

Soil moisture, carbon and nitrogen dynamics following incorporation and surface application of labelled crop residues in soil columns

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Summary

One way to increase the amount of carbon sequestered in agricultural land is to convert conventional tillage into no-tillage systems. This greatly affects the location of crop residues in soil. To investigate the impact of the location of residues on soil physical and biological properties and how the interactions between those properties influence the fate of carbon and nitrogen in soil, we did a laboratory experiment with repacked soil in columns. Doubly labelled $^{13}\text{C}^{15}\text{N}$ oilseed rape residues were incorporated in the 0–10 cm layer or left on the soil surface. The columns were incubated for 9 weeks at 20°C and were submitted to three cycles of drying and wetting, each of them induced by a rain simulator. The location of the residues affected the water dynamics and the distribution of C and N in the soil, which in turn influenced microbial activity and the decomposition rate of the added residues. After 9 weeks of incubation, $18.4 \pm 1.5\%$ of the surface applied residue-C and $54.7 \pm 1.3\%$ of the incorporated residue-C was mineralized. We observed a nitrate accumulation of $10.7 \text{ mg N kg}^{-1}$ with residues at the soil surface, 3.6 mg N kg^{-1} with incorporated residues and 6.3 mg N kg^{-1} without addition of fresh organic matter, which entailed net N mineralization in soil under mulch and immobilization of N with residue incorporation compared with the control soil. We concluded that application of oilseed rape residues at the soil surface increased the storage of fresh organic C in soil in the short term, compared with the incorporation treatment, but increased the risk of nitrate leaching.

Introduction

Most of the observed global warming over the last 50 years has almost certainly been caused by increased concentrations of greenhouse gases in the atmosphere as a result of human activities. The projected rate and magnitude of this warming can be lessened by diminishing the emissions of carbon dioxide, nitrous oxide and methane. In addition, we can try to sequester carbon to mitigate CO_2 emissions (Paustian *et al.*, 1997). We can do so by increasing the input of organic matter, by decreasing the loss of carbon due to mineralization, or both (Arrouays *et al.*, 2002). The return of crop residues to the soil, in combination with conservation tillage, has been shown to be effective in increasing the C content of soils (Paustian *et al.*, 1997; West & Marland, 2002).

Reducing tillage has an impact both on soil physical properties, such as structure and stability, water infiltration rate and soil temperature, and biological properties such as nutrient

availability and the diversity of soil biota (Blevins & Frye, 1993; Tebrügge & Düring, 1999; Holland, 2004). The combined effect of those properties, together with climatic factors and their interactions, determines the biotransformations of organic matter in soil. To understand interactions between physics and biology following changes in tillage practice, we must distinguish the influence of the modified location of crop residue from the mechanical effect of soil disturbance.

Several authors have examined the effect of the location of crop residues on the decomposition rate (Douglas *et al.*, 1980), CO_2 emission (Curtin *et al.*, 1998), N mineralization (Corbeels *et al.* 2003) or microbial activity (Holland & Coleman, 1987). However, residues left at the soil surface also retard the initial evaporation of water (Bond & Willis, 1969) and hence change the soil water regime during decomposition. As far as we know, there have been no studies that combine biological and physical processes, but such studies are essential to analyse the interactions of residue location with the carbon and nitrogen cycles. For example, Abiven *et al.* (2002) did an incubation experiment in the laboratory with sieved soil under constant temperature and moisture conditions and with

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ample nitrogen. They found that carbon mineralization was unaffected by the location of residues. An important factor that could explain this lack of response is the absence of the effect of residue location on water transport in the experiment. Under field conditions, however, rain modifies the distribution of carbon and nitrogen derived from the fresh organic matter in the soil, creating gradients in nutrient concentrations through the soil profile (Cannavo *et al.*, 2004). Also, the subsequent cycles of wetting and drying in field situations are not taken into account in the simplified laboratory incubation experiments.

We therefore designed an experiment to assess the interactions between physical and biological processes in the soil that are affected by the initial location of crop residues in the soil. The main research questions of this work were as follows.

- 1 How does the location of crop residues influence soil water dynamics and the distribution of carbon and nitrogen in soil?
- 2 How do these effects in turn influence soil microbial activity and the residue decomposition rate?

Materials and methods

Soil

The soil used in the experiment is a silt loam, Orthic Luvisol (clay 13.4%, silt 81.6%, sand 5.0%), obtained from the experimental site of INRA (Institut National de la Recherche Agronomique), Mons-en-Chaussée, in northern France. The soil had not been cropped since 1994. The top 25 cm soil layer was sampled, sieved (to pass 2 mm) at field moisture content (0.17 g g^{-1}) and stored in plastic bags at 4°C prior to use. The soil pH in water was 8.2, and the soil contained 8.5 g kg^{-1} organic carbon and 0.9 g kg^{-1} nitrogen, resulting in a C:N ratio of 9.4. The organic matter content was 13.3 g kg^{-1} , the total carbonate content 7.0 g kg^{-1} and the cation exchange capacity $8.1 \text{ cmol}_c \text{ kg}^{-1}$. The soil was pre-incubated for 2 weeks at 20°C before the start of the experiment.

Crop residue

The fresh organic matter used in this experiment was mature oilseed rape (*Brassica napus* L.), labelled with ^{13}C and ^{15}N . We obtained the plant residues by growing a rape crop under hydroponic conditions in a labelling growth chamber, with enriched $^{13}\text{CO}_2$ atmosphere (^{13}C atom percentage excess = 3.13%) and in a nutrient solution with ^{15}N labelled KNO_3 (^{15}N atom percentage excess = 9.8%). This resulted in a homogeneous labelling of all plant parts. The overall plant growth conditions are described by Trinsoutrot *et al.* (2000b). The residues applied consisted of a mixture of leaves, stalks, branches and pods, chopped into pieces 1 cm long. The C and N content of the mixture was 42.2% C and 1.45% N, resulting in a C:N ratio of 29. The atom percentage excess was 2.88% for ^{13}C and 9.73% for ^{15}N . Its biochemical composition was determined by proximate analysis (Van Soest, 1963), and was 45.6% soluble compounds, 14.4% hemicellulose, 33.2% cellulose and 6.7% lignin-like fractions. Details of the C and N content of each fraction are given in Table 1.

Soil column preparation

Plastic cylinders (PVC, 15.4 cm inner diameter and 30 cm high) with perforated bases were used, each to contain 25 cm of soil, compacted at 1.3 g cm^{-3} . To this end, 6.0 kg (dry weight) of the pre-incubated soil was divided into three subsamples, which were successively compacted homogeneously to a predefined volume of the cylinder. The surface of a compacted soil layer was loosened before addition of the next soil layer to maintain continuity in the arrangement of soil particles over the 25-cm soil profile. Residues of oilseed rape were applied at the surface (referred to as SURF) or homogeneously mixed in the upper 10 cm before compaction (referred to as INC) at a rate of $13.8 \text{ g dry matter column}^{-1}$, equivalent to a return of $7.4 \text{ t dry matter ha}^{-1}$. Control columns (referred to as CTRL) were prepared without residues. There were thus three main treatments; for CTRL

Table 1 Carbon and nitrogen content of the biochemical fractions of the applied oilseed rape residue

	Analyses for oilseed rape residue				Analyses for biochemical fractions	
	Mass /%	C /% ^a	N /% ^a	C/N	C /% ^b	N /% ^b
Total residue	99.9	42.2	1.45	29		
Soluble fraction (CWE) ^c	25.7	29.4	2.12	14	17.8	47.2
Soluble fraction (NDS) ^c	45.6	35.5	1.75	20	39.0	71.0
Hemicellulose	14.4	36.4	0.75	49	12.6	9.6
Cellulose	33.2	48.3	0.39	125	38.7	11.4
Lignin + ash	6.7	59.2	1.33	44	9.6	8.0

^a%C and N in mass of biochemical fraction.

^bPercentage of total residue-C and residue-N.

^cThe cold water-extractable fraction (CWE) is part of the fraction extracted with neutral detergent solution (NDS) (Van Soest, 1963).

and INC, we prepared 11 columns, and for SURF, 14 columns. The three extra columns were used for the determination of the moisture content of the mulch, making a total of 36 in all. When residues were incorporated, a certain volume of soil was replaced by the equivalent volume of the added residues to obtain the same apparent bulk density over the whole soil profile for all treatments.

Experimental conditions

At the start of the incubation period, artificial rain was applied with a rainfall simulator to all of the soil columns for 2.5 hours at a rate of 12 mm hour⁻¹. The simulator consisted of 380 capillary tubes (inner diameter 0.5 mm) equally distributed over a surface of 1 m², at 4 m above the soil surface. We controlled the rain intensity by adjusting the water pressure. A wire mesh placed 1 m below the capillary tubes led to a homogeneous distribution of the raindrops. The applied water was obtained from filtered rain water, with a pH of 6.5 and a composition of 1.5 mg dissolved organic carbon litre⁻¹, 1.6 mg dissolved inorganic carbon litre⁻¹, 1.3 mg NO₃⁻-N litre⁻¹ and < 0.1 mg NH₄⁺-N litre⁻¹. After this treatment, the volumetric water content of the soil increased from 0.22 to 0.34 cm³ cm⁻³, and no drainage was observed. Subsequently, the 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, the columns were again placed under the rainfall simulator until the water lost by evaporation was replaced. The applications of rain allowed us to extract the soil solution and to determine the final concentrations of dissolved organic C and mineral N. Therefore, the four rain events, further referred to as R0, R3, R6 and R9, defined three cycles of wetting and drying.

Experimental design

At the end of each of the three evaporation periods (before re-wetting), three columns of CTRL, SURF and INC were used for destructive analysis. This accounts for 27 of the columns. The soil in these columns was sliced into four layers: 0–5 cm, 5–10 cm, 10–17.5 cm and 17.5–25 cm. For each treatment (CTRL, SURF, INC), another two columns were equipped with probes to provide information about the transport of water and solutes during the 9-week incubation period (this accounted for an additional six columns). These columns were also used for CO₂-flux measurements. Finally the three extra columns, assigned to the SURF treatment, were constructed with detachable mulch: the residues were placed on a 1-mm mesh to allow us to measure the mass of mulch. These data were used to calculate the gravimetric water content of the mulch, after correction of the mass for leached and mineralized C.

Destructive soil analysis

For the SURF treatment, the mulch was removed from the soil surface and dried at 60°C; for the INC treatment, the coarse residue fraction (> 2 mm) was separated from the fine fraction (< 2 mm) and also dried at 60°C. The gravimetric soil water content was determined on a 20-g subsample of the fine fraction, after 24 hours drying at 105°C. The soil pH was measured after 30 minutes shaking in water (soil/solution ratio 1/2). Soil mineral nitrogen was extracted with 1 M KCl (30 minutes' shaking, soil-to-solution ratio 1/2.5). We determined soluble carbon by extracting 30 g of soil in 160 ml 0.03 M K₂SO₄ (30 minutes' shaking). Soil extracts for mineral N and soluble C were centrifuged (20 minutes at 5800 g), filtered and stored at -20°C until analysis. The mineral nitrogen (NO₃⁻-N and NH₄⁺-N) was measured by continuous flow colorimetry (TRAACS 2000, Bran & Luebbe, Norderstedt, Germany). We distinguished dissolved inorganic and organic C in the soluble extract: acidification of the extract by 5% H₃PO₄ (for the inorganic C) was followed by chemical oxidation in a persulfate medium (for the organic C), the CO₂ produced being measured by infrared spectrometry (1010, O.I. Analytical, College Station, TX, USA). Soluble carbon determined in K₂SO₄ extracts of the soil at the end of each evaporation period (before re-wetting) was considered as 'potentially available' dissolved organic carbon (DOC_{pa}).

The total C and N content of the soil and the recovered plant residues, with their atom percentage excess, were determined with an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) coupled to a mass spectrometer (Fisons Isochrom, Manchester, UK). The soil adhering to the recovered residues was detached and analysed separately for C and N.

We measured the microbial activity in each layer of soil using the substrate induced respiration technique (SIR) (Anderson & Domsch, 1978; Lin & Brookes, 1999). We prepared a glucose solution to add 500 µg glucose-C g⁻¹ soil by bringing the gravimetric soil water content to 0.24 g g⁻¹. A subsample of 3 g soil was incubated for 6 hours at 20°C in a sealed 10-ml tube. The amount of CO₂ produced was measured on an infrared gas analyser (UNOR 610, Maihak, Hamburg, Germany).

Semi-continuous measurements

At 6 and 14 cm depth, the volumetric water content was measured by horizontally inserted TDR-probes (three rods, 8 cm long). The measurements were recorded every hour on a TRASE system (Soilmoisture Equipment Corporation, Santa Barbara, CA, USA). At the opposite side of the columns, at 6 and 14 cm depth, the soil water potential was determined by tensiometers with porous cups 5 cm long and 3 mm diameter (RhizoCera, Rhizosphere Research Products, Wageningen, The Netherlands) which were connected to differential pressure sensors (CZ5022/2, EuroSensor, Towcester, UK). Measurements of the soil water potential were stored on a CR10X data acquisition centre (Campbell Scientific Ltd., Courtaboeuf, France).

Mass loss of the columns was used to calculate daily evaporation rates.

Soil solution (10 ml) was sampled 12 hours after each application of rain at 2, 10 and 18 cm depth (Rhizon MOM 10 cm, Rhizosphere Research Products). The pH was determined immediately on the 10 ml samples, which were further stored at -20°C until analysis for mineral nitrogen (TRAACS 2000, Bran & Luebbe) and soluble carbon (1010, O.I. Analytical). Soluble carbon determined in the soil solution sampled after each rainfall was considered as 'mobile' dissolved organic C (DOC_m).

The flux of CO_2 from the soil surface to the atmosphere was calculated from the accumulation rate of CO_2 in the headspace of the columns. During a 3-minute period, the columns were sealed with a cover that was connected to an infrared gas analyser (UNOR 610, Maihak). A fan on the inside of the 'closed chamber' mixed the air to make it homogeneous. The slope of a linear regression of the increase of CO_2 concentration over time was used to calculate the CO_2 flux. Measurements were made daily at the beginning of every evaporation cycle; the frequency of periodic measurements was decreased after the initial flush of microbial activity.

Statistical analysis

The data were analysed as a complete randomized design with repeated measurements using the MIXED procedure from the SAS statistical package. For the independent variables 'gravimetric soil water', 'potentially available DOC', 'mineral nitrogen content', 'soil microbial activity', ' ^{13}C in fine fractions', ' ^{13}C in coarse fractions', ' ^{15}N in fine fractions' and ' ^{15}N in coarse fractions' sampling occurred destructively over time. These were analysed with 'treatment' and 'time' as uncorrelated main effects, and depth as a longitudinal (correlated or repeated measurements) variable, with the soil column as subject. Time was not considered as longitudinal as the samples were taken destructively for these analyses. All possible interactions between main effects were tested for their significance by the type III sum of squares. Covariance structures considered for the repeated measurement variable 'depth' were (i) unstructured, (ii) heterogeneous first-order autoregressive, and (iii) first-order autoregressive (Verbeke & Molenberghs, 2000). The different covariance structures were compared by a likelihood ratio test at $P < 0.05$. The independent variables 'mobile DOC (non-destructive)', and 'nitrate concentration (non-destructive)' were analysed by a multivariate repeated measurements design. The 'treatment' variable was considered as a (uncorrelated) main effect, and variables 'time' and 'depth' as longitudinal (correlated) variables. This time, the variable 'time' was a longitudinal variable, as the samples were not taken destructively. The covariance structures considered were direct products of an unstructured, and an unstructured, first-order autoregressive or compound symmetry, as these are the only ones supplied by the mixed procedure from SAS. The

' CO_2 flux' variable was analysed with treatment as a main (uncorrelated) effect and time as a longitudinal variable with a heterogeneous first-order autoregressive covariance structure.

Results

The main factors, treatment, depth and time, had a significant effect on all of the variables, except for the microbial activity, which did not differ significantly with time (Table 2). The effect of treatment varied significantly with depth, except for ^{13}C and ^{15}N in the coarse fraction. The effect of treatment also varied significantly with time, except for microbial activity, ^{13}C and ^{15}N in the coarse fraction and ^{15}N in the fine fraction. Treatment, depth and time had a significant three-way interaction effect on soluble C and nitrate (both measured destructively and non-destructively), indicating that the effect of treatment on soluble carbon and nitrate in soil varied with time and depth, and that the variation with time depended on depth.

Influence of crop residue location on water dynamics

Leaving crop residues at the soil surface reduced water loss by 47–59% compared with the control, whereas incorporation of residues did not affect the total evaporation. The differences in evaporation between CTRL and INC on the one hand and SURF on the other hand strongly influenced the distribution of water in the profile (Figure 1). The greater water loss in CTRL and INC compared with SURF resulted in the development of a more pronounced gradient in the water content, which increased significantly from 0.15 g g^{-1} at the top to 0.20 g g^{-1} at the bottom of the columns. The water content for SURF was significantly larger and equally distributed over all layers (0.225 g g^{-1}). The small difference in water content between CTRL and INC could have arisen from the construction of the soil columns, where the soil for INC required longer manipulation and might have lost some water.

Figure 2 shows the change in the water content of the mulch during the 9-week incubation, combined with the change in residual mulch mass. The first rainfall event (R0) increased the water content of the mulch from 0.08 to 2.10 g g^{-1} . After 7 days of evaporation, a constant content of 0.33 g g^{-1} was reached. After R3 and R6, there was a small increase in water storage capacity (up to 2.25 g g^{-1}) and a faster reduction of the water content in the mulch until a constant was observed, while the mass of the mulch decreased over time.

We obtained water retention curves of the soil for CTRL, SURF and INC by combining the experimental results of TDR probes and tensiometers. Within treatments, a small shift in the water retention curve was measured over each evaporation period, towards a smaller volumetric water content for the same matric potential. The destructive impact of rain on soil aggregates and the transport of soil particles

Table 2 Analysis of variance to investigate the interactive effects of treatment (TRT), depth (D) and time (T) on selected variables

	Transformation	TRT	D	T	TRT × D	TRT × T	D × T	TRT × D × T	Covariance structure (depth)	Covariance structure (time)
Gravimetric soil water (destructive)	Log	***	**	***	***	***	**	NS	Autoregressive	NR
Potentially available DOC (destructive)	Log	***	***	***	***	***	*	***	Autoregressive	NR
Mineral nitrogen content (destructive)	–	***	***	***	***	***	***	***	Heterogeneous autoregressive	NR
Soil microbial activity (destructive)	Log	***	***	NS	***	NS	**	NS	Autoregressive	NR
Mobile DOC (non-destructive)	–	*	*	***	*	***	***	***	Compound symmetry	Unstructured
Nitrate concentration (non-destructive)	–	*	***	***	*	***	**	***	Compound symmetry	Unstructured
CO ₂ flux (non-destructive)	Log	***	NR	***	NR	***	NR	NR	NR	Heterogeneous autoregressive
¹³ C in fine fractions (destructive)	–	***	***	***	***	***	NS	NS	Heterogeneous autoregressive	NR
¹³ C in coarse fractions (destructive)	–	***	***	***	NS	NS	NS	NS	Unstructured	NR
¹⁵ N in fine fractions (destructive)	Log	***	***	***	***	NS	***	NS	Heterogeneous autoregressive	NR
¹⁵ N in coarse fractions (destructive)	Log	***	***	**	NS	NS	NS	NS	Unstructured	NR

TRT = treatment; D = depth; T = time; NS = not significant; NR = not relevant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

with infiltrating water are assumed to be responsible for this change. This effect was less pronounced for SURF, where the soil surface was protected from direct rain impact. No significant differences in water retention due to the presence of fresh

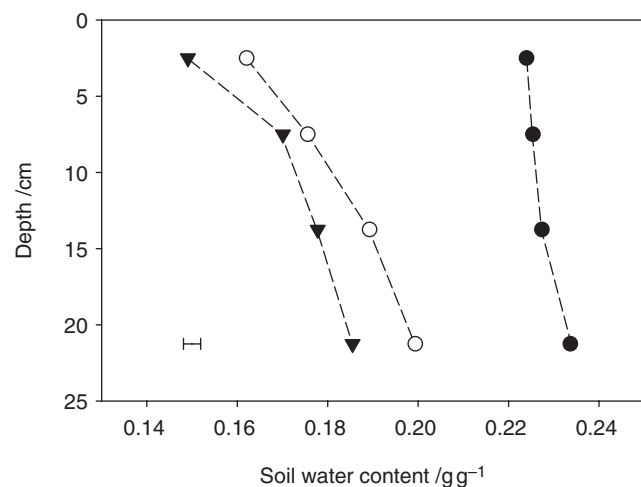


Figure 1 Distribution of the gravimetric soil water content through the soil profile of the control (CTRL ○), surface (SURF ●) and incorporation treatments (INC ▼) at the end of a 3-week evaporation period. Values are the average of the three cycles of wetting and drying. The error bar is of length equal to 2× the standard error of the means.

organic matter could be observed, irrespective of the mode of application.

PH in soil and soil solution

Addition of crop residues (SURF and INC) decreased the pH of the soil and soil solution at all sampling depths after the

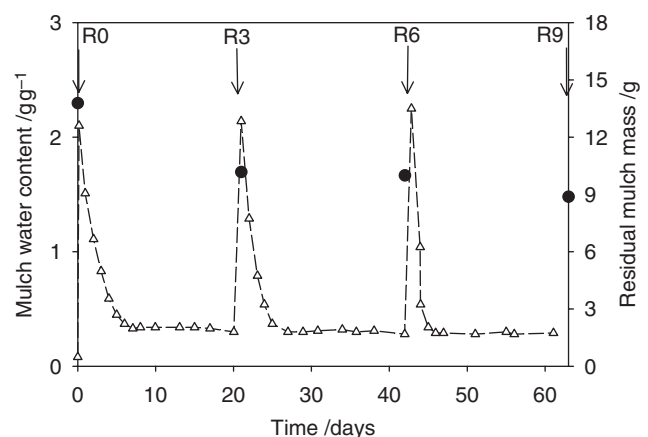


Figure 2 Change in the gravimetric water content of residues at the soil surface over the three cycles of wetting and drying (Δ) and the residual mass of surface applied residues (SURF ●). Arrows indicate the application of artificial rain (R0-R9).

first rainfall (R0). This decrease in pH ranged from -0.3 to -0.6 units for SURF and between -0.8 and -1.2 units for INC compared with the control, which had a pH between 8.2 and 8.4. The greater partial pressure of CO_2 in SURF and INC, caused by the increased microbial activity compared with CTRL, resulted in an increased amount of dissolved CO_2 and consequently in an acidification of the soil solution. This effect was less pronounced after the later rainfall events. No clear change with depth or time was observed in the pH measured in soil extracts at the end of the three evaporation periods.

Distribution of soluble carbon

The concentration of mobile dissolved organic C (DOC_m) in the soil solution of CTRL was fairly constant over time and distributed homogeneously through the column, with an average concentration of $9 \text{ mg DOC}_m \text{ litre}^{-1}$ (Figure 3). After the first rain (R0), concentrations of mobile dissolved C at 2 and 10 cm depth were significantly larger for SURF and INC than for CTRL. In addition, the distribution of this form of C in the columns was different in SURF from INC; the maximum concentration for SURF was at 2 cm depth ($110 \text{ mg DOC}_m \text{ litre}^{-1}$) and for INC at 10 cm depth ($117 \text{ mg DOC}_m \text{ litre}^{-1}$). Although smaller concentrations of the dissolved C were obtained after the later rain events, the same pattern in distribution over the soil profile was observed.

At week 3, there was a significantly larger amount of potentially available dissolved organic C (DOC_{pa}) in the 0–5 cm and 5–10 cm layers of SURF than in CTRL and INC (Figure 4). At this time, the amount of this form of C left in the 0–10 cm layer of INC was only 46.5% of the amount measured for SURF. At week 6, the amount of DOC_{pa} in the 0–5 cm and 5–10 cm layers had significantly decreased in SURF and increased in INC compared with week 3. Below 10 cm, no significant differences were observed between treatments. At week 9, the amounts and distribution of DOC_{pa} were similar to those in week 6. For CTRL, the amounts of DOC_{pa} were stable over time and distributed homogeneously in the columns.

Microbial activity: substrate-induced respiration

The application of crop residues stimulated the substrate-induced respiration (SIR) in the soil, irrespective of the mode of addition (INC or SURF) (Figure 5). Respiration induced by the substrate in the 0–5 cm and 5–10 cm layers of SURF was significantly greater than for CTRL. For the INC treatment, respiration was greater down to the 10–17.5 cm layer than in the control. In these layers with stimulated SIR (0–5 cm and 5–10 cm for SURF; 0–5 cm, 5–10 cm and 10–17.5 cm for INC), there was no significant change over time. Maximum rates of respiration were found in the 0–5 cm layer of SURF ($5.3 \mu\text{g C g}^{-1} \text{ soil hour}^{-1}$) and in the 5–10 cm layer of INC ($5.6 \mu\text{g C g}^{-1} \text{ soil hour}^{-1}$). Over the total soil profile (0–25 cm), a larger average SIR was obtained for INC

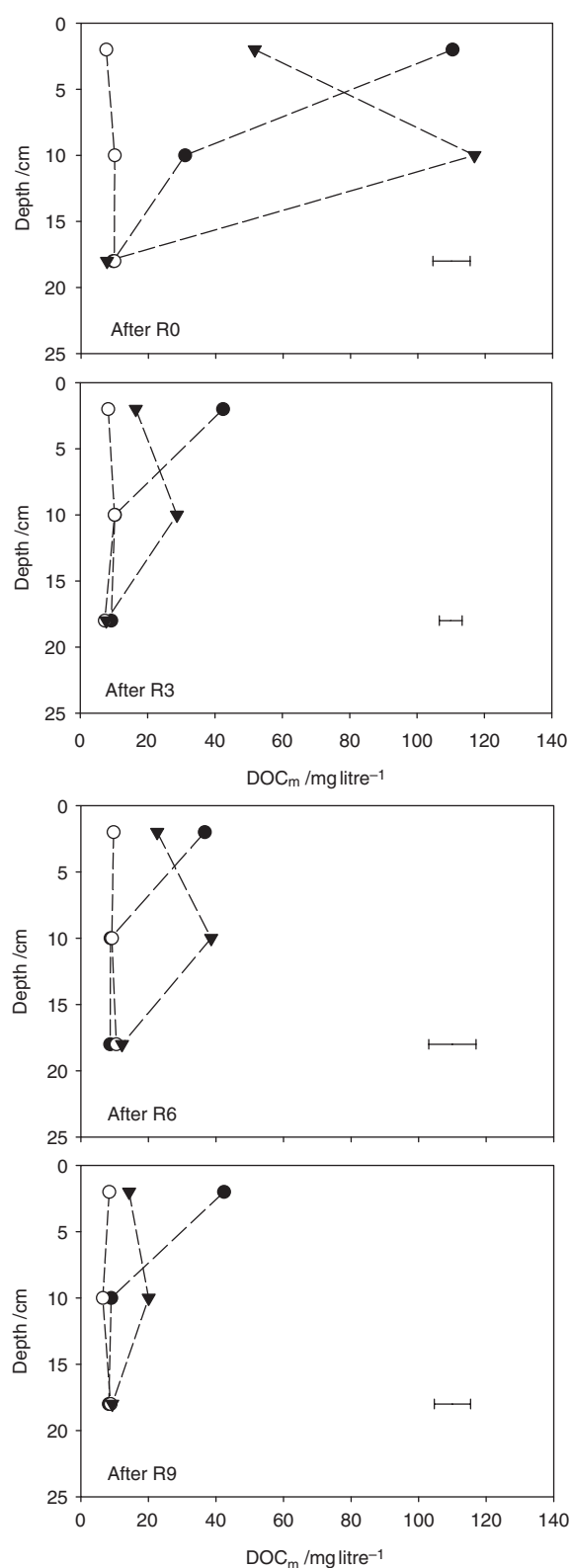


Figure 3 Distribution of the 'mobile' dissolved organic carbon concentration (DOC_m) in the soil solution after rainfall R0, R3, R6 and R9 of the control (CTRL ○), surface (SURF ●) and incorporation treatments (INC ▼). The error bars are of length equal to $2 \times$ the standard errors of the means.

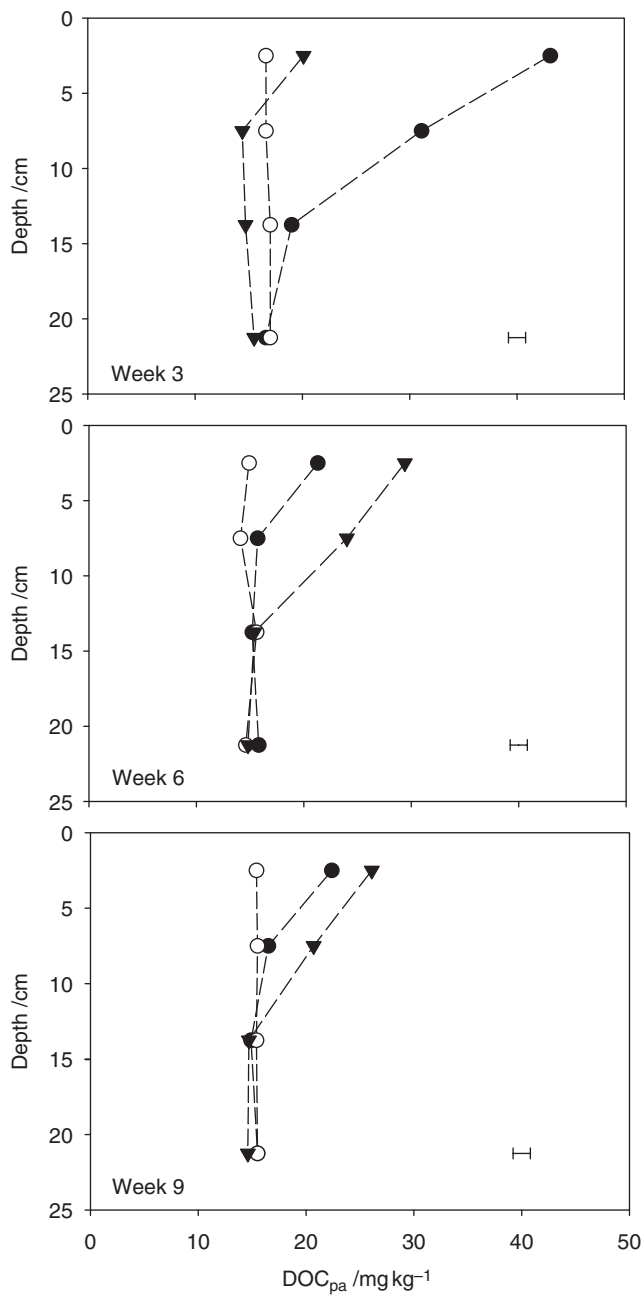


Figure 4 Distribution of the amount of ‘potentially available’ dissolved organic carbon (DOC_{pa}) with depth after 3, 6 and 9 weeks of incubation (before re-wetting) in the control (CTRL ○), surface (SURF ●) and incorporation treatments (INC ▼). The error bars are of length equal to $2 \times$ the standard errors of the means.

($21.1 \text{ mg C hour}^{-1}$ per column) than for SURF ($17.9 \text{ mg C hour}^{-1}$ per column).

Residue decomposition

For the SURF treatment, 64% of the initial mulch mass was recovered after 9 weeks of incubation (Figure 2). For the INC

treatment, only 19% of the added residue mass was found in the coarse fraction ($>2 \text{ mm}$) at the same date. The unrecovered part of the residue mass was mineralized or had entered the fine soil fraction ($<2 \text{ mm}$). For the INC treatment, we also observed different rates of decomposition at different depths: at week 3, 49% of the initial residue mass was recovered in the

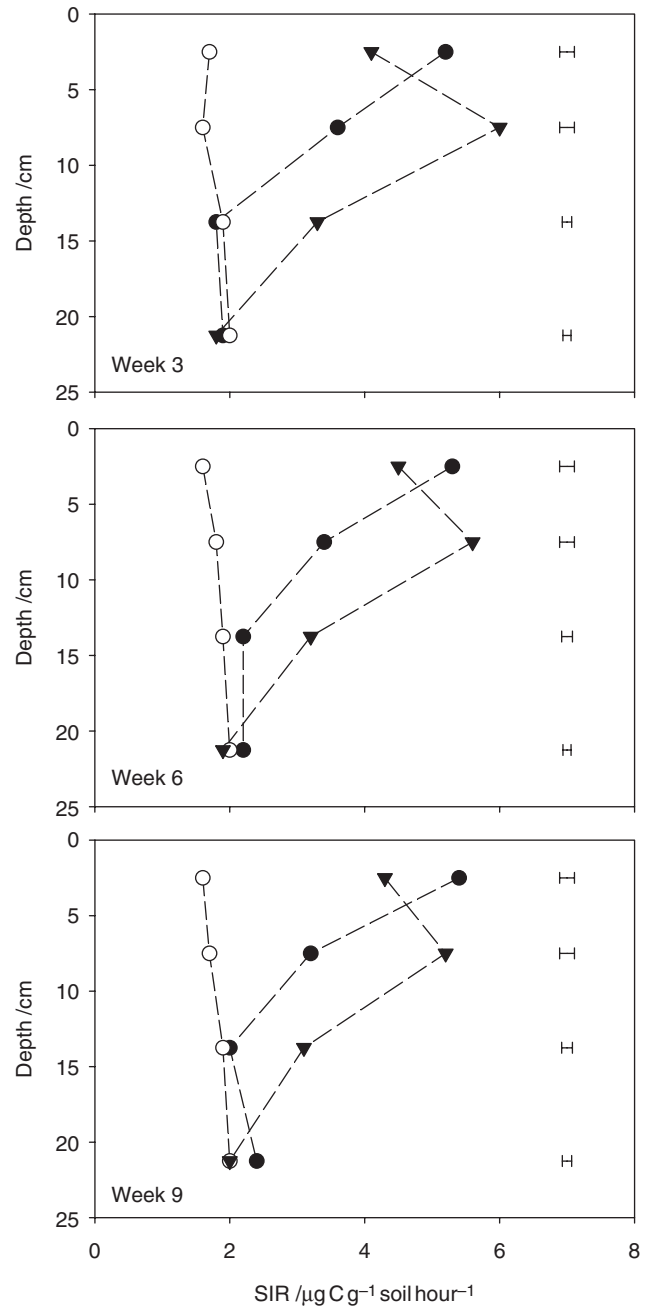


Figure 5 Distribution of the soil microbial activity with depth, measured by substrate induced respiration, after 3, 6 and 9 weeks of incubation (before re-wetting) of the control (CTRL ○), surface (SURF ●) and incorporation treatments (INC ▼). The error bars are of length equal to $2 \times$ the standard errors of the means.

0–5 cm soil layer and only 28% in the 5–10 cm soil layer. At week 9, no significant difference in decomposition was observed between the two layers.

The rate of mineralization of organic C was calculated from the CO₂ flux emitted from the soil surface (Figure 6). In general, the larger flux of CO₂ from INC was greater than from SURF. After a rain event, however, the increased water content diminished the emission of CO₂ from CTRL and INC. This is probably due to slower diffusion of CO₂ to the atmosphere (Jensen *et al.*, 1996) or trapping of mineral C in the soil solution as indicated by an increased amount of dissolved inorganic carbon in the soil over time, or both. This effect was less for SURF, where CO₂ emitted from the mulch did not interact with the soil, and there was an increased flux after each rain event. For SURF and INC, the rate of mineralization of C decreased over time, which corresponded to the depletion of the substrate during its decomposition.

We did not use our measurements of the CO₂ flux to estimate cumulative mineralization of residue-C, because integrating non-continuous measurements induced inaccuracies and the results did not take into account the consequences of a potential priming effect. Instead, we calculated the cumulative mineralization of residue-C from the difference in residual ¹³C in the soil. After 9 weeks of incubation 18% of the added C was mineralized in SURF and 55% in INC.

Distribution of residue-C and residue-N in soil

At the end of each evaporation period, the residual C and N from the rape in the coarse and fine fractions was calculated from total C and N measurements with their atom percentage excess (Table 3). The INC treatment resulted in more transfer of

residue-C and residue-N into the fine fraction than in the SURF treatment. This transfer took place mainly in the first 3 weeks of the incubation. In the bulk soil, 21.3% of the residue-C and 63.9% of the residue-N was found. We considered the amount of ¹³C not recovered in soil and residue as having mineralized. Losses of ¹⁵N could be due to denitrification.

Whereas initially no residue-C was present in the fine soil fraction of the SURF treatment, 6.6% of the residue-C had leached down to 10 cm at week 3. This corresponded to 37.1% of the initial water-extractable residue-C. For the INC treatment, no residue-C was present initially in the layers below 10 cm, but 1.9% of the residue-C was measured in the 10–17.5 cm layer after 3 weeks. For both treatments, residue-N was more mobile than residue-C. At week 3, 39.1% of the residue-N had leached down from the top to 17.5 cm soil depth for the SURF treatment, corresponding to 82.8% of the water-extractable residue-N. For the INC treatment, 10.2% of the residue-N was measured in the 10–17.5 cm soil layer after 3 weeks. Amounts of residue-N observed in the 10–25 cm soil layer generally increased over time for SURF and INC.

During the incubation, there was an isotopic dilution of the excess of ¹⁵N of the residues and we attribute this to ‘contamination’ with non-labelled soil-N by the decomposers growing on the residue particles. Dilution with nitrogen from soil particles was calculated to be negligible. For SURF, 4% of the nitrogen measured in the residues after 9 weeks of incubation originated from the soil. For INC, the amount of soil-N found in the residues was about 25%. At the first sampling time, the isotopic dilution of residues in the 0–5 cm layer of INC was half of the dilution in the 5–10 cm layer, which confirms a slower colonization and decomposition of the residues incorporated in the upper layer, as mentioned above.

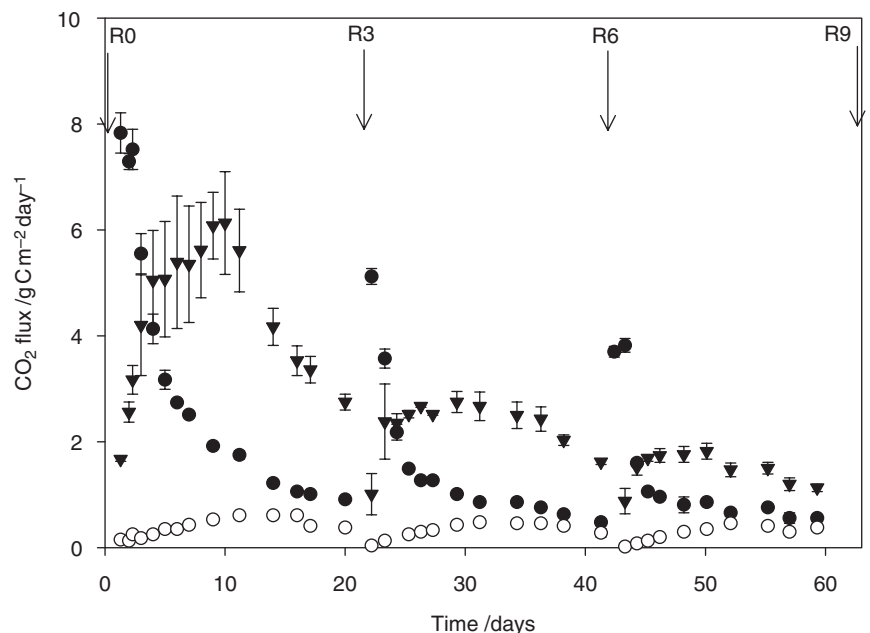


Figure 6 Change in the CO₂ flux for soil without residues (CTRL ○), residues left at the soil surface (SURF ●) or residues incorporated in the 0–10 cm soil layer (INC ▼). Arrows indicate the application of artificial rain (R0–R9). Bars are of length equal to 2× the standard errors of the means.

Table 3 Distribution of ^{13}C and ^{15}N in the fine (< 2 mm) and coarse (> 2 mm) plant residue fraction with surface applied residues (SURF) or residues incorporated in the 0–10 cm soil layer (INC), expressed as percentage of added ^{13}C and ^{15}N . Values are given \pm the standard errors of the means

		SURF					
		^{13}C			^{15}N		
		Week 3 /%	Week 6 /%	Week 9 /%	Week 3 /%	Week 6 /%	Week 9 /%
Surface	> 2 mm	84.3 \pm 0.15	79.2 \pm 0.15	73.8 \pm 0.15	55.9 \pm 0.62	51.2 \pm 0.55	43.1 \pm 0.52
0–5 cm	< 2 mm	5.0 \pm 0.69	6.2 \pm 0.69	6.3 \pm 0.69	26.0 \pm 0.81	29.7 \pm 0.81	31.2 \pm 0.81
5–10 cm	< 2 mm	1.6 \pm 0.69	1.6 \pm 0.69	1.5 \pm 0.69	10.5 \pm 0.31	9.7 \pm 0.31	10.1 \pm 0.31
10–17.5 cm	< 2 mm	0.0 \pm 0.69	0.1 \pm 0.69	0.0 \pm 0.69	2.6 \pm 0.34	3.3 \pm 0.34	4.2 \pm 0.34
17.5–25 cm	< 2 mm	0.0 \pm 0.69	0.0 \pm 0.69	0.0 \pm 0.69	0.0 \pm 0.13	0.6 \pm 0.13	3.2 \pm 0.13
0–25 cm	Total	90.9	87.1	81.6	95.0	94.5	91.8
		INC					
		^{13}C			^{15}N		
		Week 3 /%	Week 6 /%	Week 9 /%	Week 3 /%	Week 6 /%	Week 9 /%
0–5 cm	> 2 mm	29.9 \pm 0.15	15.0 \pm 0.15	11.2 \pm 0.15	9.0 \pm 0.62	6.9 \pm 0.55	6.3 \pm 0.55
	< 2 mm	10.7 \pm 0.69	13.1 \pm 0.69	11.9 \pm 0.69	24.8 \pm 0.80	29.4 \pm 0.80	30.8 \pm 0.80
5–10 cm	> 2 mm	15.8 \pm 0.15	9.9 \pm 0.15	9.5 \pm 0.15	8.4 \pm 0.62	6.8 \pm 0.55	7.1 \pm 0.52
	< 2 mm	10.7 \pm 0.69	12.4 \pm 0.69	10.5 \pm 0.69	32.2 \pm 0.77	35.7 \pm 0.77	32.5 \pm 0.77
10–17.5 cm	< 2 mm	1.9 \pm 0.69	2.3 \pm 0.69	2.3 \pm 0.69	9.9 \pm 0.62	10.9 \pm 0.62	11.5 \pm 0.62
17.5–25 cm	< 2 mm	0.0 \pm 0.69	0.1 \pm 0.69	0.0 \pm 0.69	0.3 \pm 0.14	1.8 \pm 0.14	3.1 \pm 0.14
0–25 cm	Total	69.0	52.8	45.4	84.6	91.5	91.3

Nitrogen mineralization

The distribution of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the soil solution was determined after each rain event at 2, 10 and 18 cm depth (Figure 7). The amount of $\text{NH}_4^+\text{-N}$ was negligible ($0.1 \text{ mg litre}^{-1}$). After the first rain (R0) there was a steep gradient of $\text{NO}_3^-\text{-N}$ through the columns of CTRL from 2 mg litre^{-1} at 2 cm depth to 69 mg litre^{-1} at 18 cm. The presence of residues increased the amount of $\text{NO}_3^-\text{-N}$ after R0 only at 10 cm in the SURF treatment. After R3, R6 and R9, significantly less $\text{NO}_3^-\text{-N}$ was present at 10 cm depth for the INC treatment than for the CTRL and SURF treatments.

The amount of $\text{NH}_4^+\text{-N}$ in all the layers at the end of the evaporation periods (before re-wetting) was always $< 1 \text{ mg kg}^{-1}$. The nitrate content in the 0–5 cm layer of the INC treatment was much less than in the CTRL and SURF treatments, and the difference persisted over the 9-week incubation (Figure 8). The net mean rate of mineralization of N, calculated by difference between final and initial total amounts of mineral N in the soil profile, was $0.10 \text{ mg kg}^{-1} \text{ day}^{-1}$ in CTRL, 0.17 in SURF and 0.06 in INC. The cumulative mineralization of N, expressed on an area basis, was equivalent to $20 \text{ kg NO}_3^-\text{-N ha}^{-1}$ for CTRL, $35 \text{ kg NO}_3^-\text{-N ha}^{-1}$ for SURF and $11 \text{ kg NO}_3^-\text{-N ha}^{-1}$ for INC.

Discussion

Effect of residue location on water dynamics

The soil water content is a main factor influencing the turnover of soil organic carbon (Thomsen *et al.*, 1999).

Crop residues left on the soil surface reduced the initial rate of evaporation, which resulted in the short term in a larger average moisture content in the soil. We found a significantly smaller gradient in water content in the mulched soil than in the bare one. The larger water content of soil under mulch contributed to the increased microbial activity in the upper layers and stimulated mineralization of native organic matter.

Less is known about the water content of residue mulch during successive periods of wetting and drying. After each rain event, the mulch gained water but lost mass of residue. The increased water storage capacity of the mulch suggests a change in its physical properties (e.g. increased porosity, less hydrophobic character). We know from Trinsoutrot *et al.* (2000a) that the different components of the oilseed rape (leaves, stalks, branches and pods) decompose at different rates. Therefore, we can assume that the composition of the residue mulch, with its physical properties, has changed at each application of rain. In addition, microbial attack might have modified the porosity of the residue particles and affected their water storage capacity. These physical changes also increased the rate of water loss of the mulch during the following evaporation period. The water loss in the mulch was accompanied by a decrease in the flux of CO_2 , which confirms the hypothesis that water content of the residues is an important factor controlling their decomposition.

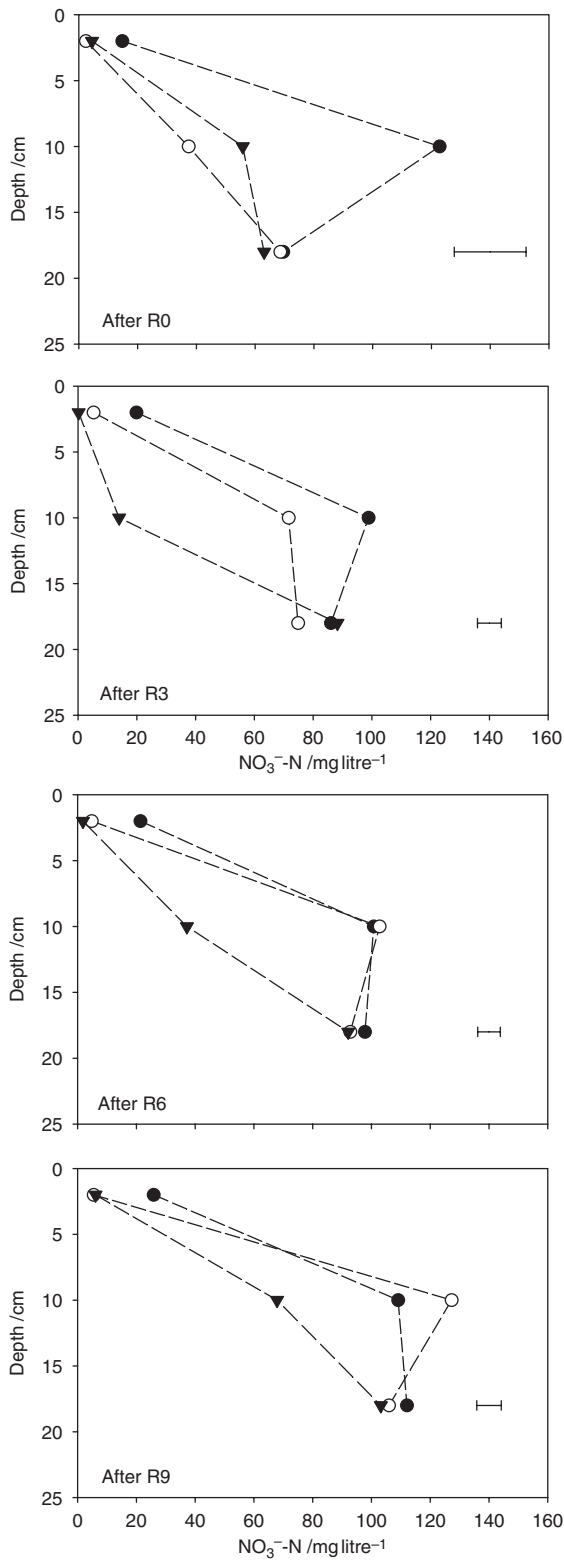


Figure 7 The concentration of NO₃⁻-N in the soil solution against depth after rainfall R0, R3, R6 and R9 for soil without residues (CTRL ○), residues left at the soil surface (SURF ●) or residues incorporated in the 0–10 cm soil layer (INC ▼). The error bars are of length equal to 2× the standard errors of the means.

Influence of residue location on decomposition dynamics

Land use and management practices can significantly influence the amount and the composition of dissolved organic matter, but the processes involved remain largely unknown, and observations are often contradictory (Chantigny, 2003). After the first rain, there was more mobile dissolved C in the soil with residues incorporated than in the soil with mulch. However,

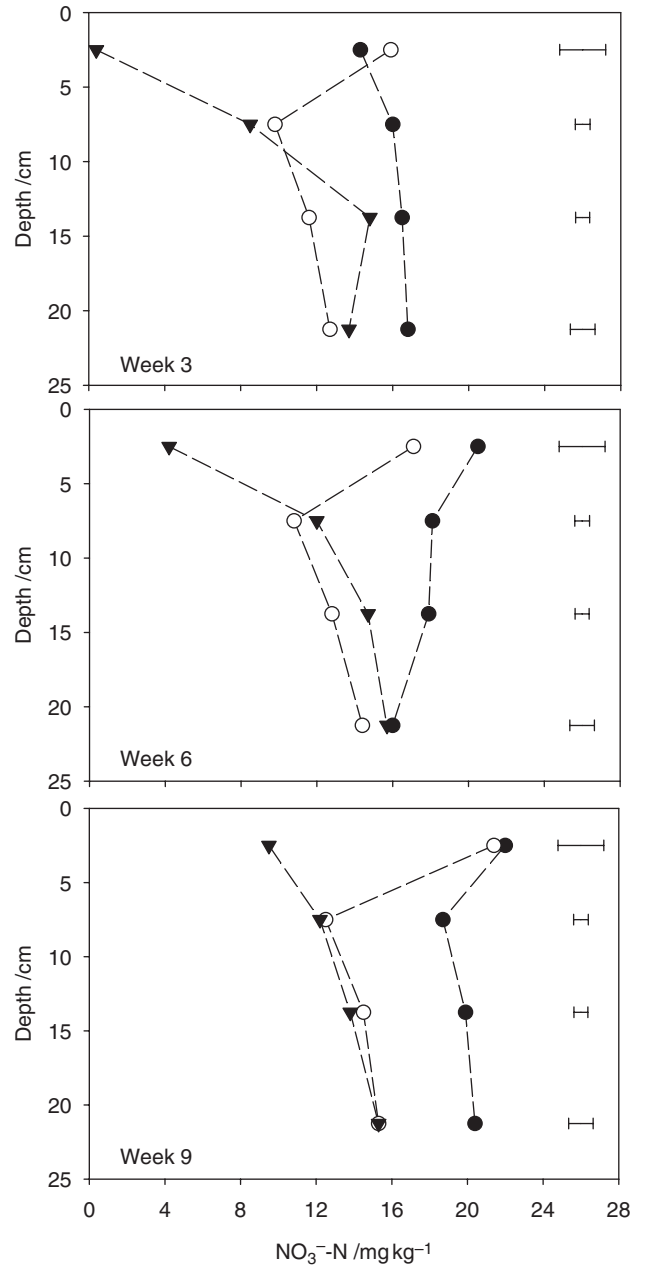


Figure 8 Distribution of NO₃⁻-N with depth after 3, 6 and 9 weeks of incubation (before re-wetting), for soil without residues (CTRL ○), residues left at the soil surface (SURF ●) or residues incorporated in the 0–10 cm soil layer (INC ▼). The error bars are of length equal to 2× the standard errors of the means.

after 3 weeks of evaporation, the total potentially available organic C in the soil profile with incorporated residues was only 61% of the amount in the soil under mulch (0–25 cm). This suggests that more soluble carbon was liberated from the incorporated residues, but that it was decomposed faster than that in residues left on the surface. The combined effect of the location of the residues and water transport regulated the distribution of nutrients for the microbial biomass in the soil profile.

The predominant effect of the location of crop residues on the dynamics of C and N was the slower decomposition when residues were left on the surface. After 9 weeks of incubation, the amount of ^{13}C mineralized from surface residues was only 66% of the amount of ^{13}C mineralized from incorporated residues. The water content of the residues was probably the main factor responsible for this difference, as mentioned above. Another factor that influenced the decomposition rate of the residues was probably the area of contact between soil and residue. When incorporated, all residue particles were surrounded by soil, whereas we estimate that only 10% of the surface mulch was in direct contact with the soil. Depending on its biochemical quality, Henriksen & Breland (2002) also observed faster decomposition with increased contact between soil and residue.

Rain falling on surface residues resulted in a spatial separation of residue-C and residue-N, and in a change of the mulch properties. The maximum mobile dissolved organic C after the first rain was measured near the soil surface, while nitrate in the soil solution, originating from the mulch, accumulated at 10 cm depth. Transport of residue-C into the soil was less important than the transport of residue-N, resulting in an increased C:N ratio of the mulch. In addition, mineral nitrogen from the upper soil layer was leached down with the rain. In this case, fungal decomposition of residues may be favoured due to the capacity to translocate soil N to the surface litter C by the hyphal network (Frey *et al.*, 2000). The greater assimilation efficiency of C and slower turnover of the fungal biomass compared with the bacterial biomass (Holland & Coleman, 1987) might have contributed to the slower mineralization of the C on the surface.

Homogeneous incorporation of residues in the upper 10 cm of the soil profile did not lead to an equal decomposition in this layer. Leaching of nutrients from the 0–5 cm layer and the more favourable water content for microbial activity in the 5–10 cm layer resulted in faster decomposition of the residues in the 5–10 cm layer. A greater SIR measured in the 5–10 cm soil layer confirmed these findings.

Effect of residue location on distribution of C and N in soil

The location of residues determined the initial distribution of C and N in the soil. With mulch, all of the C and N originating from residues was concentrated at the soil surface, whereas when incorporated, it was distributed in the upper 10 cm. We

should expect differences in the rate of biodegradation, soil respiration and nitrogen immobilization. Gaillard *et al.* (2003) showed in a microcosm incubation experiment that C from residues diffused 4–5 mm into the soil surrounding the residues (detritosphere), where microbial activity was intensified. Because there was no infiltration, they only found a negligible migration of C out of the detritosphere by diffusion. In our experiment, however, rain passing through the soil redistributed the added C and N down the profile, creating large zones of increased microbial activity.

The location of residue affected the depth to which C and N moved with the rain. To our knowledge, this effect has not been reported before. Further experiments under a rain simulator, or computer modelling with various rain scenarios, could help us to understand the influence of water regimes on the fate of C and N in soil.

After each rain event mineral nitrogen was leached out of the uppermost layer of soil. Concentrations at 2 cm depth were larger with residue mulch than with residue incorporation, because the soil under mulch was replenished with soluble nitrogen leached from the mulch itself. The larger soil water content maintained under mulch compared with a bare soil favoured mineralization of soil N. The physical separation of native soil N and C in the residues at the surface probably decreased the immobilization of N in the 0–5 cm layer compared with the incorporation treatment. This resulted in a larger net accumulation of N in the uppermost soil layer under mulch than with residue incorporation. In the latter, the stimulated microbial activity in the upper 10 cm due to the incorporation of fresh organic matter enhanced the immobilization of N compared with the control soil. This agrees with results of Corbeels *et al.* (2003), who found that more N was immobilized when residues were ground and mixed with the soil than when they were cut and left on the surface. They hypothesized that grinding and mixing of the residues made the N more accessible to microbes, which could favour immobilization. In our study, the direct effect of residue location on the dynamics of water is an additional factor to explain changes in the mineralization-immobilization balance for nitrogen.

Conclusions

The location of crop residues strongly influenced the dynamics of water in the soil: the evaporation was reduced when residues were left on the surface, so the soil remained wetter than when the residues were incorporated. At the same time, the residue mulch dried fast after each rewetting. The flux of CO_2 from the soil surface with residue mulch decreased as the mulch dried. Therefore the decomposition dynamics of residues left on the soil surface was tightly driven by the rain-evaporation sequences. The interactions between C location and water dynamics resulted in significant differences in C and N mineralization between residue incorporation and surface

application. In the 9 weeks of the experiment, emission of CO₂ from the soil with mulch was less and more residue-C was left in the soil than where residues were incorporated. As expected, the location of residues provoked a large difference in the subsequent distribution of residue-derived C and N into the soil, with all the C and N from residues near the soil surface under mulch. We showed that the mulch of residues enhanced both soil water content and net mineralization of N. This suggests that the presence of mulch on the soil surface might increase the risks of nitrate leaching.

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