

# Impact of crop residue location on carbon and nitrogen distribution in soil and in water-stable aggregates

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## Summary

One of the main effects of soil tillage is that it determines the distribution of crop residues in the soil profile. Little information is available on how this affects the distribution of carbon (C) and nitrogen (N) in the soil and more specifically in soil aggregates, as in general the contribution of other effects of tillage (i.e. mechanical disturbance) cannot be distinguished. A laboratory experiment was conducted with repacked soil columns to improve our understanding of the mechanisms of soil aggregate stabilization and storage of organic C in relation to residue location. Double-labelled oilseed rape residues (<sup>13</sup>C and <sup>15</sup>N) were incorporated in the 0–10 cm soil layer or left on the soil surface. The soil columns were incubated for 33 weeks at 20°C. Artificial rain events followed by evaporation imposed several dry-wet cycles on the soil-residue system. Spatial separation of surface-placed residues from the soil slowed down the decomposition rate compared with incorporated residues. This resulted, in the short term, in a large fraction of residue fragments at the soil surface (equivalent to 52% of carbon applied). In contrast, initial crop residue location did not significantly affect incorporation of residue-C in soil aggregates (only 10–12% of carbon applied), as new C entered the aggregate fractions mainly as soluble C. However, concentrations of residue-derived C and N were greater and aggregation increased near the soil surface with mulch, which in the longer term can modify physical and biological properties of the upper cm of the soil.

## Effet de la localisation des résidus végétaux sur la distribution du carbone et de l'azote dans le sol et dans les agrégats stables à l'eau

### Résumé

Les modalités de travail du sol influencent la distribution du carbone (C) et de l'azote (N) organique dans les sols cultivés et dans les agrégats stables à l'eau. L'influence de l'action mécanique du travail du sol est relativement bien connue dans de tels systèmes, mais l'information disponible permet rarement de dissocier cet effet de celui de la localisation initiale des résidus. Une expérience de laboratoire a été réalisée sur des colonnes de sol reconstituées, dans lesquelles des résidus de colza doublement marqués (<sup>13</sup>C, <sup>15</sup>N) ont été soit incorporés dans la couche 0–10 cm soit laissés à la surface du sol. Les colonnes ont été incubées pendant 33 semaines à 20°C et ont été soumises à des cycles d'humectation-dessiccation créés par des pluies artificielles suivies de périodes d'évaporation. La séparation spatiale sol-résidu a ralenti la décomposition des résidus à la surface du sol, comparée à celle des résidus incorporés. Ceci s'est traduit au cours des 33 semaines par la présence d'une large quantité de fragments de résidus à la surface du sol (équivalent à 52% du C apporté). Par contre la quantité de C dérivé des résidus qui a été incorporée dans les agrégats de sol est faible (10–12% du C apporté) et n'a pas été affectée par la localisation initiale. L'hypothèse est que ce carbone incorporé dans les fractions agrégées est principalement d'origine soluble, et transporté par l'eau. Cependant l'agrégation et la distribution de C et N issus des résidus a été modifiée, ce qui s'est traduit par une concentration plus importante près de la surface du sol dans le cas du mulch. Sur le long-terme, ceci peut modifier les propriétés physiques et biologiques des premiers centimètres de sol.

## Introduction

Tillage or the absence of it has a dominant influence on the distribution of carbon through the soil profile (e.g. Balesdent *et al.*, 2000). First of all, tillage determines where residues are located and consequently decomposed. Second, tillage entails mechanical disruption of soil aggregates, with well-documented effects on C dynamics and aggregate formation.

Stable soil macroaggregates are enriched in, and probably stabilized by, recently deposited organic matter (Puget *et al.*, 1995; Angers & Giroux, 1996). In turn, macroaggregates are assumed to protect fresh organic matter from decomposition, in particular in no-till soils (Beare *et al.*, 1994), where the turnover time of macroaggregates is longer than in conventionally tilled soils (Six *et al.*, 1998). A slower turnover allows the formation of stable microaggregates within macroaggregates, in which carbon can be stabilized and sequestered in the long term (Six *et al.*, 2000). Most often, however, these effects of tillage on aggregate and organic matter dynamics have been attributed mainly to the absence of mechanical disruption of soil aggregates in a no-till system.

Less is known about the specific contribution of the location of the crop residues to aggregate stability and distribution of residue-C and residue-N in and between aggregates in soils, when comparing conventional and no-till systems. Residue location obviously influences important physical and biological soil properties. Coppens *et al.* (2006) found that the soil water dynamics, the distribution of residue-C and -N in soil and the activity of the soil microbial biomass, which may be important factors in controlling aggregate dynamics, all depend on the initial residue location. In short, it was found that evaporation from columns with surface mulch was much reduced, which resulted in, on average, a wetter soil. At the same time, the surface mulch dried rapidly after each rain event, thus reducing its decomposition. As a consequence, more residue carbon was retained at the surface in this treatment, while this accumulation of carbon at the surface left plenty of opportunity for mineral nitrogen accumulation, which was much more extensively immobilized when residues were incorporated.

First, as concerns aggregation, the wetting of a soil is known to entail disruption of aggregates through slaking (Adu & Oades, 1978), while subsequent drying and rewetting of a soil may increase the strength and stability of the remaining aggregates (e.g. Denef *et al.*, 2001a). As the intensity of wetting-drying cycles and the development of water gradients through the soil profile are likely to be more important with incorporated than with surface applied residues, we anticipated that aggregate stability would be most influenced by water dynamics with residue incorporation. Second, aggregate stability has been related to soil organic carbon content (e.g. Hermawan & Bomke, 1996), acting as a binding agent for soil aggregates (Tisdall & Oades, 1982). Given the concentration of organic carbon near the soil surface with residue

mulch, it is hypothesized that more stable macroaggregates are formed in the upper soil layer than with residue incorporation. Finally, microbial activity, especially of fungi, plays an important role in the formation and stabilization of macroaggregates (e.g. Bossuyt *et al.*, 2001). Fungal biomass has been reported to be more abundant with residue mulch than with residue incorporation (e.g. Holland & Coleman, 1987), which also contributes to an increased aggregate stability near the soil surface under mulch. The interactions of all those factors influence soil aggregation and the fate of C and N in the different aggregate fractions.

As part of a larger study that aimed at investigating the specific role of crop residue location on soil water dynamics and on the biotransformation of residue-C and -N (Coppens *et al.*, 2006), this paper documents the short- and medium-term incorporation of residue-C and -N in the different soil aggregate size-fractions, as we hypothesize this is key to understanding the long-term effect on C storage in soils. This level of understanding is required for modelling and predicting the fate of C in soils. To do so, we compared the fate of recently added organic matter in soil with incorporated and surface applied oilseed rape residues, labelled with  $^{13}\text{C}$  and  $^{15}\text{N}$ . The main questions addressed were: (i) Does the presence of a large mass of residue at the soil surface (i.e. the residue mulch) and the slower decomposition rate of this mulch modify the dynamics and concentration of larger macroaggregates in the adjacent soil layer, thus leading to a larger C stabilization in the long term? (ii) How does the way the new C enters the soil with surface application (leaching of soluble C) versus incorporation (diffusion of soluble C and residue colonization) modify the distribution of residue-C in the various soil aggregate fractions?

We acknowledge that with this experimental approach, we exclude other important drivers in aggregate formation and degradation, such as roots (Gale *et al.*, 2000a,b). However, we chose to do so as we wanted to exclude root-associated effects such as drying and/or compacting of the rhizosphere soil and enrichment of the soil with substances with beneficial or adverse effects on aggregate formation.

## Materials and methods

### Soil

Soil was sampled from the experimental site of INRA, Domaine de Brunehaut, Estrées-Mons (northern France). The soil was a silt loam, Orthic Luvisol (FAO/ISRIC/ISSS, 1998) and has not been cropped since 1994. The 0–25 cm soil layer was sampled, sieved (< 2 mm) at field moisture content ( $0.17 \text{ g g}^{-1}$ ) and stored in plastic bags at  $4^\circ\text{C}$  prior to use. Selected soil properties are given in Table 1. The soil was pre-incubated for 2 weeks at  $20^\circ\text{C}$  before the start of the experiment.

**Table 1** Selected properties of the soil (Orthic Luvisol) sampled at the experimental site in Estrées-Mons (northern France)

Soil texture	Clay	/g kg <sup>-1</sup>	134
	Fine silt	/g kg <sup>-1</sup>	320
	Coarse silt	/g kg <sup>-1</sup>	496
	Fine sand	/g kg <sup>-1</sup>	38
	Coarse sand	/g kg <sup>-1</sup>	12
Organic C		/g kg <sup>-1</sup>	6.7
Total nitrogen		/g kg <sup>-1</sup>	0.9
Total carbonate		/g kg <sup>-1</sup>	7.0
CEC		/cmol <sub>c</sub> kg <sup>-1</sup>	8.1
pH <sub>H2O</sub>			8.2

### Crop residues

The fresh organic matter added to the soil was mature oilseed rape (*Brassica napus* L.), double-labelled with <sup>13</sup>C and <sup>15</sup>N. The oilseed rape crop was grown under hydroponic conditions in a plant growth chamber with an enriched <sup>13</sup>C-CO<sub>2</sub> atmosphere (<sup>13</sup>C atom percentage excess = 3.13%) and a nutrient solution with <sup>15</sup>N-KNO<sub>3</sub> (<sup>15</sup>N atom percentage excess = 9.8%). More details of the plant growth conditions are given in Trinsoutrot *et al.* (2000). The applied residue consisted of a mixture of leaves, stalks, secondary stems and pods, chopped at 1 cm before application to the soil. The C and N contents of the residues with their atom percentage excess and the biochemical composition determined by proximate analysis (Van Soest, 1963) are presented in Table 2.

### Experimental conditions

Plastic cylinders (PVC, 15.4-cm inner diameter, 30-cm length) with perforated bases were filled with 25 cm of soil, compacted to 1.3 g cm<sup>-3</sup>. Oilseed rape residues were applied at the soil surface (referred to as SURF) or homogeneously mixed in the 0–10 cm soil layer before compaction (referred to as INC) at a rate of 13.8 g dry matter per column, equivalent to a return of 7.4 t dry matter ha<sup>-1</sup>. Control columns without addition of fresh organic matter (referred to as CTRL) were also prepared. At the start of a 9-week incubation period, a rain event with an intensity of 12 mm hour<sup>-1</sup> was applied with a rainfall simulator

on all the soil columns. Rain events were imposed to induce a more natural way of rewetting and hence create a more natural water movement throughout the soil, while also entailing natural aggregate disruptive forces. The volumetric water content of the soil (0.22 cm<sup>3</sup> cm<sup>-3</sup>) was raised to 0.34 cm<sup>3</sup> cm<sup>-3</sup> after 2.5 hours of rain. Subsequently, the soil columns were transferred to a climate chamber and left uncovered at 20°C and 70% relative air humidity to allow evaporation. After 3, 6 and 9 weeks of incubation, soil columns were again subjected to the rainfall simulator until the water lost by evaporation, as measured by weight loss, was replenished. At each sampling time (3, 6 and 9 weeks), three replicate columns of each treatment were used to study organic matter dynamics and aggregate stability; two replicates of CTRL, SURF and INC were analysed after 33 weeks. For the latter soil columns, soil water content was adjusted to 0.34 cm<sup>3</sup> cm<sup>-3</sup> at week 25 and kept constant until week 33 to stimulate residue decomposition. Details of soil column preparation and the rain simulator are given by Coppens *et al.* (2006).

### Aggregate separation

After 3, 6 and 9 weeks of incubation, 150 g of soil was sampled from the 0–5 cm and 5–10 cm soil layer of all treatments. The soil was crumbled over an 8-mm aperture sieve along the natural planes of weakness and air-dried at 20°C. A 30-g subsample was used to separate four aggregate fractions by a wet-sieving method adopted from Elliott (1986). First,

**Table 2** Selected chemical and biochemical properties of the oilseed rape residues

	Mass /%	C /% <sup>a</sup>	C /% <sup>b</sup>	<sup>13</sup> C /% a.e.	N /% <sup>a</sup>	N /% <sup>b</sup>	<sup>15</sup> N /% a.e.	C:N
Total residue	99.9	42.2	99.9	2.88	1.45	100.0	9.73	29
Soluble fraction	45.6	35.5	39.0	ND	1.75	71.0	ND	20
Hemicellulose	14.4	36.4	12.6	ND	0.75	9.6	ND	49
Cellulose	33.2	48.3	38.7	ND	0.39	11.4	ND	125
Lignin + ash	6.7	59.2	9.6	ND	1.33	8.0	ND	44

<sup>a</sup>% C and N in mass of biochemical fraction.

<sup>b</sup>% of total residue-C and -N.

the soil was submerged for 5 minutes in deionised water to allow slaking. Then, the soil was successively passed through a series of three sieves to separate four fractions: large macroaggregates ( $> 2000 \mu\text{m}$ ), small macroaggregates ( $2000\text{--}250 \mu\text{m}$ ), microaggregates ( $250\text{--}53 \mu\text{m}$ ) and the silt and clay fraction ( $< 53 \mu\text{m}$ ). To obtain the large and small macroaggregates, the sieve was gently moved up and down in the water for 50 cycles in 2 minutes. To separate the microaggregates from the silt and clay fraction, the soil fraction on the  $53\text{-}\mu\text{m}$  sieve was washed. We previously checked that the washing water was clear by using 1 litre of water. The aggregate fractions were decanted to remove organic matter present between aggregates, which floated on the water, and this light fraction (LF) was analysed separately. All aggregate fractions were oven-dried at  $60^\circ\text{C}$ . Three replicates of the soil were sieved before incubation, to measure the initial aggregate size distribution. The mean weight diameter, an approximation of the median size of the aggregates, was calculated as:

$$MWD = \frac{\left(\frac{4000+2000}{2}\right)A\% + \left(\frac{2000+250}{2}\right)B\% + \left(\frac{250+53}{2}\right)C\% + \left(\frac{53+0}{2}\right)D\%}{100} \quad (1)$$

where MWD is the mean weight diameter in  $\mu\text{m}$ , A% the fraction  $> 2000 \mu\text{m}$  in wt.%, B% the fraction  $250\text{--}2000 \mu\text{m}$  in wt.%, C% the fraction  $250\text{--}53 \mu\text{m}$  in wt.% and D% the fraction  $< 53 \mu\text{m}$  in wt.%.

#### Soil analysis

The soil of four separate soil layers (0–5 cm, 5–10 cm, 10–17.5 cm, 17.5–25 cm), which remained after sampling of the aggregates, was passed through a 2-mm aperture sieve to separate large organic debris ( $> 2 \text{ mm}$ ) from the soil fraction. Soil and residues were dried, crushed and analysed for total C and N with their atom percentage excess, using an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) coupled to a mass spectrometer (Fisons Isochrom, Fisons, Manchester, UK). Before subjecting soil samples to dry combustion in the elemental analyser, they were weighed in Ag-capsules and exposed for 6 hours to HCl vapour in a desiccator to remove carbonate species. The gravimetric soil water content in each soil layer was determined for all treatments. Total C and N, with atom percentage excess, were also measured in the crushed aggregate fractions (NA 1500, Carlo Erba; Fisons Isochrom, Fisons).

Microbial activity in the 0–5 cm and 5–10 cm soil layer was measured after 3, 6 and 9 weeks of incubation, using the substrate induced respiration technique (SIR) (Lin & Brookes, 1999). A glucose solution was prepared so as to add  $500 \mu\text{g}$  glucose-C  $\text{g}^{-1}$  soil while raising the gravimetric soil water content to  $0.24 \text{ g g}^{-1}$ . A subsample of 3 g soil was incubated for 6 hours at  $20^\circ\text{C}$  in a sealed 10-ml tube. The concentration of  $\text{CO}_2$  produced was measured with an infrared gas analyser (UNOR 610, Maihak, Hamburg, Germany).

Soluble carbon of the 0–5 cm and 5–10 cm soil layer was obtained by extracting 30 g of soil in 160 ml  $0.03 \text{ M K}_2\text{SO}_4$  (30 minutes' shaking). Soil extracts were centrifuged (20 minutes at  $5800 \text{ g}$ ), filtered and stored at  $-20^\circ\text{C}$  until analysis. Dissolved organic carbon was determined by chemical oxidation in a persulphate medium, after acidification of the extract by 5%  $\text{H}_3\text{PO}_4$  to remove carbonate species. The  $\text{CO}_2$  produced was measured by infrared spectrometry (1010, O.I. Analytical, College Station, Texas, USA).

## Results

### Characteristics of the soil samples

The soil water content, substrate induced respiration rate and dissolved organic carbon (DOC) were determined on soil subsamples at the end of each evaporation period, before aggregate separation (Table 3). The soil water content in the 0–5 cm and 5–10 cm soil layers of SURF was larger than the soil water content of CTRL and INC at the first three sampling points, without a significant difference in water content between the two layers. For CTRL and INC, soil water content increased with increasing soil depth in the first 9 weeks of incubation. No differences between treatments and soil layers were observed at week 33, where soil was kept artificially at constant water content.

The application of fresh organic matter stimulated the substrate induced respiration rate of SURF and INC for both soil layers compared with CTRL. For SURF, the maximum respiration was measured in the 0–5 cm soil layer, while the greatest respiration rate for INC was found in the 5–10 cm soil layer. No significant differences were observed in the SIR between 3 and 9 weeks.

At week 3, the greatest concentrations of DOC were measured in the 0–5 cm and 5–10 cm soil layers of the SURF treatment with 43 and  $31 \text{ mg C kg}^{-1}$  soil, respectively, compared with  $14\text{--}20 \text{ mg C kg}^{-1}$  soil for the two other treatments, irrespective of the soil layer. Thereafter, greater concentration in DOC was observed for INC compared with SURF for both layers. The CTRL treatment showed stable values for DOC at about  $15.0 \text{ mg C kg}^{-1}$  soil, except at week 33, where the concentration was double. The general increase in DOC at week 33 compared with week 9, which was also observed for SURF and INC, was attributed to a change in the soil moisture regime between weeks 9 and 33 (see 'Materials and methods' section).

### Dynamics of residue-C and -N and its distribution in soil

Initially, all of the residue-C and -N was present on the soil surface for SURF, or equally distributed within the 0–5 cm and 5–10 cm soil layer for INC (Table 4). After 33 weeks of incubation, 52.1% of the residue-C still remained in light, organic matter (LF-C,  $> 2 \text{ mm}$ ) at the soil surface for

**Table 3** Distribution of (a) the soil water content, (b) microbial activity measured by substrate induced respiration and (c) dissolved organic carbon of CTRL, SURF and INC in the 0–5 cm and 5–10 cm soil layer at sampling. Different lowercase letters indicate significant differences over time ( $\alpha = 0.05$ ), for given soil layer and treatment. Different uppercase letters indicate significant differences in treatments ( $\alpha = 0.05$ ), for the given sampling point and soil layer (ND = not deleted)

		Week 3	Week 6	Week 9	Week 33
(a)		Soil water content/g g <sup>-1</sup>			
CTRL	0–5 cm	0.15 (a, A)	0.16 (b, B)	0.18 (c, AB)	0.24 (d, A)
	5–10 cm	0.16 (a, B)	0.18 (b, C)	0.19 (c, B)	0.23 (d, A)
SURF	0–5 cm	0.23 (a, C)	0.23 (a, D)	0.22 (b, C)	0.24 (c, A)
	5–10 cm	0.23 (abc, C)	0.23 (a, D)	0.22 (b, C)	0.23 (c, A)
INC	0–5 cm	0.14 (a, A)	0.15 (b, A)	0.16 (b, A)	0.24 (c, A)
	5–10 cm	0.16 (a, B)	0.18 (b, C)	0.17 (ab, A)	0.23 (c, A)
(b)		Substrate induced respiration/ $\mu\text{g C g}^{-1}$ soil hour <sup>-1</sup>			
CTRL	0–5 cm	1.7 (a, A)	1.6 (a, A)	1.6 (a, A)	ND
	5–10 cm	1.6 (a, A)	1.8 (b, A)	1.7 (ab, A)	ND
SURF	0–5 cm	5.2 (a, CD)	5.3 (a, D)	5.4 (a, D)	ND
	5–10 cm	3.6 (a, B)	3.4 (a, B)	3.2 (a, B)	ND
INC	0–5 cm	4.1 (a, BC)	4.5 (a, C)	4.3 (a, C)	ND
	5–10 cm	6.0 (a, D)	5.6 (a, D)	5.2 (a, D)	ND
(c)		Soluble carbon/mg kg <sup>-1</sup>			
CTRL	0–5 cm	16.6 (a, B)	14.9 (a, A)	15.3 (a, A)	31.4 (b, A)
	5–10 cm	16.6 (b, B)	14.1 (a, A)	15.5 (ab, A)	31.9 (c, A)
SURF	0–5 cm	43.1 (c, E)	21.3 (a, B)	22.4 (a, B)	37.9 (b, B)
	5–10 cm	31.1 (b, D)	15.7 (a, A)	16.5 (a, A)	33.3 (b, A)
INC	0–5 cm	20.1 (a, C)	29.3 (c, C)	26.1 (b, C)	38.7 (d, BC)
	5–10 cm	14.4 (a, A)	24.0 (b, B)	20.7 (b, B)	39.1 (c, C)

SURF, while only 5.2% of the residue-C was recovered as LF-C for INC (0–10 cm). For INC, LF-C decreased at a faster rate in the 5–10 cm compared with the 0–5 cm soil layer, but at week 33 no significant difference was observed between the two soil layers.

For both residue treatments, the amount of residue-C in the soil fraction < 2 mm found after 33 weeks was to a large extent already present after 3 weeks of incubation. As a result of the initial residue location, a larger amount of residue-C was measured in the soil fraction < 2 mm of the 0–5 cm soil layer compared with the 5–10 cm soil layer for SURF, while the amounts of residue-C in the soil fraction < 2 mm were comparable in both soil layers for INC. Residue-C in the 10–25 cm soil layer was negligible for SURF, while 2.5% of residue-C was transferred to the 10–25 cm soil layer for INC. By difference with the total amount of initially added C, cumulative carbon mineralization was calculated as 40.0% for SURF and 74.1% for INC after 33 weeks of incubation at 20°C.

At week 33, 30.9% of the residue-N was still present as LF-N > 2 mm on the soil surface for SURF, while only 2.1

and 3.3% of the residue-N was recovered as LF-N in the 0–5 cm and 5–10 cm soil layer for INC. Although there was no significant difference in the amount of residue-N between the two soil layers of INC at week 33, a faster initial transfer of residue-N into the soil fraction < 2 mm was observed for the 5–10 cm than for the 0–5 cm soil layer (data not shown). The percentage of residue-N transferred to the 10–25 cm soil layer increased over time to 17.1% and 25.3% for SURF and INC, respectively. Lack of recovery of residue-N was small, on average 9% of the added N, and attributed to denitrification losses or sampling errors.

The residue recovered as LF was, however, not ‘undecomposed’: the C:N ratio of the LF over time (Table 4) shows an early increase in the C:N ratio due to the fast release of residue-N into the soil, followed by a continuous decrease due to the microbial colonization of the residue particles. In parallel, a decrease in atom percentage excess <sup>15</sup>N of the LF was observed (data not shown), indicative of a dilution of labelled N by unlabelled soil N immobilized by decomposers. Consequently, the N recovered in this fraction was not uniquely residue-N any longer. At week 33 the relative

**Table 4** Evolution over time of the distribution of residue-C and -N in the light fraction (LF) (> 2 mm) and the bulk soil (< 2 mm), for SURF and INC. Values are given  $\pm$  the standard errors of the means

		Week									
		0	3	6	9	33	0	3	6	9	33
Residue-C		SURF /%					INC /%				
Surface	> 2 mm	100.0 $\pm$ 0.0	84.3 $\pm$ 1.6	79.2 $\pm$ 1.0	73.8 $\pm$ 0.7	52.1 $\pm$ 1.1	0.0 $\pm$ 0.0				
0–5 cm	> 2 mm	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	50.0 $\pm$ 0.0	29.9 $\pm$ 1.4	15.0 $\pm$ 0.9	11.2 $\pm$ 0.3	2.4 $\pm$ 0.9
	< 2 mm	0.0 $\pm$ 0.0	5.0 $\pm$ 0.1	6.2 $\pm$ 0.7	6.3 $\pm$ 0.2	6.5 $\pm$ 0.3	0.0 $\pm$ 0.0	10.7 $\pm$ 0.2	13.1 $\pm$ 0.5	11.9 $\pm$ 1.3	10.0 $\pm$ 0.8
5–10 cm	> 2 mm	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	50.0 $\pm$ 0.0	15.8 $\pm$ 2.5	9.9 $\pm$ 0.5	9.5 $\pm$ 0.4	2.8 $\pm$ 1.4
	< 2 mm	0.0 $\pm$ 0.0	1.6 $\pm$ 0.1	1.6 $\pm$ 0.2	1.5 $\pm$ 0.0	1.4 $\pm$ 0.0	0.0 $\pm$ 0.0	10.7 $\pm$ 0.3	12.4 $\pm$ 0.6	10.5 $\pm$ 0.5	8.2 $\pm$ 0.2
10–25 cm	< 2 mm	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.0	0.0 $\pm$ 0.0	1.9 $\pm$ 0.3	2.4 $\pm$ 0.2	2.3 $\pm$ 0.4	2.5 $\pm$ 0.4
Not accounted for		0.0 $\pm$ 0.0	9.0 $\pm$ 1.5	12.9 $\pm$ 1.7	18.4 $\pm$ 0.8	40.0 $\pm$ 1.4	0.0 $\pm$ 0.0	30.9 $\pm$ 1.5	47.2 $\pm$ 1.3	54.7 $\pm$ 0.8	74.1 $\pm$ 1.4
Residue-N		SURF /%					INC /%				
Surface	> 2 mm	100.0 $\pm$ 0.0	55.9 $\pm$ 3.9	51.2 $\pm$ 3.4	43.1 $\pm$ 2.3	30.9 $\pm$ 3.5	0.0 $\pm$ 0.0				
0–5 cm	> 2 mm	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	50.0 $\pm$ 0.0	9.0 $\pm$ 1.0	6.9 $\pm$ 0.3	6.3 $\pm$ 0.1	2.1 $\pm$ 0.9
	< 2 mm	0.0 $\pm$ 0.0	26.0 $\pm$ 0.9	29.7 $\pm$ 2.4	31.2 $\pm$ 0.8	30.0 $\pm$ 1.8	0.0 $\pm$ 0.0	24.8 $\pm$ 0.8	29.4 $\pm$ 0.5	30.8 $\pm$ 2.7	31.2 $\pm$ 2.0
5–10 cm	> 2 mm	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	50.0 $\pm$ 0.0	8.4 $\pm$ 1.0	6.8 $\pm$ 0.4	7.1 $\pm$ 0.1	3.3 $\pm$ 1.8
	< 2 mm	0.0 $\pm$ 0.0	10.5 $\pm$ 0.2	9.7 $\pm$ 1.0	10.1 $\pm$ 0.2	10.8 $\pm$ 0.6	0.0 $\pm$ 0.0	32.2 $\pm$ 0.4	35.7 $\pm$ 1.1	32.5 $\pm$ 0.8	30.5 $\pm$ 0.9
10–25 cm	< 2 mm	0.0 $\pm$ 0.0	2.6 $\pm$ 0.4	4.0 $\pm$ 0.5	7.4 $\pm$ 0.5	17.1 $\pm$ 0.2	0.0 $\pm$ 0.0	10.2 $\pm$ 1.7	12.7 $\pm$ 1.0	14.6 $\pm$ 1.4	25.3 $\pm$ 0.9
Not accounted for		0.0 $\pm$ 0.0	4.9 $\pm$ 3.9	5.4 $\pm$ 1.3	8.2 $\pm$ 1.6	11.3 $\pm$ 4.7	0.0 $\pm$ 0.0	15.4 $\pm$ 1.8	8.5 $\pm$ 3.0	8.8 $\pm$ 2.1	7.6 $\pm$ 0.9
C:N		SURF /-					INC /-				
Surface	> 2 mm	29.0 $\pm$ 0.0	43.6 $\pm$ 3.1	43.5 $\pm$ 3.2	47.9 $\pm$ 2.9	41.9 $\pm$ 1.2	–	–	–	–	–
0–5 cm	> 2 mm	–	–	–	–	–	29.0 $\pm$ 0.0	87.3 $\pm$ 6.1	52.4 $\pm$ 4.4	39.2 $\pm$ 1.2	22.7 $\pm$ 0.2
	> 2 mm	–	–	–	–	–	29.0 $\pm$ 0.0	43.2 $\pm$ 2.8	33.2 $\pm$ 0.4	29.0 $\pm$ 1.3	18.2 $\pm$ 0.1

contribution of soil N represented 35% (INC, 0–5 cm), 28% (INC, 5–10 cm) and 15% (SURF) of the measured LF-N. This also means that part of the LF-C recovered in this fraction was microbial biomass-C, but because the almost unique source of C for decomposers was the residue itself, the LF atom percentage excess  $^{13}\text{C}$  did not change over time and the relative contribution of microbial biomass C to the LF-C fraction can only be indirectly estimated from the N data.

#### Aggregate dynamics

Changes in the mean weight diameter (MWD) resulted mainly from a decrease in the microaggregate fraction (250–53  $\mu\text{m}$ ) in favour of the small macroaggregate fraction (2000–250  $\mu\text{m}$ ) (Figure 1). The amount of large macroaggregates (> 2000  $\mu\text{m}$ ) was very small, irrespective of the time, with at most 1.4% and 0.2% of large macroaggregates formed for INC and SURF, respectively, and their contribution to MWD was negligible.

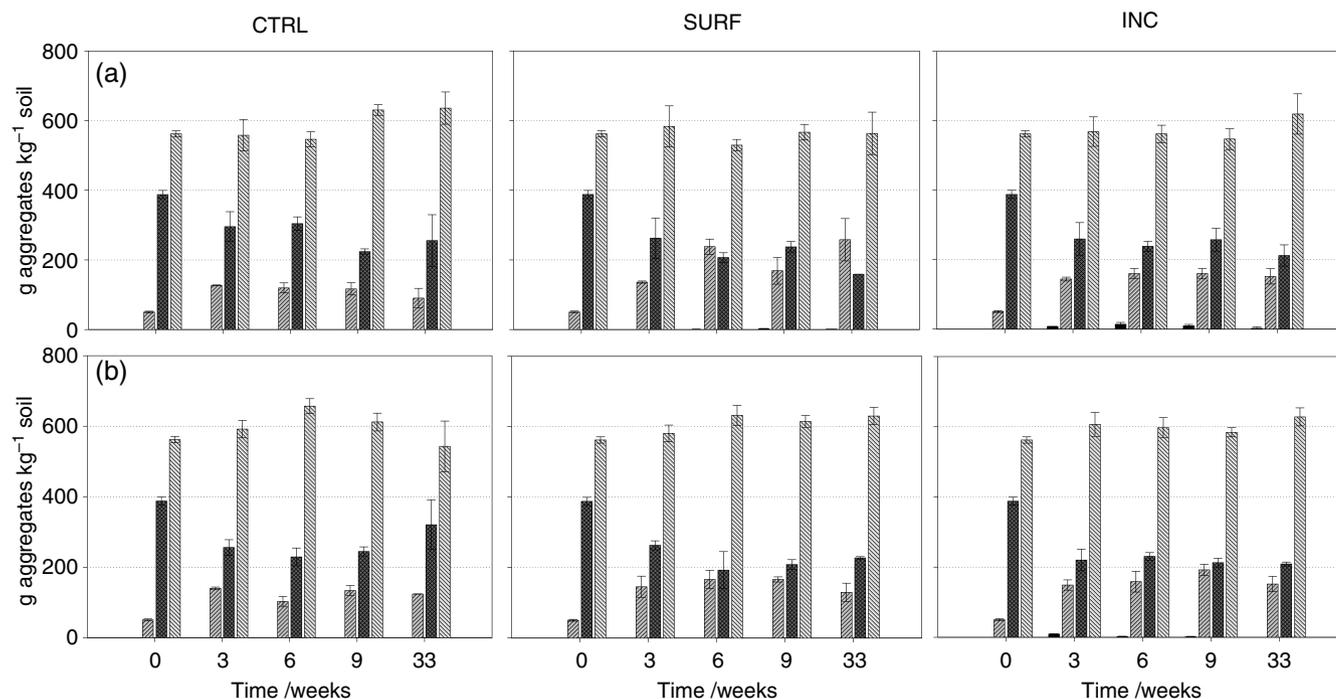
In the 0–5 cm soil layer, the initial mean weight diameter (MWD) increased for all treatments by at least 56% during the first 3 weeks of incubation, with a significantly larger MWD for INC compared with CTRL and SURF (Table 5). From week 6, both residue treatments had a significantly larger

MWD compared with CTRL. This difference was maintained until week 33. In the 5–10 cm soil layer, the initial MWD also increased for all treatments during the first 3 weeks of incubation, with a significantly larger MWD only for INC compared with CTRL. While at weeks 6 and 9, the residue treatments were still resulting in MWD values above those of the CTRL, after 33 weeks these differences disappeared.

In both soil layers, and for all treatments, the MWD after 33 weeks was still larger than at time zero. In CTRL and INC, no significant differences in MWD existed between the two soil layers, while a larger MWD could be calculated for the top 5 cm layer of the SURF treatment compared with the 5–10 cm soil layer at weeks 6 and 33.

#### Aggregate enrichment with $^{13}\text{C}$ and $^{15}\text{N}$

In the 0–5 cm soil layer of SURF, an enrichment in  $^{13}\text{C}$  was measured in the small macroaggregate (2000–250  $\mu\text{m}$ ), microaggregate (250–53  $\mu\text{m}$ ) and silt and clay fraction (< 53  $\mu\text{m}$ ) right from week 3 (Figure 2). This enrichment increased slightly up to week 33 without a significant difference between the three fractions. Conversely at week 6, the large macroaggregate fraction (> 2000  $\mu\text{m}$ ) appeared and immediately became strongly enriched in  $^{13}\text{C}$  compared with the other fractions, up to 1%



**Figure 1** Evolution of the distribution of aggregate fraction mass over time for the large macroaggregates ( $> 2000 \mu\text{m}$ ) (■), small macroaggregates ( $2000\text{--}250 \mu\text{m}$ ) (▨), microaggregates ( $250\text{--}53 \mu\text{m}$ ) (▩) and the silt and clay fraction ( $< 53 \mu\text{m}$ ) (□), for (a) the 0–5 cm and (b) the 5–10 cm soil layer of CTRL, SURF and INC. Error bars show the 95% confidence interval.

$^{13}\text{C}$  atom excess, which indicates that about one-third of the large macroaggregate-C was residue derived C. In the 5–10 cm soil layer of SURF, as expected from the small total residue C recovered (cf. Table 4), the percentage  $^{13}\text{C}$  atom excess was very small for all fractions without any significant change over time. No large macroaggregates were formed in this layer.

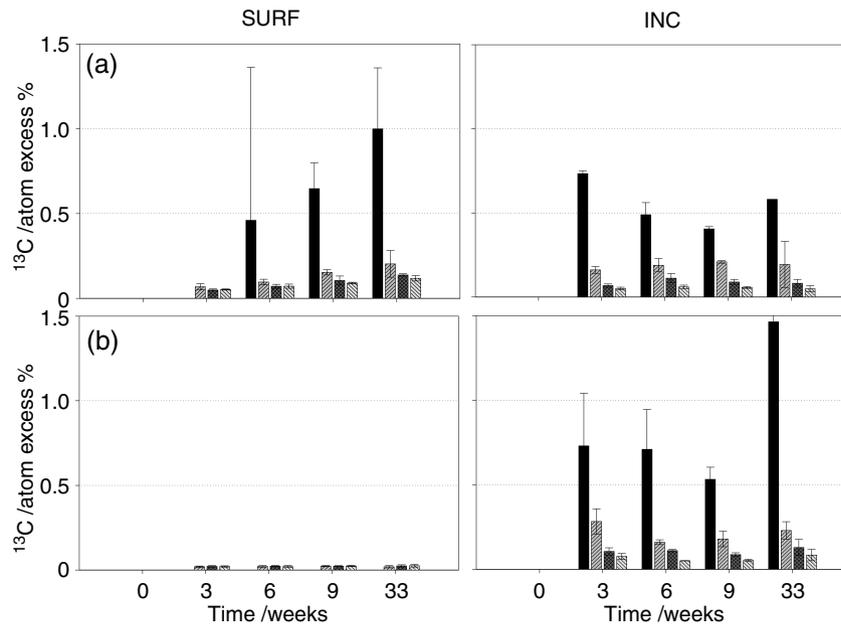
For the INC treatment, the situation was rather different than for SURF. All fractions including the large macroaggregate fraction ( $> 2000 \mu\text{m}$ ) were already labelled at week 3, with a decrease in the percentage atom excess  $^{13}\text{C}$  with decreasing aggregate size. This reflected the smaller relative contribution of labelled residue-C to the larger amounts of C in the fraction  $< 53 \mu\text{m}$ . There were no significant differences in enrichment between week 3 and week 33 for each fraction,

thus indicating little or no redistribution over time of initial  $^{13}\text{C}$  into the different aggregate sizes, or that mineralized  $^{13}\text{C}$  from aggregates was replaced over time. Similar patterns were observed for the 5–10 cm layer, except that the large macroaggregate fractions tended to be slightly more enriched in  $^{13}\text{C}$  than the corresponding 0–5 cm fraction.

Rather similar patterns as for the  $^{13}\text{C}$  enrichment were observed for  $^{15}\text{N}$  enrichment across treatments and layers. However, in contrast to  $^{13}\text{C}$  enrichment, a significant increase in  $^{15}\text{N}$  percentage atom excess was obtained over time for all fractions of the SURF in the 0–5 cm layer. In both treatments and layers, the large macroaggregate fraction ( $> 2000 \mu\text{m}$ ) again showed a strong enrichment in  $^{15}\text{N}$ , indicating that 20–30% of this fraction N was residue derived.

**Table 5** Evolution of the mean weight diameter (MWD) of soil aggregates in the 0–5 cm and 5–10 cm soil layer of CTRL, SURF and INC. Values are given  $\pm$  the standard errors of the means

		MWD / $\mu\text{m}$				
		Week 0	Week 3	Week 6	Week 9	Week 33
CTRL	0–5 cm	130 $\pm$ 1	203 $\pm$ 4	195 $\pm$ 9	182 $\pm$ 11	157 $\pm$ 13
	5–10 cm	130 $\pm$ 1	212 $\pm$ 5	168 $\pm$ 7	204 $\pm$ 9	202 $\pm$ 23
SURF	0–5 cm	130 $\pm$ 1	208 $\pm$ 5	315 $\pm$ 13	248 $\pm$ 25	334 $\pm$ 39
	5–10 cm	130 $\pm$ 1	219 $\pm$ 19	233 $\pm$ 13	235 $\pm$ 5	197 $\pm$ 16
INC	0–5 cm	130 $\pm$ 1	233 $\pm$ 6	273 $\pm$ 16	261 $\pm$ 9	226 $\pm$ 22
	5–10 cm	130 $\pm$ 1	245 $\pm$ 9	235 $\pm$ 19	269 $\pm$ 11	220 $\pm$ 14



**Figure 2** Evolution of the  $^{13}\text{C}$  atom percentage excess over time, measured in the large macroaggregates ( $> 2000\ \mu\text{m}$ ) (■), small macroaggregates ( $2000\text{--}250\ \mu\text{m}$ ) (▨), microaggregates ( $250\text{--}53\ \mu\text{m}$ ) (▩) and the silt and clay fraction ( $< 53\ \mu\text{m}$ ) (▧), for (a) the 0–5 cm and (b) the 5–10 cm soil layer of SURF and INC. Error bars show the 95% confidence interval.

#### *Distribution of residue-C and -N in aggregate fractions at week 33*

The distribution of residue-C and -N in different aggregate fractions of the 0–5 and 5–10 cm soil layer was calculated at each date and for each layer from the aggregate fraction mass, its carbon and nitrogen content and the atom percentage excess of  $^{13}\text{C}$  and  $^{15}\text{N}$ . Initial C and N distribution in the aggregate fractions of CTRL is given in Table 6. As expected from the changes in mass (cf. Figure 1) and in enrichment (cf. Figures 2 and 3), no major evolution in the distribution of  $^{13}\text{C}$  across the various aggregate sizes was observed over time, and therefore only the data obtained at week 33 are commented on here in detail (Table 7).

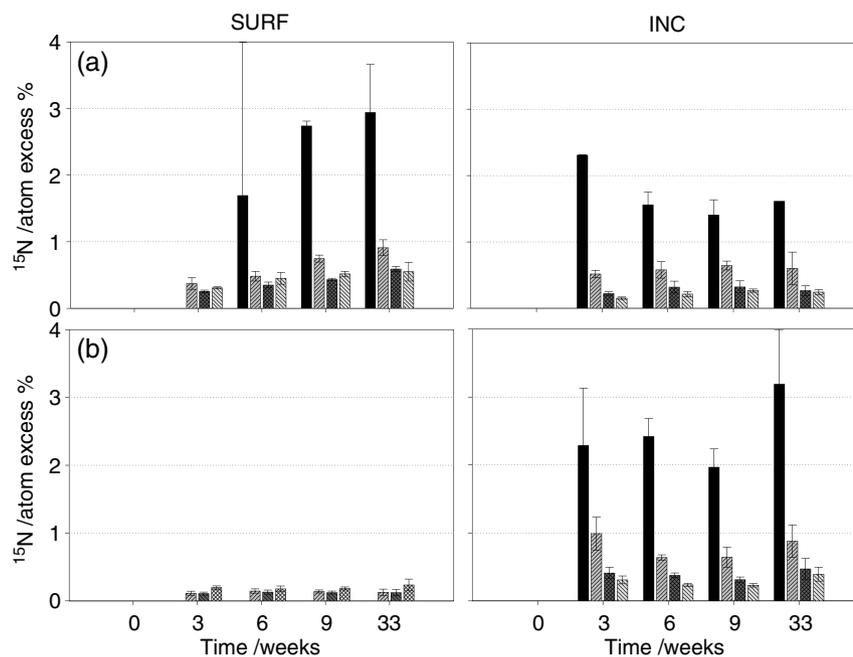
For SURF, no light fraction (LF) at all was recovered from any aggregate size fraction, indicating that  $^{13}\text{C}$  recovered in those fractions originated from soluble  $^{13}\text{C}$  leached from the mulch or that  $^{13}\text{C}$  was translocated by fungal hyphae. In total 9.5% of the added residue-C was present in the sum of the four

aggregate fractions, with a minor contribution of the large macroaggregate fraction ( $> 2000\ \mu\text{m}$ ). In the 5–10 cm soil layer of SURF, the percentage of residue-C increased with decreasing particle size, but in total only 1.4% of  $^{13}\text{C}$  was recovered in these fractions. In the 0–10 cm soil layer, 10.9% of the added C was recovered. This analysis does not take into account the amount of residue-C in the mulch still left at the soil surface (i.e. 52.1% of the added C at week 33).

For INC, the  $^{13}\text{C}$  was almost homogeneously distributed within the two layers due to the initial incorporation of half the residue-C in each layer. No significant difference in the distribution of residue-C over the small macroaggregate ( $2000\text{--}250\ \mu\text{m}$ ), microaggregate ( $250\text{--}53\ \mu\text{m}$ ) and silt and clay fraction ( $< 53\ \mu\text{m}$ ) was observed when comparing week 3 and week 33. At that time 5.4% (0–5 cm) and 7.2% (5–10 cm) of residue-C was recovered in the sum of the four aggregate fractions, with most of this residue-C being measured in the small macroaggregate fraction ( $2000\text{--}250\ \mu\text{m}$ ).

**Table 6** Initial mass distribution and total C and N in the large macroaggregates ( $> 2000\ \mu\text{m}$ ), small macroaggregates ( $2000\text{--}250\ \mu\text{m}$ ), microaggregates ( $250\text{--}53\ \mu\text{m}$ ) and the silt and clay fraction ( $< 53\ \mu\text{m}$ ) of the control soil

	Mass /g kg <sup>-1</sup> soil	C		N		C:N
		/g kg <sup>-1</sup> fraction	/g kg <sup>-1</sup> soil	/g kg <sup>-1</sup> fraction	/g kg <sup>-1</sup> soil	
$> 2000\ \mu\text{m}$	0.0	0.0	0.0	0.00	0.00	–
$2000\text{--}250\ \mu\text{m}$	50.4	11.3	0.6	1.05	0.05	10.8
$250\text{--}53\ \mu\text{m}$	387.8	10.1	3.9	1.04	0.40	9.7
$< 53\ \mu\text{m}$	561.9	7.2	4.0	0.84	0.47	8.6



**Figure 3** Evolution of the  $^{15}\text{N}$  atom percentage excess over time, measured in the large macroaggregates ( $> 2000 \mu\text{m}$ ) (■), small macroaggregates ( $2000\text{--}250 \mu\text{m}$ ) (▨), microaggregates ( $250\text{--}53 \mu\text{m}$ ) (■) and the silt and clay fraction ( $< 53 \mu\text{m}$ ) (▩), for (a) the 0–5 cm and (b) the 5–10 cm soil layer of SURF and INC. Error bars show the 95% confidence interval.

The same trends were observed in the 5–10 cm soil layer. In total for the 0–10 cm layer, the recovered  $^{13}\text{C}$  in the aggregate

fractions amounted to 20.1% of the added C. Although the distribution of residual  $^{13}\text{C}$  within the two soil layers was very

**Table 7** Summary of the distribution of residue-C and -N in soil aggregate and light organic matter fractions (LF) in the 0–5 cm and 5–10 cm soil layer, after 33 weeks of incubation. Residue-C present at the soil surface as mulch is not taken into account. Values are given  $\pm$  the standard errors of the means

			Residue-C /% added		Residue-N /% added	
			SURF	INC	SURF	INC
0–5 cm	Soil aggregates	$> 2000 \mu\text{m}$	$0.2 \pm 0.0$	$0.2 \pm 0.0$	$0.4 \pm 0.1$	$0.4 \pm 0.0$
		$2000\text{--}250 \mu\text{m}$	$4.3 \pm 0.9$	$2.6 \pm 0.9$	$15.1 \pm 2.6$	$5.7 \pm 1.6$
		$250\text{--}53 \mu\text{m}$	$1.8 \pm 0.0$	$1.2 \pm 0.3$	$6.4 \pm 0.2$	$3.4 \pm 0.7$
		$< 53 \mu\text{m}$	$3.2 \pm 0.2$	$1.4 \pm 0.2$	$15.7 \pm 2.0$	$7.3 \pm 1.2$
		Total	$9.5 \pm 1.1$	$5.4 \pm 1.4$	$37.6 \pm 4.8$	$16.8 \pm 1.3$
	LF	$> 2000 \mu\text{m}$	$0.0 \pm 0.0$	$1.6 \pm 0.8$	$0.0 \pm 0.0$	$1.7 \pm 0.8$
		$2000\text{--}250 \mu\text{m}$	$0.0 \pm 0.0$	$1.4 \pm 0.6$	$0.0 \pm 0.0$	$1.6 \pm 0.6$
		$250\text{--}53 \mu\text{m}$	$0.0 \pm 0.0$	$0.3 \pm 0.2$	$0.0 \pm 0.0$	$0.5 \pm 0.2$
		Total	$0.0 \pm 0.0$	$3.3 \pm 1.2$	$0.0 \pm 0.0$	$3.8 \pm 1.2$
		5–10 cm	Soil aggregates	$> 2000 \mu\text{m}$	$0.0 \pm 0.0$	$0.1 \pm 0.0$
$2000\text{--}250 \mu\text{m}$	$0.2 \pm 0.1$			$2.8 \pm 0.6$	$1.1 \pm 0.3$	$8.2 \pm 1.7$
$250\text{--}53 \mu\text{m}$	$0.4 \pm 0.1$			$1.9 \pm 0.3$	$1.8 \pm 0.4$	$6.1 \pm 1.0$
$< 53 \mu\text{m}$	$0.8 \pm 0.1$			$2.4 \pm 0.5$	$7.7 \pm 1.6$	$11.7 \pm 1.4$
Total	$1.4 \pm 0.2$			$7.2 \pm 1.4$	$10.6 \pm 2.3$	$26.1 \pm 4.2$
LF	$> 2000 \mu\text{m}$		$0.0 \pm 0.0$	$1.8 \pm 0.3$	$0.0 \pm 0.0$	$2.0 \pm 0.4$
	$2000\text{--}250 \mu\text{m}$		$0.0 \pm 0.0$	$2.2 \pm 2.0$	$0.0 \pm 0.0$	$2.6 \pm 2.3$
	$250\text{--}53 \mu\text{m}$		$0.0 \pm 0.0$	$0.2 \pm 0.1$	$0.0 \pm 0.0$	$0.3 \pm 0.0$
	Total		$0.0 \pm 0.0$	$4.2 \pm 1.8$	$0.0 \pm 0.0$	$4.9 \pm 2.0$
	0–10 cm		Soil aggregates + LF	Total	$10.9 \pm 1.3$	$20.1 \pm 0.5$

different for SURF and INC, the relative distribution of residual  $^{13}\text{C}$  within the aggregate fractions was similar, with 46–48% of the  $^{13}\text{C}$  in the small macroaggregate (2000–250  $\mu\text{m}$ ) fraction, 19–22% in the microaggregate fraction (250–53  $\mu\text{m}$ ) and 34–26% in the < 53  $\mu\text{m}$  fraction at week 33.

More residue-N entered the soil aggregates compared with residue-C; however, the same trends are observed (Table 7). For SURF, the amount of residue-N incorporated in aggregates increased from 16.9% (week 3) to 37.6% (week 33) in the 0–5 cm soil layer but did not change significantly over time in the 5–10 cm soil layer (8.8–10.6% of residue-N). For INC, the percentage of residue-N present in soil aggregates after 33 weeks of incubation was 16.8% in the 0–5 cm and 26.1% in the 5–10 cm soil layer.

## Discussion

### Soil aggregation

Previous studies concerning aggregate dynamics and distribution of C and N in aggregates often used soils that are recently brought into cultivation and consequently still relatively rich in organic C (e.g. Six *et al.*, 1998). In contrast, agricultural soils of northern France have been cultivated for several hundreds of years and are often poor in native organic matter (e.g. Angers *et al.*, 1997; Puget *et al.*, 2000). Soils from this region are very susceptible to stresses induced by rapid wetting of air-dried aggregates (Le Bissonnais, 1988), which results in the collapse of most macroaggregates by slaking. In addition, the silt loam soil used in this study did not receive organic inputs since 1994 and was strongly depleted in organic C. The initial aggregate MWD of this soil was small and formation of large macroaggregates was weak compared with other aggregation studies (e.g. Deneff *et al.*, 2001b; Six *et al.*, 2001). A single application of fresh organic matter only resulted in small and short-term effects, although the hierarchical model of aggregate build-up (Tisdall & Oades, 1982) could be confirmed. Macroaggregates were formed out of microaggregates, and particulate organic matter was mainly associated with large macroaggregates.

Summarizing the observed effects in the two soil layers over time, the addition of oilseed rape residues resulted in an increased MWD in the 0–10 cm soil layer compared with the control soil after 33 weeks of incubation, without significant differences between SURF and INC. However, in the 0–5 cm soil layer of SURF, more small macroaggregates (2000–250  $\mu\text{m}$ ) and a larger MWD were found than for INC.

Several factors contributed to the increase in MWD over time. The influence of physical processes is demonstrated by the evolution of the MWD in CTRL, which was not affected by the addition of residue-C. Aggregate disruption and redistribution of soil particles after a rain event (Adu & Oades, 1978) and 'soil aging' during subsequent wetting and drying of the soil (e.g. Shainberg *et al.*, 1996) have an impact on the

stability of aggregates. Those physical processes may be responsible for the initial increase in MWD from week 0.

In addition, microbial activity and residue decomposition are biological processes favouring aggregate stability (Tisdall, 1994; Martens, 2000), with a particular role of fungi in the formation of macroaggregates (Molope *et al.*, 1987; Guggenberger *et al.*, 1999; Deneff *et al.*, 2001b). Although the maximum microbial activity for SURF and INC was not significantly different, it has been shown that important variations exist in the ratio of bacterial/fungal biomass (Bossuyt *et al.*, 2001). In particular, the fungal biomass has been reported to be more abundant when residues are placed on the surface compared with being incorporated in the soil (Holland & Coleman, 1987), because of their capacity to translocate soil-N to the surface residue with their hyphal network (Frey *et al.*, 2000). The observed dilution here of residue-N by N originating from the soil supports this hypothesis. Therefore, based on the large concentration of organic debris or LF at the soil surface of the SURF treatment we expected greater macroaggregate formation in the 0–5 cm soil layer of SURF compared with CTRL and INC.

After the first dry/wet cycle, however, no significant differences were found between the MWD of CTRL and SURF, where macroaggregates were not yet slaking resistant. For INC, the large macroaggregates that could be recovered were found attached to the fresh organic matter, which is in agreement with Buyanovsky *et al.* (1994), who suggested that large macroaggregates are organized around plant residue particles. At the end of the second dry/wet cycle, the MWD of SURF had increased significantly in the 0–5 cm soil layer, which can be related to the pulse of microbial activity after the application of rain at week 3, as reported earlier (Coppens *et al.*, 2006). For SURF, the long incubation period between 9 and 33 weeks without aggregate disruptive forces (i.e. rain events) and with favourable conditions for decomposition, is supposed to make the aggregates more slaking resistant. This idea was supported by our observation of higher MWD for SURF (0–5 cm) at week 33. In contrast, the MWD for INC tends to decrease between weeks 9 and 33 and this was probably due to the more advanced decomposition state of the incorporated residues (74% of residue-C mineralized for INC compared with 40% for SURF), which already implied a reduction in microbial activity.

As residue-C for SURF is concentrated at the soil surface, observed differences in aggregation between SURF and INC are diluted by the relevant depth of the upper soil layer (5 cm). Consequently, differences between treatments would be more pronounced when reducing the depth of the upper soil layer. It is expected that those differences in C and aggregate distribution also significantly modify physical and biological properties of the upper cm of soil (e.g. soil structural stability, hydraulic conductivity, microbial activity and oxygen concentration), which thereby determine environmental impacts other than total C storage.

### Residue-C and -N stored in aggregate fractions

The initial location of crop residues affected dramatically the dynamics of residue decomposition as discussed in detail by Coppens *et al.* (2006). This resulted in slower decomposition and a large amount of residue particles left at the soil surface for SURF, which represents 52% of the added C at week 33, while almost all the residue particles were decomposed in the INC treatment. However, as discussed earlier, this mulch of residue particles was not 'untouched': (i) the soluble C and N were leached by rain at an early stage and entered the adjacent soil layer as shown by the dynamics of  $^{13}\text{C}$  and  $^{15}\text{N}$  in the soil fractions; and (ii) the mulch particles were colonized by the decomposing microbial biomass, as shown by the changes in C:N ratio and  $^{15}\text{N}$  labelling of the residual mulch. The total residual  $^{13}\text{C}$  in the INC treatment (20.1% of added  $^{13}\text{C}$ ) after 33 weeks at 20°C, which corresponds to about 1.4 years at 10°C ( $Q_{10} = 2.2$ ), is in the range of values obtained in field conditions on the same soil (e.g. Aita *et al.*, 1997). By comparing the total residual  $^{13}\text{C}$  in the soil (INC) or mulch + soil (SURF), we can conclude that, in the short term, a major result of having crop residues decomposing on the soil surface is a large LF fraction that is maintained on this soil surface, due to reduced soil-residue contact that slows down decomposition.

Basically, the two treatments differ in the way the carbon enters the soil. With the residues left on the soil surface (SURF), some of the  $^{13}\text{C}$  entered the soil, possibly by diffusion at the soil-residue interface near the soil surface (as observed by Gaillard *et al.*, 1999), but more likely by convective transport after rain, as demonstrated by the large increase in the soluble C content of the soil solution just after rainfall (Coppens *et al.*, 2006). This is corroborated by the observation that no LF-C ( $> 2000 \mu\text{m}$ ) is found within the soil or associated with aggregate fractions. For the INC treatment, all of the LF-C ( $> 2000 \mu\text{m}$ ) was initially present in the soil and it decreased rapidly as decomposition proceeded. Little residue-C remained associated with aggregate fractions at week 33 (3–4% of added  $^{13}\text{C}$  in each layer).

Although differences exist in the way residue-C enters the mineral soil fraction, the total amount of  $^{13}\text{C}$  in the 2000–250  $\mu\text{m}$ , 250–53  $\mu\text{m}$  and  $< 53 \mu\text{m}$  aggregate fractions was rather small both for SURF and INC, equivalent to 10.6–12.3% at week 33, with no significant difference between the two treatments. However, this carbon was mostly located in the upper layer for SURF, while, as expected, it was almost equally distributed over the two soil layers in which the residues were initially incorporated for INC.

Surprisingly, the relative distribution of  $^{13}\text{C}$  in the various aggregate fractions at week 33 did not differ between the two treatments, despite the completely different way for new C to enter the soil, i.e. despite the mixing of residue particles with the soil for INC. In the short term (0–9 weeks), residue-derived organic matter may have been incorporated into the

macroaggregates of INC, in addition to diffusion of soluble carbon and nitrogen into the aggregates as observed for SURF. This initially resulted in larger amounts of residue-C and -N in the larger aggregate fractions of INC compared with SURF, but this effect was only transient. Our findings confirm the hypothesis of Smucker *et al.* (2003), who suggested that diffusion of soluble carbon is the main process for enriching soil aggregates with recently deposited residue-C. Furthermore, over time (3–33 weeks) little change was observed for the residue-C recovered in aggregates, which suggests that the association of residue-C with the soil aggregates was established very early, during the first 3 weeks of its decomposition, after which it was protected against mineralization. This supports the concept of a two-compartment system with a soluble fraction of crop residues that diffuses early and is stabilized (before or after being assimilated by the microbial biomass) and a non-soluble residue fraction (in the soil or at the soil surface) that supports the growth of colonizers, contributes more to net C mineralization and has little interaction with the soil matrix. This conceptual schema was already proposed by, for example, Gaillard *et al.* (2003).

As observed by Angers *et al.* (1997), more of the added residue-N than -C was recovered in the soil aggregates, for both residue treatments. Except from small denitrification losses, all residue-N remained in the soil while residue-C disappeared as  $\text{CO}_2$  through mineralization. Where residue-C for SURF was mainly preserved as LF or organic debris on the soil surface, the largest part of residue-N at week 33 was found in the soil aggregate fractions. The larger amount of N in the soluble residue fraction (71% N compared with 39% C) resulted in a larger accumulation of residue-N than -C in the soil aggregates. This also corresponded with Angers *et al.* (1997), who attributed the initial difference in distribution of residue-N to the presence of soluble N in the residues.

### Conclusion

Under experimental conditions that simulate wetting-drying conditions of field situations, oilseed rape residues were applied as mulch or incorporated in the 0–10 cm soil layer. Spatial separation of soil and surface placed residues slowed down the decomposition rate compared with residue incorporation. This resulted in the short term, in a large fraction of organic debris (LF) at the soil surface, while almost all of the LF had disappeared with residue incorporation. The distribution of residue-C and the associated microbial activity determined the dynamics and concentration of macroaggregates in soil: residue addition increased the aggregate mean weight diameter (MWD) compared with the control soil and a larger MWD was obtained in the 0–5 cm soil layer under mulch than with residue incorporation.

In contrast to the larger amount of residual LF for soil with residue mulch than with residue incorporation, the total amount of  $^{13}\text{C}$  recovered in the aggregate fractions did not

differ significantly between treatments and was rather small, about 11% of added C. However, this C was mostly located in the upper soil layer with mulch, while it was almost equally distributed over the two soil layers with residue incorporation. Despite the different way for new C to enter the soil (i.e. leaching of residue-C after rainfall with mulch versus diffusion of residue-C and residue colonization with incorporation), the relative distribution of  $^{13}\text{C}$  in the various aggregate fractions did not differ between the two treatments at week 33, probably because in both treatments residue-C mainly entered the soil aggregate fractions as soluble C.

In the medium to long term, the observed distribution of residue-C in soil aggregate fractions at week 33, suggests the following scenario:

**1** Residue-C enters the aggregate fractions as soluble C, either after being transported from the residue mulch by convective transport (after rain), or by diffusion and convective transport from those residue particles surrounded by soil in the case of incorporation. The incorporation of C in soil fractions occurs early and does not change much over time, and the decomposition of residue-derived organic matter (LF, > 2000  $\mu\text{m}$ ) in the soil does not seem to have any significant impact on C storage in aggregates.

**2** Therefore, as the main factor determining the storage of residue-C in the soil in the medium to long term would be the initial quality of the crop residue (i.e. the amount of soluble C), the initial residue location has no significant impact on long-term C storage in soil, at least not through differential incorporation into stable aggregate-fractions. In the longer term, the fate of mulch-C accumulated at the soil surface should be examined.

**3** Although the effect of crop residue location on soil aggregation was negligible when expressed as a function of the amount of residue added, our results clearly showed a greater concentration of the residue-C and -N and increased aggregation near the soil surface with residue mulch. This enhanced aggregation at the soil surface may contribute to increased stabilization of residue-C in soil in the longer term.

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