

Formation of millimetric-scale aggregates and associated retention of ^{13}C – ^{15}N -labelled residues are greater in subsoil than topsoil



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ABSTRACT

Decomposition of fresh crop residues quickly leads to the formation of water-stable (WS) soil macroaggregates. How the initial soil organic carbon (SOC) concentration affects macroaggregation is still unclear, and the consequences for short-term retention of crop-residue C and N are unknown. A 51-day laboratory incubation study investigated the C and N incorporation in WS macroaggregates of SOC-rich topsoil and SOC-poor subsoil when amended with ^{13}C – ^{15}N -labelled corn residues at rates of up to 40 g residue-C kg^{−1} soil. Water-stable macroaggregate formation per unit of residue-C added was greater in the SOC-poor subsoil than in the SOC-rich topsoil. Large macroaggregates (>1000 μm) in the SOC-poor subsoil were enriched in ^{13}C and ^{15}N in both particulate organic matter (POM, >50 μm) and fine particle-size (<50 μm) fractions compared to the SOC-rich topsoil. We postulate that the retention of residue-C and -N in both POM and fine fractions within WS macroaggregates is due to the large-scale occlusion of coarse material and small-scale adsorption of organic substances occurring concomitantly in soil. Although the mass of WS macroaggregates levelled off at high residue input rate, accumulation of ^{13}C and ^{15}N in both POM and fine fractions continued throughout the incubation, and WS macroaggregates did not become saturated with C and N in the short-term. Possible mechanisms occurring at increasing input rates include 1) coating of macroaggregates with residue decomposition products, 2) continual turnover of macroaggregates and 3) greater stability and formation of larger macroaggregates. In this heavy clay soil, macroaggregates represent a dynamic soil fraction that accumulates POM and organic compounds from decomposing plant material. We conclude that the presence of unsaturated mineral surfaces in the SOC-poor subsoil favoured the formation of millimetric WS macroaggregates and the short-term retention of residue-C and -N.

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1. Introduction

Surface soil horizons generally have higher SOC concentration than subsurface soil horizons (Paul et al., 2001; Rasmussen et al., 2005). Therefore, the subsoil should have more available sorption sites on mineral surfaces (Kaiser and Guggenberger, 2003; Rasse et al., 2005). The presence of unsaturated mineral surfaces provides capacity for retention of SOC, either by direct adsorption of molecules on sorption sites or through occlusion of coarse material (Köbl et al., 2007; Kimetu and Lehmann, 2010). Occlusion may also be important for C retention in SOC-poor subsoil by restraining the

access of decomposers to the substrate (Salomé et al., 2010). This could explain the old age of occluded subsoil SOC reported by Rasmussen et al. (2005) and Schrumph et al. (2013). Macroaggregation is a crucial process influencing SOC stabilization (Six et al., 2004), but its role in C retention within SOC-poor subsoil, particularly in regards to macroaggregates from different size classes, has not been studied explicitly.

SOC-poor soils may exhibit substantial macroaggregate formation following the addition of organic amendments (Fortún et al., 1996; Grosbelle et al., 2011). Browning and Milam (1944) showed that the addition of corn stover in a clay loam soil increased the soil mass in macroaggregates by 30% in SOC-poor subsoil compared to 10% in SOC-rich topsoil. The addition of free organic material such as crop residues to the soil can serve as a substrate for the microbial production of organic substances that can act as binding agents, stabilizing soil aggregates (Oades, 1967). Short-term changes in aggregation induced by labile C inputs are

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usually seen first in the macroaggregate fraction (Oades, 1984; Angers et al., 1997), and binding agents involved at these spatio-temporal scales are roots, fungal hyphae (Tisdall and Oades, 1982; Gupta and Germida, 1988; Oades and Waters, 1991) and microbial by-products (e.g. extracellular polysaccharides) of both fungal and bacterial origin (Harris et al., 1964; Gupta and Germida, 1988; Miller and Jastrow, 1990; Jastrow et al., 1998). Stable macroaggregates represent hot-spots of microbial processes (transformations) where finer scale stabilization of residue is believed to take place [e.g. micro within macroaggregate model (Oades, 1984; Six et al., 2000)]. Therefore, the OM accumulating in stable macroaggregates should be found in both particulate ($>50\ \mu\text{m}$) and fine physical fractions.

Incubating subsoil with labelled organic residues or compounds has proven useful to study the mechanisms controlling SOC mineralization, stabilization and saturation in SOC-poor soil that comes in contact with fresh organic substrate (Fontaine et al., 2007; Stewart et al., 2008; Salomé et al., 2010). It is common for SOC-poor soils or soil layers to come in contact with high amount of organic matter. This can be the case when residues are added to a degraded soil to initiate regeneration and restructuration process (Grosbellet et al., 2011; Larney and Angers, 2012) or in ploughed soils when residues are buried deeper in the soil profile (Angers et al., 1995). Other situations include forest soils when aboveground litter falls in tree throws with exposed subsoil, when macro biota (ex: earthworms, rodents) bury organic residues deep in the soil profile, or around decomposing plant roots.

The objective of this study was to evaluate how the initial SOC concentration (i.e., in topsoil vs subsoil) would affect 1) macroaggregate formation following incorporation of fresh crop residue and 2) its subsequent effect on the short-term retention of crop residue-C and -N within aggregate-associated POM and fine fractions. To achieve this objective, we incubated SOC-rich topsoil and SOC-poor subsoil from a heavy clay soil with a wide range of ^{13}C – ^{15}N labelled residue inputs for 51 days.

2. Materials and methods

2.1. Soils, residues and incubation

The topsoil (0–20 cm) and subsoil (30–70 cm) horizons of a heavy clay under barley (*Hordeum vulgare* L.) cropping were collected in 2007 from Lévis, Québec, Canada ($46^{\circ}48'\text{N}$, $71^{\circ}23'\text{W}$). The soil was classified as a Haplic Gleysol according to the World Reference Base for Soil Resource system (IUSS Working Group WRB, 2006) and as an Orthic Humic Gleysol according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998). Soil horizons had similar texture (281 g silt kg^{-1} and 656 g clay kg^{-1} in topsoil; 271 g silt kg^{-1} and 675 g clay kg^{-1} in subsoil) and mineralogy (dominated by quartz, feldspar, amphibole, chlorite, vermiculite and muscovite). However, topsoil and subsoil had contrasting SOC and total N concentrations (31.3 g SOC kg^{-1} and 2.5 g total N kg^{-1} in topsoil; 4.5 g SOC kg^{-1} and 0.5 g total N kg^{-1} in subsoil). Soil pH (1:2 soil-to- CaCl_2 0.01 M ratio) values were 5.2 and 6.3 in topsoil and subsoil, respectively. After collection, topsoil and subsoil horizons were gently crumbled by hand and large organic fragments and rocks were removed. Soils were air-dried and sieved through 6 mm mesh prior to the incubation, to preserve macroaggregates and minimize SOC loss from soil preparation.

Air-dried soils were incubated without and with ^{13}C – ^{15}N -labelled corn (*Zea mays* L. cv. Cargill 2610-L) residues. Corn plants were grown in a greenhouse and pulse-labelled with $^{13}\text{CO}_2$ -C and fertilized with K^{15}NO_3 weekly. Corn leaves and stems harvested at V10–V12 vegetative stage were ground; residue that passed

through a 1 mm sieve and was retained on a $100\ \mu\text{m}$ sieve was used for the incubation. The C/N ratio of corn residue was 26, the $\delta^{13}\text{C}$ isotopic signature was 69.7‰ relative to the Pee Dee Belemnite standard and the atom % ^{15}N (At% ^{15}N) was 7.40%.

Each experimental unit was a 1 L glass jar containing 150 g of air-dried soil (topsoil or subsoil) mixed with corn residue at rates of 0 (control), 2.5, 5, 10, 20 and 40 g residue-C kg^{-1} soil (air-dry basis). Each treatment was replicated four times for a total of 48 experimental units, which were organized in a completely randomized factorial design with initial SOC level (topsoil or subsoil) and residue input rate as the two factors tested. Jars were left partly opened and incubated in a Fabien climate chamber for 51 d in the dark under controlled temperature ($25\ ^{\circ}\text{C}$), moisture ($-38\ \text{kPa}$) and nutrient (C/N = 10) conditions. Further details on soils, residue and the incubation experiment were reported in Poirier et al. (2013).

2.2. Soil aggregate fractions

Soil from the incubation experiment was first separated into 3 fractions: large macroaggregates (LM, $>1000\ \mu\text{m}$), small macroaggregates (SM, $250\text{--}1000\ \mu\text{m}$) and microaggregates plus unaggregated particles (m + UP, $<250\ \mu\text{m}$). Forty grams of air-dried soil was placed on top of a set of 2 sieves (1000 and $250\ \mu\text{m}$) in a 4 L bucket filled with $\sim 3.5\ \text{L}$ of distilled water, submerged for 5 min to allow capillary wetting, then wet-sieved with total immersion for 10 min on a wet-sieving apparatus similar to the one described by Kemper and Rosenau (1986). This procedure allowed isolation of slaking-resistant WS macroaggregates (Puget et al., 1995; Angers and Giroux, 1996). The apparatus was calibrated to raise and lower the top sieve 3.7 cm, 29 times per min. The LM and SM fractions from the 40 g soil sample were transferred onto aluminium plates with distilled water, and the procedure was repeated with a second 40 g soil sample, yielding a total of 80 g of soil wet-sieved per treatment. The m + UP fraction was recovered by centrifugation at 670 g for 15 min. The pellet formed by the m + UP fraction was transferred from the centrifuge bottles onto aluminium plates with distilled water. Soil aggregate fractions (LM, SM and m + UP) were dried at $50\ ^{\circ}\text{C}$ for at least 24 h and weighed. The weight distribution of individual aggregate fractions in soil was expressed as g of LM, SM or m + UP per kg dry soil. The weight distribution of macroaggregates ($>250\ \mu\text{m}$) in soil was calculated as the sum of LM + SM fractions and expressed as g macroaggregates per kg soil. For each C input rate, the rate of macroaggregate formation per unit C added, expressed as (g macroaggregates kg^{-1} dry soil) g^{-1} C added, was calculated according to the following equation:

macroaggregate formation rate

$$= \left[\Delta \text{ increase in g(LM + SM) kg}^{-1} \text{ soil} \right] / [\Delta \text{g C input}]$$

Each soil aggregate fraction underwent a second separation step to remove the free light fraction (FLF) containing unaggregated and unprotected residues. A subsample (4–30 g, depending on the mass of material available) of each aggregate fraction was weighed in a 1 L beaker and slowly humidified with water vapour for 2 h to minimize aggregate disruption. When less than 4 g of an aggregate fraction was available, replicates were combined to make composite samples. Once the soil was completely wet, $\sim 100\ \text{ml}$ of water was slowly added and the beaker was swirled gently to allow unaggregated and unprotected residues to detach from the soil and float on the water surface. Floating residues were recovered by syphoning, dried at $50\ ^{\circ}\text{C}$ for at least 24 h and weighed.

The remaining soil from each aggregate fraction was then submitted to a final separation step. Wet soil (up to 30 g) was

transferred into 250 ml plastic bottles, distilled water added (1:4 soil:water ratio) and shaken overnight with 10–15 glass balls (6 mm diameter) to disrupt aggregates. Thereafter, the soil and water mixture was washed over a 50 μm sieve to separate particulate organic matter (POM, >50 μm) from the fine particle-size (<50 μm) fraction. Both fractions were dried at 50 °C for at least 24 h and weighed.

This study focused on the mechanisms of OM retention in POM and fine fractions through WS macroaggregation. Thus, in each aggregate-size class, the FLF was removed (data measured but not shown) and only results regarding C and N retention in POM and fine fractions are presented.

2.3. Carbon and nitrogen analyses

The presence of inorganic C was not detected in topsoil or subsoil upon carbonate acidification reaction analysis (SSM-5000A, Shimadzu, Kyoto, Japan), and the SOC concentration in soil fractions was considered equivalent to the total C concentration analysed by dry combustion (CNS-1000, LECO Corp. St. Joseph, MI). The concentrations of SOC in POM (POM-C) and fine (fine-C) fractions within LM and SM (unit: g POM-C or fine-C kg^{-1} LM or SM) were calculated as follows:

$$\text{POM-C}_{\text{LM or SM}} = (\text{g SOC kg}^{-1} \text{ POM}) * (\text{kg POM kg}^{-1} \text{ LM or SM})$$

$$\text{fine-C}_{\text{LM or SM}} = (\text{g SOC kg}^{-1} \text{ fine}) * (\text{kg fine kg}^{-1} \text{ LM or SM})$$

Total N concentration in soil fractions was analysed by dry combustion (CNS-1000, LECO Corp. St. Joseph, MI). The concentrations of total N in POM (POM-N) and fine (fine-N) fractions within LM and SM were calculated similarly as for POM-C and fine-C.

Residue-C concentration in soil fractions was calculated from the ^{13}C isotopic signature ($\delta^{13}\text{C}$) given by isotope ratio mass spectrometry analysis (Stable Isotope Facility, UC Davis, CA) and calculated according to:

$$\delta^{13}\text{C} = \left[\left(\frac{^{13}\text{R}_{\text{sample}}}{^{13}\text{R}_{\text{standard}}} - 1 \right) \right] * 1000$$

where $^{13}\text{R} = ^{13}\text{C}/^{12}\text{C}$ and the standard is the international Pee Dee Belemnite. In POM and fine fractions within LM or SM, the proportion of SOC coming from residue-C (f_C , in g residue-C g^{-1} POM-C or fine-C) was calculated as follows:

$$f_C = [(\delta_{\text{tr}} - \delta_c) / (\delta_r - \delta_c)]$$

where $\delta_{\text{tr}} = \delta^{13}\text{C}$ of the POM or fine fraction within LM or SM in the residue-amended soil, $\delta_c = \delta^{13}\text{C}$ of the POM or fine fraction within LM or SM in the unamended control soil, and $\delta_r = \delta^{13}\text{C}$ of the corn residues, respectively.

Residue-N concentration in soil fractions was calculated from the atom % ^{15}N (At% ^{15}N) given by isotope ratio mass spectrometry analysis (Stable Isotope Facility, UC Davis, CA) and calculated as:

$$\text{At}\%^{15}\text{N} = [\text{no. of } ^{15}\text{N} \text{ atoms} / (\text{no. of } (^{15}\text{N} + ^{14}\text{N}) \text{ atoms})] * 100$$

In POM and fine fractions within LM or SM, the proportion of total N coming from residue-N (f_N , in g residue-N g^{-1} POM-N or fine-N) was calculated as follows:

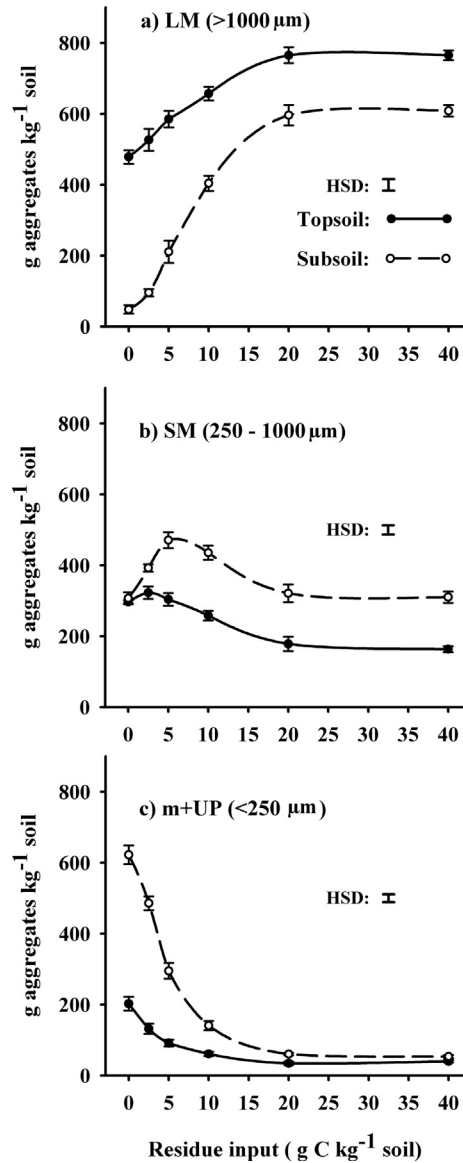


Fig. 1. Distribution of a) large (LM, >1000 μm) and b) small (SM, 250–1000 μm) water-stable macroaggregates, and c) microaggregates plus unaggregated particles (m + UP, <250 μm) in topsoil (0–20 cm depth) and subsoil (30–70 cm depth) from a heavy clay soil incubated with ^{13}C – ^{15}N -labelled corn residues for 51 d. Vertical bars on data points represent the standard deviation of the mean. HSD = Tukey's Honestly Significant Difference at $\alpha = 0.05$.

$$f_N = [(At_{\text{tr}} - At_c) / (At_r - At_c)]$$

where $At_{\text{tr}} = \text{At}\%^{15}\text{N}$ of the POM or fine fraction within LM or SM in the residue-amended soil, $At_c = \text{At}\%^{15}\text{N}$ of the POM or fine fraction within LM or SM in the unamended control soil, and $At_r = \text{At}\%^{15}\text{N}$ of the corn residues, respectively.

The amounts of residue-C retained in POM (POM-Cres) and fine (fine-Cres) fractions within LM or SM (unit: g POM-Cres or fine-Cres kg^{-1} LM or SM) were calculated as follows:

$$\text{POM-Cres}_{\text{LM or SM}} = f_C * \text{POM-C}_{\text{LM or SM}}$$

$$\text{fine-Cres}_{\text{LM or SM}} = f_C * \text{fine-C}_{\text{LM or SM}}$$

The amounts of residue-N retained in POM (POM-Nres) and fine (fine-Nres) fractions within LM or SM were calculated similarly as POM-Cres and fine-Cres.

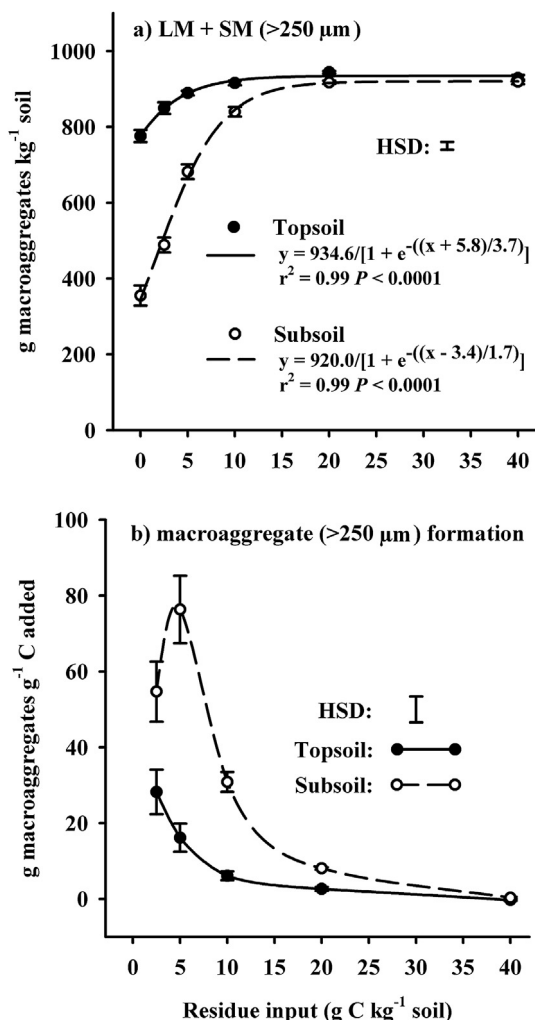


Fig. 2. a) Proportion of water-stable macroaggregates [large (LM, >1000 μm) + small (SM, 250–1000 μm)] and b) water-stable macroaggregate formation per unit of C added (i.e., Δ increase in >250 μm aggregates/ Δ C input) in topsoil (0–20 cm depth) and subsoil (30–70 cm depth) from a heavy clay soil incubated with ^{13}C – ^{15}N -labelled corn residues for 51 d. Vertical bars on data points represent the standard deviation of the mean. HSD = Tukey's Honestly Significant Difference at $\alpha = 0.05$.

Table 1

Soil organic C (SOC) and total N concentrations in particulate organic matter (POM, >50 μm) and fine particle-size (<50 μm) fractions within large (LM, >1000 μm) and small (SM, 250–1000 μm) water-stable macroaggregates in unamended topsoil (0–20 cm) and subsoil (30–70 cm) from a heavy clay soil incubated for 51 d.

	LM		SM	
SOC	POM-C (g SOC kg ⁻¹ LM)	Fine-C (g SOC kg ⁻¹ LM)	POM-C (g SOC kg ⁻¹ SM)	Fine-C (g SOC kg ⁻¹ SM)
Topsoil	2.7 ^{Aa*}	28.3 ^{Aa}	2.4 ^{Aa}	26.1 ^{Ab}
Subsoil	0.7 ^{Ba}	8.1 ^{Ba}	0.4 ^{Bb}	4.0 ^{Bb}
Total N	POM-N (g total N kg ⁻¹ LM)	Fine-N (g total N kg ⁻¹ LM)	POM-N (g total N kg ⁻¹ SM)	Fine-N (g total N kg ⁻¹ SM)
Topsoil	0.2 ^{Aa}	2.4 ^{Aa}	0.2 ^{Aa}	2.2 ^{Aa}
Subsoil	0.05 ^{Ba}	0.9 ^{Ba}	0.03 ^{Ba}	0.5 ^{Bb}
C/N	POM	Fine	POM	Fine
Topsoil	15.6 ^{Aa}	11.9 ^{Aa}	16.0 ^{Aa}	11.9 ^{Aa}
Subsoil	14.1 ^{Aa}	9.0 ^{Ba}	11.4 ^{Bb}	8.2 ^{Ba}

Means followed by distinct uppercase letters within particle-size fraction (POM and fine) and aggregate-size class (LM and SM) and means followed by distinct lowercase letters within soil horizon (topsoil and subsoil) and particle-size fraction (POM and fine) are significantly different at $\alpha = 0.05$.

The amount of macroaggregate-associated residue-C retained in POM or fine fractions was expressed on a whole soil basis and calculated as the sum of POM-Cres or fine-Cres in LM plus POM-Cres or fine-Cres in SM. The amount of macroaggregate-associated residue-N retained in POM or fine fractions was calculated similarly.

2.4. Statistical analysis

Normal distribution of residuals and homogeneity of variances were verified using the PLOT and UNIVARIATE procedures of the SAS 8.2 software (SAS Institute, 2001) and no transformation was done prior to analysis of variance (ANOVA). The ANOVA was performed with the general linear model (GLM) procedure of SAS to test the effect of initial SOC concentration (i.e., SOC-rich topsoil vs SOC-poor subsoil), residue input, and their interactions on the dependent variables. All dependent variables were analysed separately. When ANOVA yielded significant differences among treatments at $\alpha = 0.05$, we used the Honestly Significant Difference (HSD) of Tukey's test to separate treatment means. Graphical representations and regression analysis were performed with the Sigmaplot 9.0 software (Systat Software Inc., 2004).

3. Results

3.1. Distribution of aggregate fractions

Unamended topsoil contained greater amount of LM, similar amount of SM and lower amount of m + UP than unamended subsoil (Fig. 1a–c). Residue additions up to 20 g residue-C kg⁻¹ soil positively influenced LM formation in both soils. The increase in LM formation was two-times greater in the subsoil than in topsoil, but the maximum amount of LM was greater in topsoil than subsoil (Fig. 1a). The addition of 2.5 and 5 g residue-C kg⁻¹ soil did not affect SM formation in the topsoil, but promoted SM formation in subsoil. The mass of SM decreased in both soils with the addition of 10 and 20 g residue-C kg⁻¹ soil and there was more SM in subsoil than topsoil (Fig. 1b). In both soils, residue input reduced the mass of the m + UP fraction and the effect was more pronounced in the subsoil than topsoil (Fig. 1c).

The combined macroaggregate fractions (LM + SM) had a significant ($P < 0.001$) nonlinear relationship with residue input in topsoil and subsoil (Fig. 2a). There was no significant difference in the mass of WS macroaggregates between topsoil and subsoil receiving 20 and 40 g residue-C kg⁻¹ soil. Overall, more new WS macroaggregates were formed in residue-amended subsoil than topsoil (relative to the unamended soils), providing a greater macroaggregation formation rate per unit C added in subsoil (Fig. 2b). In topsoil, WS macroaggregate formation was highest (28.2 g of >250 μm aggregates per gram of C added) with the lowest residue input (2.5 g residue-C kg⁻¹ soil). In the subsoil, WS macroaggregate formation increased to 76.3 g of >250 μm aggregates per gram of C added with residue input of 5 g residue-C kg⁻¹ soil and decreased thereafter (Fig. 2b).

3.2. Soil organic C and total N concentrations in macroaggregates of unamended soils

The POM-C, fine-C, POM-N and fine-N concentrations within LM and SM in unamended topsoil and subsoil are presented in Table 1. The fine-C concentration within LM was greater than within SM for both soils. In the topsoil, the fine-C concentration of LM was 1.1-fold greater than the fine-C concentration of SM. In the subsoil, the POM-C and fine-C concentrations within LM were 2.0 and 1.8-fold greater, respectively, than within SM. Total N concentrations

Table 2

Percentage of residue-C added retained in large (LM, >1000 μm) and small (SM, 250–1000 μm) water-stable macroaggregates in topsoil (0–20 cm) and subsoil (30–70 cm) from a heavy clay soil incubated for 51 d with increasing amounts of ^{13}C - ^{15}N -labelled corn residues.

Residue-C input g kg^{-1} soil	LM (>1000 μm)		SM (250–1000 μm)		Not retained in macroaggregates	
	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
	% Residue-C input (SD)		% Residue-C input (SD)		% Residue-C input (SD)	
2.5	11.3 (3.3)	12.4 (2.1)	12.6 (2.6)	12.2 (3.2)	76.1 (0.7)	75.4 (2.6)
5	12.3 (2.5)	18.2 (6.8)	15.0 (11.5)	14.7 (2.4)	72.5 (8.5)	67.1 (5.3)
10	25.0 (2.8)	20.9 (2.7)	7.7 (1.2)	11.9 (0.7)	67.3 (2.8)	67.3 (2.5)
20	22.9 (4.4)	27.8 (4.4)	9.8 (0.4)	9.4 (3.8)	67.2 (4.4)	62.8 (5.1)
40	29.9 (1.0)	27.1 (0.8)	8.8 (1.6)	12.6 (1.5)	61.3 (1.3)	60.3 (2.2)

SD = standard deviation from the mean.

followed similar trends as SOC concentrations. The C/N ratios were greater in topsoil than subsoil within most macroaggregate-size classes and particle-size fractions, except for the POM fraction within LM (Table 1). In both soils, the C/N ratios were higher in the POM than in fine fractions. In the topsoil, the C/N ratios of the POM and fine fractions were similar within LM and SM. However, in the subsoil, the C/N ratio of the POM was greater in LM than SM (Table 1).

3.3. Residue-C and -N concentrations in macroaggregate fractions of amended soils

Water-stable macroaggregates (LM + SM) retained approximately 25–40% of the residue-C added in both soils with the greatest proportions observed at the highest input rates. LM retained between 11 and 30% of the residue-C added in both soils, also with greatest proportions observed at the highest input rates (Table 2). SM retained about 8–15% of the residue added, but the greatest proportions were observed at the lowest input rates, especially in the topsoil (Table 2). Within LM, we found that POM-Cres, POM-Nres, fine-Cres and fine-Nres concentrations were significantly higher in subsoil than topsoil for every residue treatment (Fig. 3a–d). Within SM, we observed that POM-Cres and fine-Cres concentrations were significantly higher in topsoil than subsoil when 20 and 40 g residue-C kg^{-1} soil were added (Fig. 4a and b). However, at lower residue addition rates in SM, a tendency (not significant) towards higher fine-resC concentration was noted in subsoil than topsoil (Fig. 4b). Within both LM and SM, about 70 and 60% of the residue-C was retained as POM in topsoil and subsoil, respectively (Figs. 3a and 4a). However, within LM and SM, about 66% of the residue-N was retained in the fine fraction in both soils (Figs. 3d and 4d).

3.4. Accumulation of residue-C and -N in soil macroaggregates

When both WS macroaggregate fractions were combined, the residue-C retained in the POM fraction of soil macroaggregates showed a significant ($P < 0.0001$) and positive quadratic relationship with increasing residue inputs (Fig. 5a). In the fine particle-size fraction, there was 2, 1.4 and 1.1 times more residue-C in subsoil than topsoil with additions of 5, 20 and 40 g residue-C kg^{-1} soil. Quadratic equations fitted to the data were significant ($P = 0.002$), with a positive relationship in topsoil and a negative relationship in subsoil (Fig. 5b).

The residue-N retained in the POM fraction of soil macroaggregates showed a positive linear relationship with increasing residue input with a significantly ($P = 0.0003$) greater slope in subsoil than topsoil due to 1.2 times more residue-N retained at the highest residue input rate (Fig. 5c). The same significant ($P < 0.0001$) trend was observed for the mass of residue-N retained in the fine particle-size fraction (Fig. 5d).

4. Discussion

4.1. Water-stable macroaggregate formation

Our results demonstrate that WS macroaggregate formation per unit of residue-C applied was greater in the SOC-poor subsoil than SOC-rich topsoil, which is consistent with previous studies showing enhanced macroaggregate formation after organic matter was added to low organic matter soil (Browning and Milam, 1944; Fortún et al., 1996; Kimetu and Lehmann, 2010). With increasing C inputs up to 20 g residue-C kg^{-1} soil, the gain in LM was achieved at the expense of SM and m + UP fractions fraction in SOC-rich topsoil and SOC-poor subsoil. This indicates that the building blocks forming the larger macroaggregates are smaller macroaggregates, as previously observed for these soils (Angers, 1998).

As expected, adding crop residue to this heavy clay soil stimulated microbial activity in proportion to the residue input (Poirier et al., 2013). Adding labelled residues probably provided substrate for the microbial production of organic substances that act as binding agents stabilizing soil aggregates (Oades, 1967). In the present study, WS macroaggregate may have been stabilized by the direct entanglement action of fungal hyphae and by microbial by-products (e.g. extracellular polysaccharides) of fungal and bacterial origin whose interaction with inorganic soil constituents stabilize macroaggregates along planes of weakness (Harris et al., 1964; Tisdall and Oades, 1982; Gupta and Germida, 1988; Miller and Jastrow, 1990; Oades and Waters, 1991; Jastrow et al., 1998).

4.2. Residue-C and -N retention within macroaggregates

Overall, the percentage of residue-C added retained in LM was two times greater than in SM in both soils, with differences between macroaggregate fractions becoming more obvious with increasing residue inputs. This suggests that in the short-term, the retention of high amounts of crop residues in WS aggregates occurred at the millimetric scale. This is consistent with results from Bravo-Garza et al. (2010) and with the idea that large macroaggregates are stabilized in the short-term by labile SOC in these clay soils (Angers, 1998). This was observed in both SOC-rich topsoil and SOC-poor subsoil.

However, the SOC-poor subsoil retained more residue-C and -N in the POM fraction within LM at every residue input rate compared to the SOC-rich topsoil. During decomposition, POM becomes gradually encrusted with microbial products and clay particles (Golchin et al., 1994; Six et al., 2004). Direct contact of POM with unsaturated mineral surfaces in the SOC-poor subsoil might have favoured occlusion of added residues in this soil. This mechanism can be responsible for the retention of residue-derived POM inside large macroaggregates (Golchin et al., 1994). Our results for the LM fraction support the hypothesis that in soils with low SOC saturation level, POM contact with unsaturated mineral surfaces result in

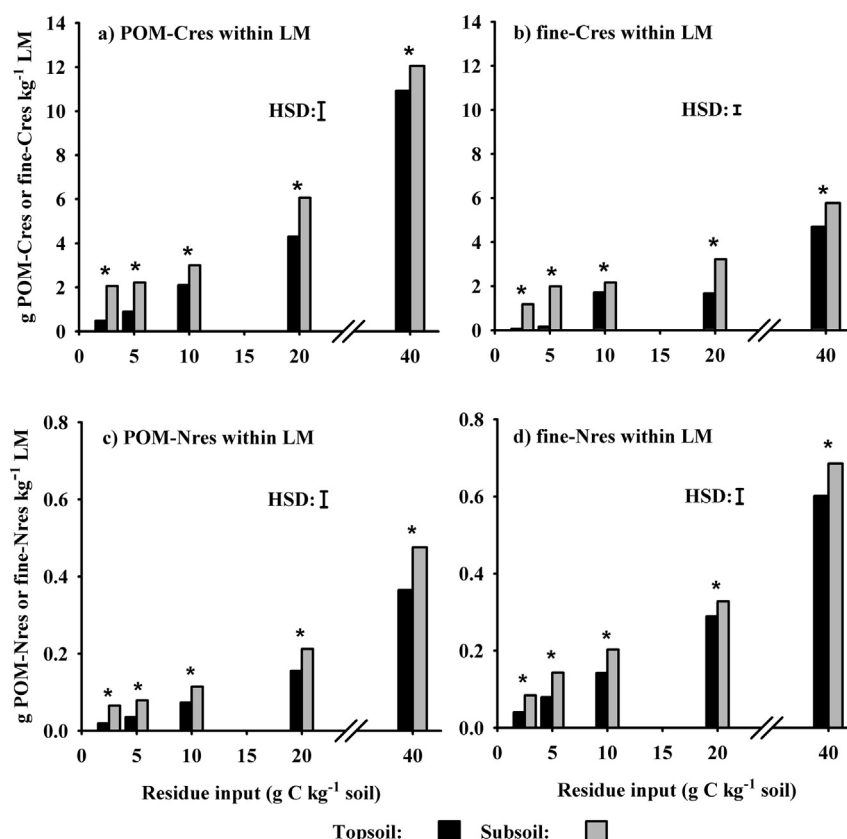


Fig. 3. Retention of residue-C and -N in particulate organic matter (POM, >50 μm) (a, c) and in fine particle-size (<50 μm) (b, d) fractions within large water-stable macroaggregates (LM, >1000 μm) in topsoil (0–20 cm depth) and subsoil (30–70 cm depth) from a heavy clay soil incubated for 51 d with increasing amounts of ¹³C–¹⁵N-labelled corn residues. Vertical bars represent Tukey's Honestly Significant Difference (HSD) at $\alpha = 0.05$. Within residue input rate, * indicates a significant difference between topsoil and subsoil according to Tukey's HSD.

greater retention of POM-associated residue-C and -N occluded inside aggregates.

The presence of unsaturated mineral surfaces in the SOC-poor subsoil also favoured the retention of residue-derived C and N in the fine fraction within LM compared to the SOC-rich topsoil. This might have occurred first through the direct diffusion of soluble compounds (Gaillard et al., 2003; Coppens et al., 2006) that can be stabilized on mineral surfaces within smaller organo-mineral complexes (Tisdall and Oades, 1982). Second, labile compounds released upon plant residue decomposition are assimilated by the microbial biomass including fungi and bacteria, and could act as binding agents in various forms such as extracellular polysaccharides forming stable macroaggregates (Degens, 1997; Puget et al., 1999). Large macroaggregates formed in the SOC-poor subsoil during this short-term experiment are stable since they resisted slaking. Thus, the lower initial level of SOC and the presence of unsaturated mineral surfaces in the subsoil favoured the retention of organic compounds, which could act as LM binding agents and become precursors of stable soil organic matter (Bradford et al., 2013; Cotrufo et al., 2013). Our results for the fine fraction within LM supports the fact that residue-derived compounds are more likely to become associated with mineral surfaces in SOC-poor subsoil than in SOC-rich topsoil and could resist decomposition in soils having lower SOC concentration, consistent with observations of Kalbitz et al. (2000) and Majumder and Kuzyakov (2010). Together, these results demonstrate that in the SOC-poor subsoil, the greater macroaggregate formation per unit C added resulted in an enrichment of residue-derived C and N in LM in both the POM and fine fractions.

Our results for the POM and fine fractions within SM do not follow the same trends as observed for LM. Differences between soils were not significant at lower residue input rate; however, the addition of large amounts of residue resulted in greater retention of C in both the POM and the fine fraction within SM in the SOC-rich topsoil. The latter was initially closer to its maximum mass of LM, which led to lower LM formation compared to the SOC-poor subsoil, and lower incorporation of labelled residues in this fraction. Consequently, labelled residues were preferentially incorporated within SM in the SOC-rich topsoil when large amounts of residues were added. This was likely facilitated by the size of the crop residues (100–1000 μm) added to soil, which roughly corresponds to the size of the SM fraction (250–1000 μm). This resulted in greater retention of residue-C within the SM particle-size fractions in the SOC-rich topsoil, in contrast to SOC-poor subsoil where residue-C was preferentially retained in LM.

Occlusion of POM was the dominant mechanism responsible for the short-term retention of residue-C in both LM and SM. Indeed, about 70% and 60% of residue-C accumulation occurred in the form of POM within macroaggregates in the SOC-rich topsoil and the SOC-poor subsoil, respectively. This was expected since not all residues could be decomposed in this short-term incubation. However, the fact that residue-C was preferentially found in a particulate form inside macroaggregates indicates that the mechanism of occlusion was active in the short-term in this heavy clay soil, regardless of the initial SOC concentration. Occlusion of POM inside macroaggregates can be an important mechanism in the retention of organic matter derived from crop residue since it could act as a first step in the formation of stable microaggregates inside

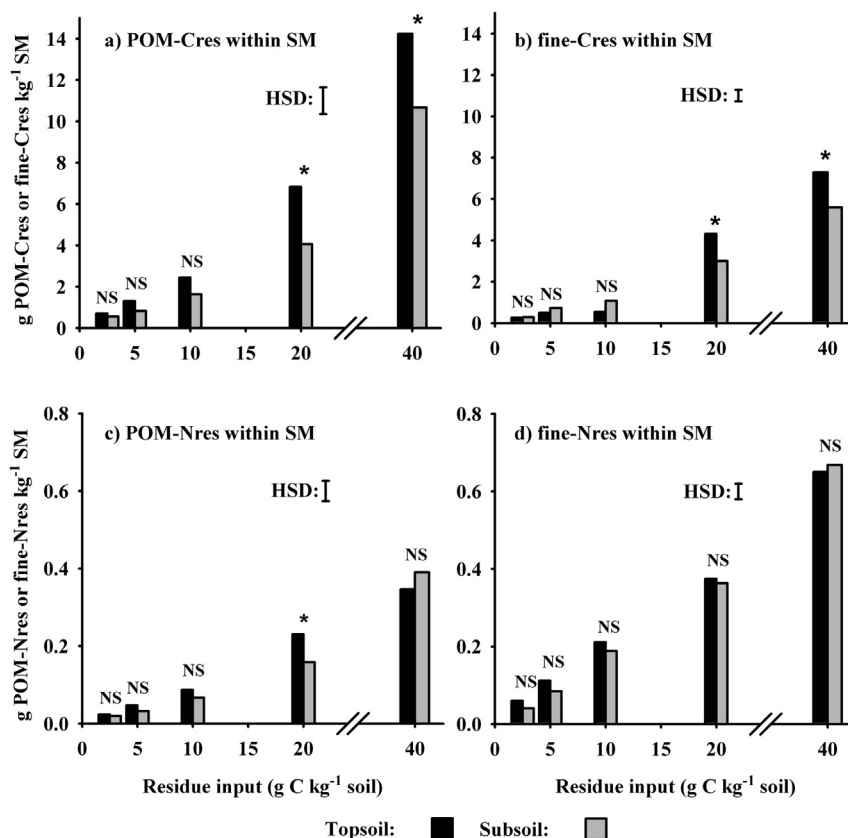


Fig. 4. Retention of residue-C and -N in particulate organic matter (POM, >50 μm) (a, c) and in fine particle-size (<50 μm) (b, d) fractions within small water-stable macroaggregates (SM, 250–1000 μm) in topsoil (0–20 cm depth) and subsoil (30–70 cm depth) from a heavy clay soil incubated for 51 d with increasing amounts of ^{13}C – ^{15}N -labelled corn residues. Vertical bars represent Tukey's Honestly Significant Difference (HSD) at $\alpha = 0.05$. Within residue input rate, * indicates a significant difference and NS indicates no significant difference between topsoil and subsoil according Tukey's HSD test.

macroaggregates (Six et al., 2000) and protect SOC against decomposition in the short-term (De Gryze et al., 2005; Goebel et al., 2009; Bravo-Garza et al., 2010).

Regardless of the initial SOC concentration, residue-N association with silt and clay particle was the dominant mechanism responsible for retention of residue-N in both LM and SM. Approximately 66% of the residue-N retained inside these aggregates was associated with the fine fraction in both the SOC-rich topsoil and SOC-poor subsoil. This could be explained by the presence of soluble N in the residues (Angers et al., 1997; Coppens et al., 2006; Helfrich et al., 2008) that was immobilized by microbes living on mineral surfaces. Residue-N could be retained as N-rich biomolecules like proteinaceous microbial compounds adsorbed onto mineral surfaces (Sollins et al., 2006; Kleber et al., 2007) or in the form of ^{15}N – NH_4^+ adsorbed onto clay surfaces as a by-product of residue-N mineralization. Indeed, NH_4^+ fixation can be up to 30% of the applied N recovered in clayey Gleysols (Chantigny et al., 2004) similar to the soil studied in this work.

4.3. Accumulation of residue-C and -N in soils

When both macroaggregate fractions were combined and expressed per unit of soil mass, we found no difference in residue-C and residue-N retention between soils. Thus, the greater enrichment of LM observed in the SOC-poor subsoil was compensated by the enrichment of SM in the SOC-rich topsoil with increasing residue additions. During this short-term experiment, residue-C and -N concentrations in macroaggregates increased linearly with increasing residue inputs despite the levelling off in

macroaggregate mass in both soils. This means that the macroaggregate fraction did not reach organic matter saturation, despite the very high amounts of residue added and suggests that in the short-term, the maximum mass of macroaggregates does not limit the soil's capacity to retain residue-C and -N through macroaggregation.

Three hypotheses are proposed to explain our results. The first explanation could be that when large amounts of residues are added to the soil, residues break down and surround existing macroaggregates with a fine film, which results in the accumulation of residue-C and -N on the exterior of macroaggregates and the formation larger macroaggregates of increased stability (Kavdir and Smucker, 2005; Grosbellet et al., 2011). The nature of this protection film, however, would remain to be determined by analysing the plant and/or microbial origin of the organic matter along the different layers forming the macroaggregates. Second, we could interpret the accumulation of residue-C and -N in macroaggregates with increasing C rates to mean that there was turnover in this aggregate fraction during the 51 d incubation. Throughout the incubation, soils were watered every 3–4 days to limit desiccation and maintain soil moisture. However, given the very high clay content of the soils, drying did occur at a low level and cracks were observed on the soil surface. The slight effect of drying and rewetting may have induced successive aggregate formation and breakdown and favoured POM incorporation inside macroaggregates (Bravo-Garza et al., 2010) as the residue was progressively fragmented by decomposition. This hypothesis could be confirmed by time-series experiments with a succession of wetting and drying cycles and destructive sampling and separation of free residue,

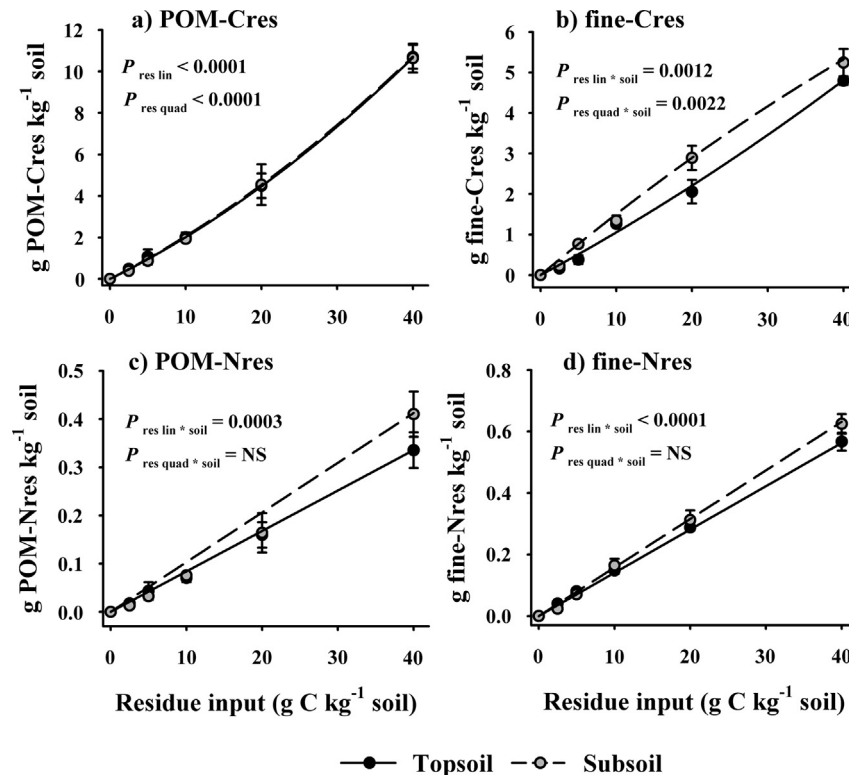


Fig. 5. Accumulation of residue-C and -N in soil water-stable macroaggregates (>250 μ m) in the form of particulate organic matter (POM, >50 μ m) (a, c) and associated with fine particles (<50 μ m) (b, d) in topsoil (0–20 cm depth) and subsoil (30–70 cm depth) from a heavy clay soil incubated for 51 d with increasing amount of ¹³C–¹⁵N-labelled corn residues. Vertical bars on data points represent the standard deviation of the mean (res lin = linear effect of residue input, res quad = quadratic effect of residue input, res lin * soil = interaction between linear effect of residue input and soil horizon, res quad * soil = interaction between quadratic effect of residue input and soil horizon, NS = not significant).

POM and fine organic matter associated with macroaggregates, ideally with ¹³C–¹⁵N-labelled residues so that retention of residue-C and residue-N can be quantified through time. Finally, a third explanation could be that larger and more stable macroaggregates were formed at increasing residue addition levels, but this was not quantified given the upper size limitation (1000 μ m) in our study. This hypothesis could be confirmed by increasing the upper size limit of the sieve during the wet-sieving procedure.

5. Conclusion

The formation of WS macroaggregates of millimetre size in the short-term, following addition of fresh crop residue to the subsoil, suggests that the structure of SOC-poor soils (e.g. deeper soil layers, degraded lands, artificial soils) can be improved rapidly. The formation of millimetric-scale aggregates was greater in the SOC-poor subsoil and was associated with greater enrichment in residue-C and -N in both POM and fine fractions compared to the SOC-rich topsoil. We postulate that two pathways – large-scale occlusion of coarse material and small-scale adsorption of organic substances derived from either decomposing residues or the associated microbial biomass – occurred concomitantly, resulting in residue-derived C and N retention in both POM and fine fractions within large WS macroaggregates. The latter continued to accumulate ¹³C and ¹⁵N tracers in POM and fine fractions despite the levelling off of macroaggregate formation (on a mass basis), indicating that this fraction does not become saturated in the short-term with C and N from high residue inputs. Three hypotheses are suggested to explain the continued accrual of residue C and N at high input rates: 1) coating of macroaggregate exterior with residue decomposition

products, 2) continual turnover of macroaggregates, and 3) increased formation and stability of aggregates larger in size than our upper limit. Overall, our results confirm that in these marine clay soils, the millimetric-scale WS aggregates were the fraction that responded noticeably to organic inputs. The high rate of macroaggregate formation in the SOC-poor subsoil following incorporation of fresh plant material favoured the short-term retention of organic C and N in this fraction.

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