate	mg C g^{-1}	mg C g^{-1} aggregate	C/N
<2 μm (occluded clay-size)		615 ± 38	41.7 ± 2.0	26.0 ± 2.8	8.2 ± 0.2	450 ± 40	42.3 ± 1.6	19.2 ± 2.1	8.4 ± 0.2
2–20 μm	Occluded μLF	16.8 ± 1.4	292.2 ± 22.5	4.82 ± 0.4	14.2 ± 0.2	19.6 ± 1.3	240.3 ± 24.9	5.96 ± 0.6	13.8 ± 0.2
	Occluded minerals	280 ± 38	24.7 ± 2.9	6.86 ± 1.3	10.4 ± 0.3	308 ± 11	17.6 ± 2.5	5.46 ± 0.9	11.5 ± 0.3
20 –50 μm	Occluded μLF					8.8 ± 1.6	253.6 ± 40.7	1.66 ± 0.3	16.6 ± 1.5
	Occluded minerals					134 ± 29	3.1 ± 0.4	0.38 ± 0.1	16.9 ± 1.2
Total, recovered		912 ± 5		37.6 ± 2.3		914 ± 8		32.7 ± 2.1	
Total, original				38.4 ± 1.6				37.5 ± 2.8	

Average ± standard error ($n=6$).

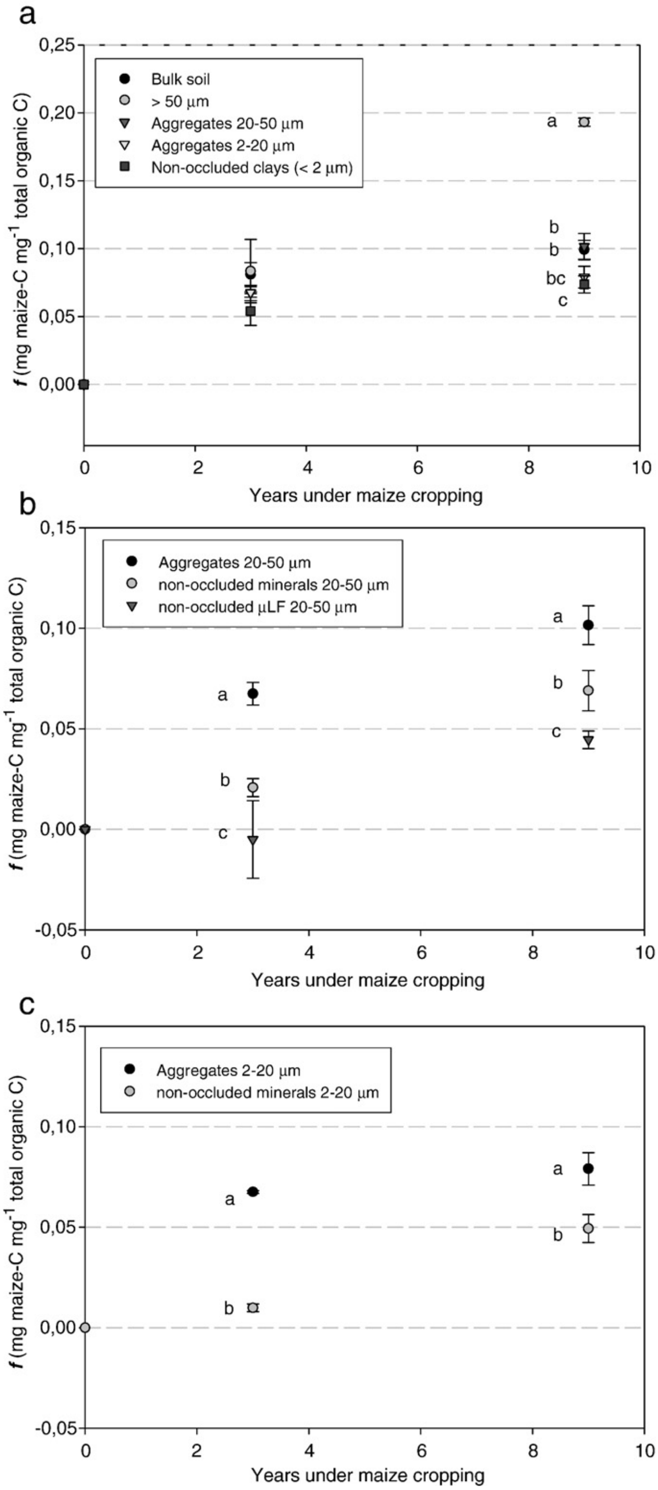

Fig. 3. Concentrations of maize-derived C (***f***) in the separates after complete dispersion of aggregates >50 µm. (a) Bulk soil, fraction >50 µm, non-occluded clay-size particles and stable aggregates 2–20 and 20–50 µm in size; (b) Aggregates, non-occluded minerals and non-occluded µLF in the fraction 20–50 mm; (c) Aggregates, non-occluded minerals and non-occluded µLF in the fraction 2–20 µm. Average ± standard errors ($n = 3$ per time point). Different letters indicated different homogeneous Duncan's groups at each time point ($P < 0.05$).

continuous maize cropping, the average enrichment in C from maize for the bulk soil was of 9.9 ± 0.7% (Fig. 3a), similar to that found by other researchers in the same soil (Dignac et al., 2005; Rasse et al., 2006; Bahri et al., 2006).

However, as maize residues were incorporated, C from maize increased at a different pace among fractions obtained from the dispersion of aggregates >50 µm and the density separation of the silt-size fraction (Fig. 3a). While in the fraction >50 µm, C from maize accounted for 19.3 ± 0.3% of the total C after 9 years, no differences were observed in the amount of C from maize incorporated into coarse and fine silt-size slaking-resistant aggregates and the average bulk soil, both after 3 and 9 years of maize cultivation, and the proportion of C from maize in the non-occluded clays was only slightly smaller after 9 years (7.4 ± 0.6% for 9.92 ± 0.7% for the bulk soil).

Overall, the calculated amount of maize-derived C in the fraction <50 µm (0.56 mg maize-C g^{-1} soil) was very similar to the one reported by Derrien et al. (2006) for the same soil (0.50 mg maize-C g^{-1} soil).

Within the silt-size fraction, the incorporation pace of C from maize was significantly faster in aggregates than in the non-occluded µLF and the non-occluded minerals, both at the coarse and fine silt-size scale (Fig. 3b and c).

Fractions obtained from the dispersion of slaking-resistant silt-size aggregates after 9 years under maize had different *f* values among them (Fig. 4a and b). These differences followed the same pattern both in coarse and fine silt-size aggregates. In both cases, occluded clay-size particles had *f* values similar to those corresponding to whole aggregates. The occluded µLF and silt-size minerals had smaller values of *f*, which were not significantly different among them in the coarse

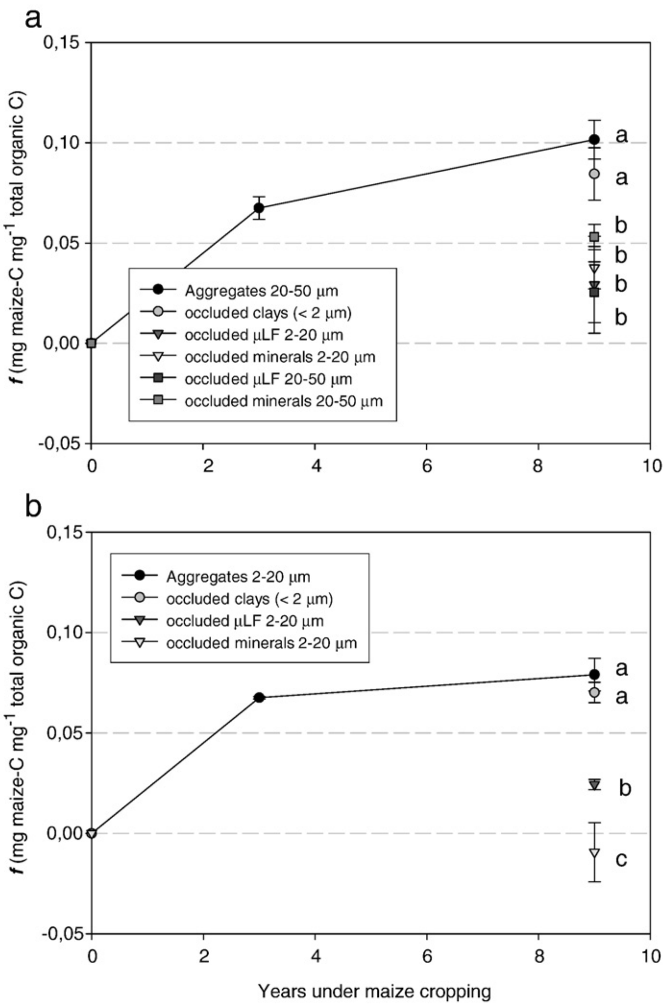

Fig. 4. Concentrations of maize-derived C (***f***) within aggregates (a) 20–50 µm, and (b) 2–20 µm. Average ± standard errors ($n = 3$ per time point). Different letters indicate different homogeneous Duncan's groups at each time point ($P < 0.05$).

silt-size fraction (Fig. 4a). The amount of C derived from maize incorporated in the fine silt-size (2–20 µm) occluded minerals within 2–20 µm slaking-resistant aggregates was not significantly different from zero.

Fig. 5 shows *f* data for clay-size particles and µLF found within and outside slaking-resistant silt-size aggregates, for comparison of each occluded fraction with their non-occluded counterparts. No differences were observed in the percentage of maize-derived C between occluded and non-occluded clay-size particles and between the occluded and non-occluded µLF (Fig. 5a and b).

The values of *k* as estimated from Eq. (2), and their corresponding mean residence time of C (*T*) in the isolated fractions are summarized in Table 3. The fastest apparent turnover time corresponded to organic C in the fraction >50 µm (42 years), and the slowest one to silt-size minerals and the µLF (125–333 years), irrespective of their location within or outside aggregates (i.e. occluded or non-occluded). Overall organic C within silt-size aggregates had turnover times similar to that of non-occluded and occluded clay-size particles (77–100 years), and close to that of bulk soil (77 years).

To evaluate the accuracy of our estimations, we compared the observed values of *k* estimated from the *f* values of the whole 20–50 µm fraction and aggregates 20–50 µm in size before fractionation (0.011 ± 0.002 and 0.013 ± 0.002, respectively) to the calculated values of *k* of these fractions using data of their constituents shown in Tables 1–3 (0.010 and 0.015, respectively).

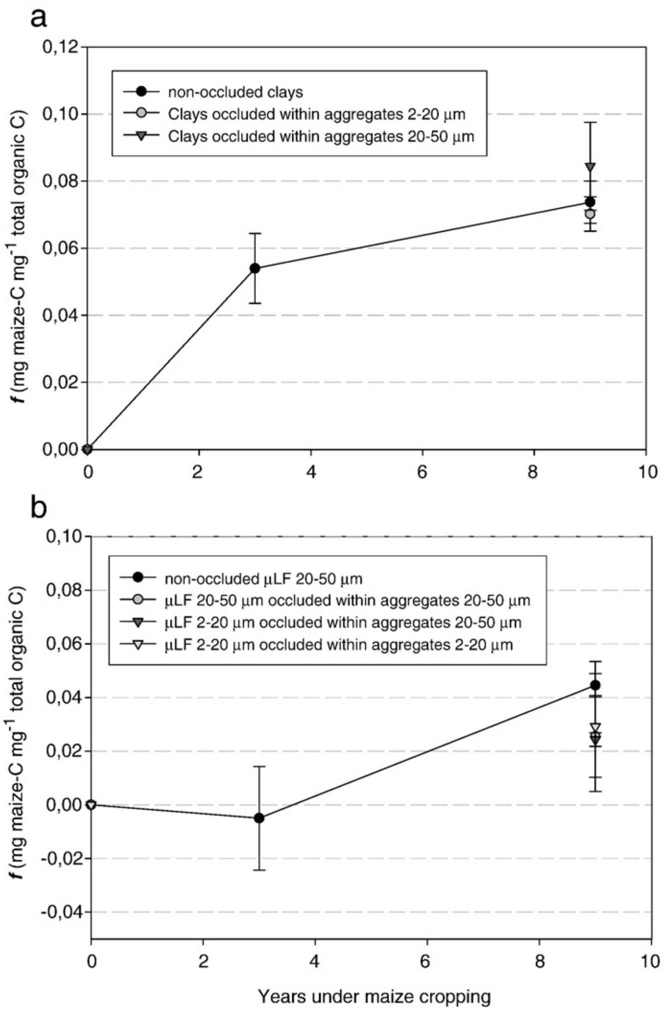

Fig. 5. Concentrations of maize-derived C (***f***) in (a) occluded vs. non-occluded clay-size particles, and (b) the µLF. Average ± standard errors ($n = 3$ per time point).

Table 3
Constant, *k*, of the incorporation of C from maize in the separated fractions and $T = 1/k$, as an estimate of the mean residence time of C.

Soil fractions (µm)	k ($f = 1 - e^{-kt}$)	T (y)
Bulk soil	0.013 ± 0.003	77
>50 (POM)	0.024 ± 0.002	42
20–50 (total)	0.011 ± 0.002	91
20–50 non-occluded µLF	0.004 ± 0.002	250
20–50 aggregates	0.013 ± 0.002	77
<2 (occluded clay-size in 20–50 µm aggregates)	0.010 ± 0.001	100
occluded µLF 2–20 in 20–50 µm aggregates	0.006 ± 0.002	167
Occluded minerals 2–20 in 20–50 µm aggregates	0.004 ± 0.001	250
Occluded µLF 20–50 in 20–50 µm aggregates	0.003 ± 0.001	333
Occluded minerals 20–50 in 20–50 µm aggregates	0.005 ± 0.001	200
20–50 non-occluded minerals	0.008 ± 0.001	125
2–20 (total)	0.010 ± 0.003	100
2–20 non-occluded µLF	N.A.	
2–20 aggregates	0.010 ± 0.003	100
<2 (occluded clay-size in 2–20 µm aggregates)	0.008 ± 0.001	125
Occluded µLF 2–20 in 2–20 µm aggregates	0.003 ± 0.001	333
Occluded minerals 2–20 in 2–20 µm aggregates	–	
2–20 non-occluded minerals	0.005 ± 0.001	200
<2 (non-occluded clay-size particles)	0.010 ± 0.002	100

Average ± standard error.
Values of *k* for bulk soil, organic matter >50 µm, non-occluded µLF, silt-size aggregates and non-occluded silts and clays estimated from *f* data after 3 and 9 years of continuous maize cropping. Values of *k* for fractions within slaking-resistant silt-size aggregates (occluded µLF, minerals and clays) estimated from *f* data after 9 years.

4. Discussion

By using the described fractionation protocol, we found almost half of the soil organic C stored within slaking-resistant silt-size aggregates, which represented only 16% of the soil mass (Table 1). This demonstrates their importance as reservoirs of soil OM in the studied soil.

Dispersion of these aggregates confirmed that organic C was present within them both in the form light uncomplexed organic particles (µLF) and in clay-size particles. Organic matter present in the silt-size mineral fraction could be due to direct absorption of OM onto silt-size minerals (Morras, 1995), or to undispersed clay coatings around silt-size mineral particles, as observed by Virto et al. (2008).

4.1. Mean residence time of organic C within silt-size aggregates

We had hypothesized silt-size aggregates as sites of long-term OM stabilization, because of their ability to store the oldest and most processed organic particles certain soils (Golchin et al., 1994). We found indeed longer turnover rates of C within slaking-resistant silt-size aggregates in comparison to POM in the fraction >50 µm (Table 3) which is in line with present knowledge (e.g., Jastrow et al., 1996). Puget et al. (1995) and Puget et al. (2000) already found younger OM in slaking-resistant macroaggregates than in microaggregates in different silty soils in France. Similarly, Monreal et al. (1997) found mean residence times of 275 years in the OM associated to aggregates <50 µm in a Canadian Gleysol, for 14 and 61 years for OM in aggregates >250 µm and 50–250 µm in size, respectively. Our results indicate that such aggregates are likely formed in part by slaking-resistant silt-size aggregates bound together with or by younger particulate organic matter.

However, our results were not consistent with the hypothesis of a preferential preservation of OM within silt-size aggregates, in two senses. First, we found similar apparent turnover times of organic C in silt-size aggregates, non-occluded clay-size particles and the bulk soil (Fig. 3a). Second, differences were observed in the incorporation kinetics of new organic C in the µLF and clay-size particles: C from maize was less abundant in the µLF than in clay-size particles, and this regardless of the location of µLF or clay-size particles within or outside slaking-resistant silt-size aggregates (Figs. 3a, 4a and b). As a result, the two types of OM contained within such aggregates had different apparent turnover times.

The absence of differences in the mean residence time of C between silt-size aggregates and non-occluded clay-size particles can be explained if we consider that most of the organic C present within aggregates was associated to occluded clay-size particles (Table 2), and that no differences were found in the turnover time of C in the occluded and in the non-occluded clay-size particles (Table 3 and Fig. 5a).

However, if slaking-resistant silt-size aggregates are conceived as structures created around organic cores that would be preserved from biotic degradation and would become less biodegradable with time, one would expect longer turnover times in the occluded µLF and clay-size particles than in the less protected non-occluded µLF and clays, which was not the case in the studied soil.

4.2. Dynamics of organic matter at the silt-size scale

Our data showed that the location of µLF and clay-size particles (within or outside aggregates) in the silt-size fraction did not affect the kinetics of the renewal of organic C associated to these fractions (Fig. 4a and b).

Considering the µLF, the two known mechanisms that can account for its preservation are chemical resistance to biodegradation and physical protection within aggregates (Lützow et al., 2006).

Biochemical recalcitrance may well account for the absence of differences between occluded and non-occluded µLF. For instance, a study on Cu accumulation in the topsoil (0–10 cm) of four calcaric cambisols in vineyards in NE France (Besnard et al., 2001) noted that smaller organic particles accumulated more Cu than bigger ones. This might also be the case of other metal ions present in the soil. Preferential accumulation of charcoal in this fraction, as described by Roscoe et al. (2001) in the LF of a Brazilian Oxisol, could also explain this uniformity in the age of µLF. Further chemical analyses on this fraction would be needed to verify this hypothesis, especially since no differences were observed either in the C-to-N ratio of occluded vs. non-occluded µLF (Tables 1 and 2).

However, the fact of having found most of the µLF occluded within silt-size aggregates in our soil supports the hypothesis that aggregation may play a protective role for this OM. In that case, only a fast turnover of silt-size aggregates would explain the similar turnover rates of µLF and organic C in clays within and outside aggregates.

Aggregate turnover rates at this scale cannot be equated to those of the OM isolated from them, because the different pools of organic C within silt-size aggregates were observed to have different turnover rates, and are likely to have different origins (John et al., 2005). The very few studies that have been developed to model aggregate dynamics in agricultural soils without using OM as a proxy for aggregate turnover agree in assigning microaggregates turnover rates in the daily scale. Plante et al. (2002) used Dy-labelled ceramic spheres to determine the turnover time of macroaggregates of different sizes after two cropping seasons in two loam soils in Canada. They estimated mean residence times of 38–95 days for aggregates <1 mm. In a later study (De Gryze et al., 2006), rare-earth oxides were used as tracers to study microaggregate (53–250 µm) turnover times on incubated reconstituted soil samples from a silt-loam Luvisol in Belgium. This allowed for quantifying the rates of both aggregate break-down and re-formation, which take place simultaneously. By doing so, they determined turnover times of 88 days.

In our soil, the fact of having found maize-derived particles within silt-size aggregates only 9 years after conversion to maize cropping indicates that these aggregates can be formed and entrap uncomplexed OM in a relatively short period of time (less than 9 years), and are thus active agents in the soil OM cycle.

We found that OM renewal in the clay-size fraction was relatively fast compared to that in the µLF (Table 3 and Fig. 4a and 4b). The average turnover times found by Derrien et al. (2006) for the silt + clay fraction as a whole (91 years), and for soil glucose (99 years) and total sugars (101 years) stored in the sil + clay fraction of the same soil were similar to our data for the clay fraction. Puget et al. (2000) found an apparent turnover time of 75 years in the silt + clay fraction of two silty cultivated French soils. This confirms a different nature of the organic C of µLF in this fraction.

These results are also in agreement with research reported by Yamashita et al. (2006), who found faster mean turnover times (102 years) in the mineral fraction than in the occluded particulate OM (166 years) of slaking-resistant aggregates <53 µm in a silty soil under maize cultivation. This is an important difference with bigger aggregates, where in general, occluded OM is younger than OM associated to the mineral particles (Puget et al., 1995; Feller and Beare, 1997).

Swanston et al. (2005), who also found a faster incorporation of young C into the dense fraction ($\rho > 1.7\ \mathrm{g\ cm^{-3}}$) than in the occluded particulate OM after dispersion of a forest soil with recently ^{14}C-enriched litter inputs, postulated the existence of two pools of OM in the dense fraction: well stabilized old OM, and younger OM arrived to this fraction in recently formed dispersion-resistant microaggregates. This cannot explain the presence of younger C in the occluded clay-size fraction in our soil, because we separated this fraction before density fractionation (Fig. 1). This means that if dispersion-resistant silt-size aggregates enriched in younger µLF were present in our soil, we would have recovered them together with the occluded silt-size minerals, which had turnover times similar to those of the occluded µLF (Table 3), and not as occluded clay-size particles. In addition, the percentage of young C in the non-occluded clay-size particles was similar to that of occluded particles of the same size (Fig. 5a).

In consequence, considering that most of the soil clay-size particles were found within slaking-resistant silt-size aggregates in our soil (Table 1), it can be said that, at this scale, the same structures that protect the µLF from degradation are simultaneously being enriched in C from crop residues.

Considering that the amount of organic C in the clay-size fraction in the form of small organic particles was negligible (Chenu and Plante, 2006), two non-mutually excluding mechanisms can explain the faster incorporation of young C onto clay-size particles, regardless of their location within or outside silt-size aggregates: (i) incorporation via microbial activity and (ii) adsorption of soluble compounds. Microbial activity is capable of transferring C from fresh organic residues from its original position to the clay particles onto which an important amount of the soil biomass is attached (Six et al., 2006). The direct adsorption of soluble organic compounds issued from the decomposition of maize residues or from maize rhizodeposition onto clays is also possible (Kaiser and Guggenberger, 2000; Kahler et al., 2004). A recent study (Lehmann et al., 2007) proposes the adsorption of organics on mineral surfaces, and not occlusion of organic debris, as the initially dominant process of C stabilization in soils. The degree to which this soluble C issued from recent crop residues is responsible for the stability of silt-size aggregates is a promising question of research, especially because clay-size particles within silt-size aggregates were more enriched in organic C than particles of the same size not forming part of such aggregates (non-occluded clay-size particles, Tables 1 and 2).

5. Conclusions

By isolating slaking-resistant silt-size aggregates of a silty cultivated temperate soil supporting a conventionally-tilled wheat-maize chronosequence, we found that OM stored within such aggregates was essentially composed by two different pools, the uncomplexed light µLF and OM in the clay-size fraction, which had different renewal kinetics, as determined by isotopic analysis of organic C.

Unlike hypothesized, we found that the apparent turnover rates of both fractions was similar within and outside silt-size aggregates, and that the µLF had longer turnover rates in average than organic C associated to clay-size particles and POM in fractions >50 µm. We conclude that if the observed preservation of µLF at the decadal scale is assumed to be due at least in part to protection by entrapment within aggregates, only a fast turnover of silt-size aggregates could explain the absence of differences in the pace of enrichment in new organic C within and outside such aggregates.

Our observations have two other important implications in relation to our vision of the silt-size fraction in temperate silty soils: the renewal of OM at the silt-size scale is not homogeneous in terms of kinetics, and more importantly, silt-size aggregates do not appear as preferential sites of OM stabilization in the medium term (decade) *per se*. Organic matter renewal at this scale seems not to be related to the environment in which it is found (i.e. within or outside slaking-resistant aggregates), but to the mechanism through which it is preserved in the soil (in the clay-size fraction or physically entrapped µLF). Modelling of OM dynamics in soils as the one studied here, which often consider the silt + clay fraction as a homogeneous pool, should take into account this heterogeneity, in order to avoid the risk of misinterpretations, as already suggested by Roscoe et al. (2001).

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