C and N fluxes of decomposing $^{13}$C and $^{15}$N Brassica napus L.: effects of residue composition and N content

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Abstract

The interactions occurring between biochemical composition and N content of crop residues while decomposing in soil, and the associated N dynamics were assessed by studying the kinetics of C and N biotransformations of different tissues of Brassica napus L. (roots, stems and pod walls). These residues were obtained by growing a rapeseed crop under low and high N nutrition, in a labeling growth chamber with enriched $^{13}$CO$_2$ atmosphere and a $^{15}$N nutritive solution. The resulting crop residues in which the C-to-N ratio varied between 22 and 135 were homogeneously labeled with $^{13}$C and $^{15}$N. Paired labeled residues ($^{13}$C$^{15}$N labeled residues with unlabeled soil inorganic N; $^{13}$C$^{14}$N residues with $^{15}$N labeled soil inorganic N) were used to determine net and gross fluxes of immobilization and mineralization. Decomposition was studied during laboratory incubations at 15°C, the initial soil N availability being non-limiting with regard to the rate of C decomposition. The rate of $^{13}$C mineralization from the residues was influenced by the biochemical composition of the tissues and particularly by their soluble C content. The N content of the tissues did not significantly affect the kinetics or the amount of C mineralized, except in the very short-term. Decomposition was rapid and after 168 days of incubation at 15°C, 82% of the C from the stems and pod walls and 69% from the roots at both low and high N contents had disappeared from the soil coarse fraction. Residue decomposition first resulted in net immobilization of soil mineral N for all the residues. The intensity and duration of this immobilization depended on the tissues and the N content of the residues. Compared to the control, the residues with low N content, still induced net N immobilization after 168 days ($-22$ to $-14$ mg N g$^{-1}$ of added C) whereas the high N residues induced little net immobilization or mineralization, at $-3$ to $+4$ mg N g$^{-1}$ of added C at the same date. The NCSOIL model was used as a tool to calculate, by fitting simulation against the data, the gross N mineralization and immobilization fluxes and also to determine the total N fluxes involved over the 168 days of decomposition. Depending on the tissues and their N content, gross cumulative immobilization ranged from 71 to 113 mg N g$^{-1}$ of added C and gross mineralization varied from 66 to 123 mg N g$^{-1}$ of added C. The differences in net mineralization, observed during decomposition of the tissues with low and high N contents, were well explained by the differences between gross mineralization fluxes which were themselves attributable to the different quantities of N mineralized from the residues. The use of modeling to calculate the total gross N fluxes demonstrates that the total amount of N involved in the decomposition of crop residues is much higher than the resulting net fluxes quantified either by N balance or by $^{15}$N tracing. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Brassica napus L; Labeled plant residues; Gross fluxes; N mineralization; N immobilization

1. Introduction

The return of crop residues to the soil represents an input of “fresh” organic matter of which the characteristics are highly variable. This variability results from the biochemical composition of the tissues being
returned to the soil (stems, leaves, roots, pod walls etc.), their physical characteristics, relative contribution to the total amount of dry matter returned and their location at the start of decomposition (i.e. left on the soil surface or incorporated). For a given crop, the nature and amount of residues returning to the soil are influenced by its growing conditions and, particularly, by N nutrition. N fertilization not only modifies dry matter production, but also the root-to-shoot ratio, and N content of the residues (Rahn et al., 1992).

Oilseed rape (Brassica napus L.) has been developed in Northern Europe for the production of biofuel (Messéan, 1996). This crop has the peculiarity of accumulating large and variable quantities of N depending on its conditions of fertilization, so that the N contents of crop residues remaining on the soil at harvest can vary considerably (Reau et al., 1994; Trinsoutrot et al., 2000a). Field studies have shown that the N from such residues is relatively unavailable during the period between crops (Thomsen and Christensen, 1998; Trinsoutrot et al., 2000a). Understanding the interactions between biochemical composition and N content of the residues and their effects on C decomposition and the associated N dynamics in soil is therefore important, and rape residues would seem suitable for the investigation of such relationships. Biochemical composition, and particularly the soluble C content, determines the initial rate of residue decomposition (Heal et al., 1997) whereas the lignin content controls the medium to long-term fate of added C. The N content of the residues is also an important factor, but its effect on C decomposition and N mineralization is complex. On the one hand, residue-N is a source of organic N that is subject to mineralization, microbial assimilation, and humification. Quantification of these processes, even by stable isotopic methods, is only partially feasible due to the direct assimilation of organic N by soil microorganisms (Mary et al., 1993; Barraclough, 1997), to assimilation by microorganisms growing on the residue itself (Cheshire et al., 1999; Gaillard et al., 1999), or to substitution effects between residue-derived N and soil N during microbial assimilation or plant uptake (Hart et al., 1986; Jenkinson et al., 1985). On the other hand, residue-N is itself a component of the overall N availability (soil mineral N + residue-N) that may control the growth of heterotrophic microorganisms, and consequently alter the kinetics of C decomposition (Recous et al., 1995). Whether the initial content of residue-N might or might not control residue decomposition, it cannot be analyzed independently of the conditions of incubation, i.e. of the soil N availability. It is therefore essential to distinguish the effect of the nature and availability of C substrates from the effect of N availability in understanding the effect of chemical characteristics of crop residues and their modeling (Trinsoutrot et al., 2000a). In the work reported here, our main objective was to analyze the interactions between biochemical composition and N content of the residues, and their effects on decomposition and soil N dynamics, under conditions where the overall N availability was not limiting the microbial demand. Various methods (N balance, 15N tracing and modeling) were used to quantify the N fluxes involved, and the contribution of different sources of N to these processes. Our study, carried out under controlled conditions, was a part of a larger program intended to characterize the dynamics of oilseed rape residue decomposition in the field (Trinsoutrot et al., 2000a) and the resulting effects on N dynamics during the intercropping period (Justes et al., 1999).

2. Materials and methods

2.1. Soil

The soil used was a highly calcareous rendosol overlying cryoturbated chalk (Ballif et al., 1995). The surface horizon (0–10 cm) of the soil was sampled and sieved (4 mm) to eliminate plant debris. The main characteristics of the soil were: clay = 9.9%, silt = 11% and sand = 0.8%; CaCO3 = 78.3%; organic C = 1.81%; organic N = 0.17%; pH = 8.3.

2.2. Labeled plant residues

Spring oilseed rape seeds (Brassica napus L., cv. Star) were germinated in the laboratory and allowed to emerge in a glasshouse. At the 2–4 true leaf stage, the rape plants were transferred to an airtight growth chamber where the growing conditions (air moisture, air temperature and photoperiod) were monitored to mimic spring conditions. Day-time light intensity was kept constant (600 μmol m−2 s−1) throughout the crop cycle. The plants were grown under hydroponic conditions using nutrient solutions as described by Devienne et al. (1994). The nutrient solutions were recycled through the nutrient troughs to ensure their constant mixing. The plants were continuously labeled with 13C by using the same 13CO2 bottles (13C isotopic excess = 3.13‰) throughout the entire growth cycle. Two sets of plants were grown: one set received unlabeled KNO3 and the other set KNO3 labeled with 15N (15N isotopic excess = 9.8‰), to ensure that the resulting plants were similar but differently labeled (13C15N or 13C14N).

A first crop was grown for 195 days to produce plants with a low-N content. The KNO3 concentration in the nutrient solution was 0.8 mM for the first 2 months and 1 mM for the next 4 months. The solutions were used without renewal until the N was
exhausted. A second crop was grown for 78 days using a nutrient solution with 3.0 mM KNO₃ to produce plants with a high-N content. The concentration was readjusted, until crop flowering, as soon as it dropped by more than 10%. From flowering to plant maturity, the N source was removed from the nutrient solution to avoid obtaining mature plants high in NO₃⁻.

The roots, stems, and pod walls were separated at harvest, dried at 80°C for 48 h, and ground to <1 mm. Leaves present at harvest were not used in this study. The characteristics of the different plant materials (determined in duplicate) are shown in Table 1.

### 2.3. Soil incubations

The treatments studied and the amounts of the different plant materials incorporated into the soil are presented in Table 2. Soil samples equivalent to 125 g of dry soil were incubated with or without residues in 500 ml polyethylene pots (4 replicates per treatment) placed in 2 l glass jars (1 pot per jar) in the presence of a CO₂ trap (30 ml 1 M NaOH). Mineral N and microbial biomass were monitored on other soil samples equivalent to 25 g dry soil (4 replicates per treatment) incubated with or without residues in 50 ml polystyrene pots placed in 2 l jars (7 pots per jar) in the presence of a CO₂ trap (30 ml 1 M NaOH). To prevent decomposition being limited by mineral N availability (Recous et al., 1995), mineral N (60 g N kg⁻¹ dry soil) was applied to all treatments in the form of K¹⁵NO₃ (for unlabeled N residues) or KNO₃ (for ¹⁵N labeled residues) (Table 2). The soil samples were incubated at 15 ± 0.5°C. The CO₂ traps were periodically replaced and the soil moisture content maintained at a matrix potential of 0.05 MPa by weighing and readjusting if necessary by the addition of deionized water.

### 2.4. Analytical measurements

The plant residues studied were characterized as follows: (i) the soluble fraction was determined by hot water extraction (100°C) for 30 min followed by extraction with a neutral detergent (100°C) for 60 min (Linères and Djakovitch, 1993); (ii) the hemicellulose, cellulose, and lignin fractions in the residual samples were then determined using the method described by Van Soest (1963); (iii) the nitrate content of the residues was determined in other samples by water extraction for 30 min at 20°C (material-to-extractant ratio 2/1000). The total C, N, ¹³C and ¹⁵N contents of plant residues and fractions were measured using an elemental analyzer (NA 1500, Carlo Erba) coupled to a mass spectrometer (Fisons Isochrom).

The amounts of CO₂ trapped in the NaOH were measured by continuous flow colorimetry (Chaussod et al., 1986) using an auto-analyzer (TRAACS 2000, Bran & Luebbe). Another aliquot of NaOH containing CO₂ was precipitated in BaCO₃ using BaCl₂. The BaCO₃ precipitate was separated by vacuum filtration (glass fiber filter, porosity 1.2 µm) and dried at 80°C.
for 15 h. The isotopic excess value was measured by mass spectrometry, after elemental dissociation of the precipitate in the presence of a catalyst (PbO₂).

The soil mineral N (NO₃⁻ and NH₄⁺) was extracted with 1 M KCl (30 min shaking at 20°C, soil-to-solution ratio 1/4). Soil extracts obtained by centrifugation (20 min at 5800 g) were stored at −20°C until analysis. The centrifuged soil was recovered and washed three times with 1 M KCl to remove all traces of mineral N (Recous et al., 1988). The organic N and its isotopic excess were measured on the soil. The mineral N in the soil and plant extracts was analyzed by continuous flow colorimetry (TRAACS 2000, Bran & Luebbe). Nitrate was measured using an adaptation of the method proposed by Kamphake et al. (1967) and ammonium by a method derived from that published by Krom (1980). Isotopic excess of inorganic N was measured on the ammonium sulfate obtained after steam distillation of the soil extracts (Keeney and Nelson, 1982).

The microbial biomass carbon was determined using the fumigation-extraction method proposed by Vance et al. (1987), using the modifications suggested by Chaussod et al. (1988): extraction of the soluble carbon from samples, fumigated or not with chloroform, by 250 mM K₂SO₄ (soil-to-extractant ratio 1/4, 30 min shaking at 20°C), followed by separation of the extracts by centrifugation (20 min, 5800 g) and storage at −40°C. The soluble carbon concentration of the extracts was measured with an auto-analyzer (1010, O.I. Analytical) using an oxidation method at 100°C in a persulfate medium, the CO₂ produced being measured by infrared spectrometry (Barcelona, 1984). The isotopic excess was measured using the procedure described by C. Aita (unpub. Ph.D. thesis, Université Pierre et Marie Curie, Paris VI, 1996) with 13C analysis of the lyophilized soil extract (GT2/S0 apparatus, Amsco/Finn-Aqua). The microbial biomass carbon was calculated by subtracting the soluble C in the fumigated soil from the soluble C of the unfumigated soil, and dividing by a coefficient $K_{EC} = 0.38$ (Vance et al., 1987).

The non-decomposed residues were extracted after dispersion of 125 g of soil with 200 ml 250 mM K₂SO₄ (30 min agitation at 20°C). Sieving under water resulted in separation of a fraction >1 mm made up of small chalk particles, a 50–1000 μm fraction containing the residues and a fine fraction (<50 μm) obtained after continuous centrifugation of the sieving water. The fraction containing the residues was decarbonated by drop addition of 2 M HCl (pH stabilized at 3–3.5), rinsed with demineralized water to remove all traces of acid, and then dried at 80°C. The carbon and N contents of this fraction and their 13C and 15N isotopic excesses were then determined. The determinations of the isotopic excess of mineral N, organic N, total N and of N in the residues extracted from the soil, those of total C, BaCO₃ precipitate, microbial biomass C and C in the residues extracted from the soil were all performed with an elemental analyzer (NA 1500, Carlo Erba) linked to a mass spectrometer (Fison Isochrom).

### 2.5. Calculation of N fluxes

**Apparent mineralization** was calculated by subtracting at a given time the amounts of inorganic N accumulated in the soil with residues from those in the soil without residues. It was expressed in mg N g⁻¹ of added C to permit comparison of treatments with different amounts added.

**Mineralization of the residue-derived N** was calculated from the net accumulation of inorganic 15N in soil incubated with the 13C15N residues.

**Net immobilization rates** were calculated from the results obtained with the 13C14N residues incubated in the presence of 15N labeled soil mineral N using the method described by Recous et al. (1995).

**Gross immobilization and mineralization fluxes** occurring during degradation of unlabeled residues (13C14N) in the presence of labeled K15NO₃ were calculated.
with the NCSoil model (Molina et al., 1983; Hadas et al., 1989). The model, which has been intensively tested under various conditions of incubation and for various residues (Nicolardot et al., 1994a; Hadas et al., 1998; Corbeels et al., 1999) was used here to calculate the gross N biotransformations over time and to identify the processes involved. The data set was not used to test the model. We, therefore, sought the best fit between the simulated and the measured data from which the model calculated the N fluxes involved using the following procedure. The NCSoil model considers five organic pools (Fig. 1): residues, zymogenous microbial biomass (pool 0), autochthonous (native) microbial biomass (pool I), humads (pool II) and stable organic matter (pool III). The parameter values assigned to pools I, II and III (decomposition, recycling and humification rates) were those published by Nicolardot et al. (1994a). The initial size and C-to-N ratio of pool II were calculated by optimization and comparing the simulated and experimental data for the kinetics of the CO2 released, the mineral N and microbial biomass in the soil without residues. The residues were described using the three fractions (soluble fraction, hemicellulose + cellulose and lignin) obtained from the biochemical characterization described earlier. In order to minimize the number of parameters requiring optimization, the decomposition rate and assimilation yield of lignin were fixed at $1.0 \times 10^{-5}$ d$^{-1}$ and 0.60, respectively. The kinetics of total CO2 release, $^{13}$C-CO2, total mineral N, $^{15}$N labeled N, biomass C and $^{13}$C biomass obtained for each $^{13}$C labeled residue (mean of four replicates) were simulated by optimizing the following parameters for each residue: C-to-N ratio of pool 0, degradation rate and assimilation yield of the soluble and (hemicellulose + cellulose) fractions. Simulated and experimental data were compared using the approach proposed by Barak et al. (1990), whereby a low figure-of-merit ($\chi^2$) indicated a good fit. The cumulated immobilization and mineralization fluxes calculated by the model for the entire incubation (168 days) were then calculated, the immobilization fluxes corresponding to all the N fluxes entering pools 0 (zymogenous biomass) and I (autochthonous biomass) and the mineralization fluxes corresponding to the fluxes leaving the residue pool and pools 0 (zymogenous biomass), I (autochthonous biomass), II (humads) and III (stable organic matter).

### 3. Results

#### 3.1. Characteristics of the crop residues: effect of N nutrition

The N nutrition of oilseed rape influenced the biochemical composition of its residues (Table 1). The organic N content of plants grown with a high amount of N was 3- to 4-fold higher than in plants grown with the low amount of N. In addition, the cellulose and lignin contents of high-N content residues were lower and the soluble compounds content higher than those of N-low residues. Some differences between tissues could also be observed. The biochemical characteristics of the stems and pod walls were almost the same, but the N and lignin contents were higher and the cellulose content lower for roots. Residue labeling ($^{13}$C$^{14}$N or $^{13}$C$^{15}$N) had no effect on the characteristics of low-N residues. However, for the high-N residues, the N contents of tissues labeled with $^{13}$C$^{15}$N were lower than corresponding plant tissues labeled with $^{13}$C$^{14}$N. The observed differences could probably be attributed to small differences in growth conditions (composition of nutrient solutions, position of the plant in the growth chamber).
3.2. ¹³C and ¹⁵N recovery in soil

The ¹³C balance at each sampling date (except day 0) was only determined for treatments in which stems were added and was calculated as the sum of the labeled ¹³C present in the non-fractionated soil and the ¹³C–CO₂ mineralized (Fig. 2). The mean ¹³C balance for these treatments and all measuring dates, was 94.9 ± 0.7%. The ¹⁵N balances were calculated only at the end of the experiment for all treatments as the sum of ¹⁵N present in soil organic and inorganic N. The mean ¹⁵N balance for all treatments was 95.9 ± 2.1% at 168 days of incubation (individual data not shown). In consequence and taking into consideration experimental errors, the ¹³C and ¹⁵N losses to unknown fluxes were considered to have been negligible during the incubation.

3.3. C fluxes

3.3.1. ¹³C–CO₂ released by the soil

The kinetics of carbon mineralization by the stems, pod walls and roots were identical for the ¹³C¹⁵N and ¹³C¹⁴N residues (results not shown), so that only the kinetics obtained with the ¹³C¹⁴N residues are shown (Fig. 3). Mineralization occurred rapidly after incorporation of the residues, representing 17–22% of the added ¹³C for the low-N residues and 21–28% for the high-N residues after 21 day. The kinetics of C mineralization was influenced both by the type of tissue and the N content of the residues. Mineralization rates were ranked in the following order: pod walls > stems > roots and high-N content > low-N content. The differences were maximum after 35 days and decreased later. At 168 day, only the differences between tissues were significant. The cumulative amounts of ¹³C mineralized were 29, 37 and 39% for the roots, stems and pod walls, respectively.

3.3.2. ¹³C labeled microbial biomass

The maximum incorporation of labeled C into the microbial biomass was attained on 7 day after incorporation of the residues (Fig. 4) for all tissues and N contents. Greater incorporation of ¹³C was found in residues with a high N content: 13.8–16.7% of the C added, compared with 11.4–13.5% of the C added for the low-N tissues. Later, the labeled carbon in the microbial biomass decreased rapidly, particularly for those residues highest in N. After 168 day incubation, the amounts of ¹³C remaining in the microbial biomass, in relation to tissues and N content were not statistically different, the mean ¹³C-biomass representing 6.5 ± 0.8% of the added ¹³C.
3.3.3. $^{13}$C present in the 50–1000 μm fraction

At time 0, the quantities of labeled C found in the 50–1000 μm soil fraction, representing the remaining residues, were only 57–61% of the added $^{13}$C (Fig. 5). They decreased rapidly during incubation with 36–39% of the $^{13}$C added in the low-N residues, and 25–41% of the $^{13}$C added in the high-N tissues present in this fraction on day 35. Those residues with the highest N content decomposed faster, except for roots which also decomposed much more slowly than the two other tissues. At day 168, 33% of the $^{13}$C from roots remained in the coarse fraction compared with ≈20% for stems and pod walls.

3.4. N fluxes

3.4.1. Soil mineral N and apparent mineralization

The evolution of the mineral N in the soil was similar for the $^{13}$C$^{14}$N and $^{13}$C$^{15}$N residues, so only the results for the former are presented (Fig. 6). The dynamics of mineral N exhibited two distinct phases. The first phase (2–3 weeks) consisted of net immobilization of soil mineral N. Its duration and intensity depended on the type of residue, and particularly on its initial N content. The second phase was a net mineralization phase that lasted until the end of the experiment. Net N immobilization was greater for the low-N residues than for the high-N residues. Maximum immobilization (43 mg N kg$^{-1}$ soil) occurred with the low-N stems. For these low-N residues, the amount of mineral N accumulated in the soil remained below that of the control soil until the end of incubation. The high-N pod walls and roots caused a smaller net immobilization and led to a higher accumulation of mineral N than in the control after 84 day. By the end of incubation, apparent mineralization of the residues was $-22$ to $-14$ mg N g$^{-1}$ of added C for low-N residues and $-3$ to $+4$ mg N g$^{-1}$ of added C for high N residues.

3.4.2. Net mineralization of residue-N

The treatments with the $^{13}$C$^{15}$N residues enabled the net release of $^{15}$N from the different tissues to be estimated (Fig. 7). The small quantities of $^{15}$N found in mineral form at time 0, corresponding to 0.5–2.4 mg residue-N kg$^{-1}$ soil, were attributed to the soluble N initially present in the residues. $^{15}$N release was faster with the high-N residues: 4.2–8.0% of the $^{15}$N added in the high-N tissues was mineralized within 28 days, whereas the corresponding values for the low-N tissues were 1.9–3.7%. The fraction of $^{15}$N released was also greater at 168 day, except for the stems. The proportion of N from the stems accumulated in the soil was the same at 168 day, i.e. 23% of the N added, but this represented very different amounts, i.e. about 3 and 12 mg N kg$^{-1}$ soil for low-N and high-N stems, respectively. The $^{15}$N released from pod walls and roots, represented 3.2 and 7.0 mg N kg$^{-1}$ soil for low-N pod walls and roots respectively, and 12.4 and 13.8 mg N kg$^{-1}$ soil for the corresponding high N tissues. Less mineralization occurred in the roots than in other tissues.

3.4.3. Net immobilization of soil N

The treatments which had received $^{13}$C$^{14}$N residues and mineral $^{15}$N were used to calculate soil net N immobilization (Fig. 8). N immobilization was very low in the control soil (0.7–0.9 mg N kg$^{-1}$ soil by day 168). Net immobilization in the treatments with added residues, reached a maximum at 49 day of incubation and represented 24–40 mg N kg$^{-1}$ soil for the low-N tissues and 17–26 mg N kg$^{-1}$ soil for the high-N tissues. After 49 day, the amounts of N immobilized fell progressively, indicating re-mineralization of the recently immobilized N.
3.5. Gross N fluxes simulated with the NCSOIL model

The optimized parameter values and associated $\chi^2$ values for each simulated treatment are shown in Table 3. The $\chi^2$ values indicate that the simulations made with the NCSOIL model were not entirely satisfactory. Residue application, in fact had a considerable priming effect on mineralization of the non-labeled soil C and N (results not presented) which was not simulated by the model. A large part of this priming effect might be due to the amounts of CO$_2$ and NO$_3^-$ produced during the decomposition of residues which influence carbonate dissolution in chalky soil (Salomons and Mook, 1976; Jacquin et al., 1980). However, the kinetics of the labeled C and N added to the soil were satisfactorily simulated and were then used to calculate the gross immobilization and mineralization fluxes (Table 4).

The gross mineralization rates cumulated over the period 0–168 day varied from 66 to 123 mg N g$^{-1}$ of added C, depending on the treatment. Gross mineralization was greater for the high-N than the low-N stems and pod walls, but was similar for the low-N and high-N roots.

The cumulative gross N immobilization varied from 71 to 113 mg N g$^{-1}$ of added C. The amounts of N immobilized with stems were the same regardless of N content. Gross N immobilization was higher for the high-N roots than the low-N roots. In the case of pod walls, the large difference between gross immobilization for the low-N tissues (76 mg N g$^{-1}$ of added C) and the high-N ones (113 mg N g$^{-1}$ of added C) might be an artifact resulting from the poor agreement between experimental values and the model values simulated for the high-N pod walls residue ($\chi^2 = 0.51$).
The net mineralization simulated by the model was calculated as the difference between the simulated fluxes of gross mineralization and immobilization. The simulations reproduced the observed differences between residues. The decomposition of low-N residues always resulted in net immobilization at day 168, whereas the high-N residues caused a small positive net effect. The simulated values were, however, always slightly higher than those measured (+5 to +8 mg N g⁻¹ C).

4. Discussion

4.1. Residue decomposition

The decomposition of plant residues was estimated in two ways: (i) by measuring $^{13}$C remaining in the soil coarse fraction (50–1000 μm) and (ii) by measuring the $^{13}$C–CO₂ emissions.

The $^{13}$C–CO₂ produced by the soil corresponds to mineralization of C from the actual residue and from turnover of the soil organic matter fractions (e.g. microbial biomass, metabolites) into which the labeled carbon from the residue had been incorporated. The $^{13}$C present in the 50–1000 μm fraction quantifies the added C which is still present in the form of residue. The amount of residue C which has been degraded is then calculated from the difference. However, at day 0, all the $^{13}$C added in residue form was not found in the 50–1000 μm soil fraction, mainly because of the presence of water-soluble C in the residues which was then eliminated during the separation procedure. This is confirmed by measurement of $^{13}$C between 0 and 7 days for the low-N residues. In these treatments, the amount of labeled C present in this soil fraction
remained constant during this period, whereas a release of $^{13}$C–CO$_2$ was measured, indicating degradation of the applied residues.

Measurement of the C fluxes through the different compartments shows that the decomposition of oilseed rape residues in the soil was rapid. After 168 day, 82% of the C had disappeared from the residue (coarse fraction) for the stems and pod walls and 69% for the roots. At this time, 37% of the added $^{13}$C had been mineralized as $^{13}$CO$_2$ for the stems and pod walls and 30% for the roots. Aita et al. (1997) and Trinsoutrot et al. (2000a) found similar rates of C decomposition during in situ experiments but over a longer period, 71% and 74% of the C being decomposed 421 and 594 day, respectively, after incorporation of wheat and rape straw at a reference temperature of 15°C. The more rapid decomposition of rape residues observed in our laboratory study can be attributed to more favorable conditions of temperature and soil moisture content. This result may also be due to the non-limiting availability of N and finer grinding of residues which optimized the contact between soil and residues; these latter two factors often enhance decomposition (Recous et al., 1995, 1998).

The initial rate of C mineralisation was strongly dependent upon the amounts of soluble C initially present in the residues. The C mineralized at 7 day was correlated with the initial soluble C content of the residue at 20°C ($r = 0.93$, $P = 0.01$) and the size of the soluble fraction defined by Van Soest ($r = 0.95$, $P = 0.01$). From 28 day onwards, C mineralization was largely related to the lignin content ($r = 0.91$, $P = 0.01$). This result agrees with the observations of Collins et al. (1990) and Trinsoutrot et al. (2000b). The roots, which are richer in lignin and N than the stems and pod walls, decompose more slowly than the stems and pod walls, this confirming the work of Saini et al. (1984) with residues of mustard, rice and wheat, and Amato et al. (1984) with Medicago. According to Camiré et al. (1991), the lower degradation rate of roots can be attributed to the formation of lignin-N derivatives resulting from the simultaneous presence of large amounts of N and lignin.

The decomposition of rape residues was ac-

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**Table 3**

Optimized values of the parameters with associated chi-squared values ($\chi^2$) obtained for the simulation of experimental data with $^{13}$C residues

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<td>$\chi^2$</td>
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**Table 4**

C and N mineralization, gross and net N fluxes measured and simulated by the NCOSIL model accumulated during the 168 d for $^{13}$C$^{14}$N residues incubated with K$^{15}$NO$_3$.

<table>
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<th>Variables</th>
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<th>High-N residues</th>
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<tr>
<td>Measured soil inorganic $^{15}$N</td>
<td>mg N kg$^{-1}$ soil</td>
<td>24.4</td>
<td>27.7</td>
</tr>
<tr>
<td>Simulated soil inorganic $^{15}$N</td>
<td>mg N kg$^{-1}$ soil</td>
<td>24.8</td>
<td>33.7</td>
</tr>
<tr>
<td>Simulated gross N immobilization</td>
<td>mg N g$^{-1}$ of added C</td>
<td>103</td>
<td>84</td>
</tr>
<tr>
<td>Simulated gross N mineralization</td>
<td>mg N g$^{-1}$ of added C</td>
<td>86</td>
<td>79</td>
</tr>
<tr>
<td>Simulated net N mineralization</td>
<td>mg N g$^{-1}$ of added C</td>
<td>$-17$</td>
<td>$-5$</td>
</tr>
<tr>
<td>Measured net N mineralization</td>
<td>mg N g$^{-1}$ of added C</td>
<td>$-22$</td>
<td>$-14$</td>
</tr>
</tbody>
</table>

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* Calculated from the difference between simulated gross N mineralization and gross N immobilization.
accompanied by C assimilation in the microbial biomass: after 7 days of incubation, about 15% of the C added was found in this compartment, regardless of the residue. This value is in good agreement with other studies showing that 15–20% of the added C was present in the microbial biomass shortly after the start of decomposition, e.g., by Ladd et al. (1995) for Medicago residues, by Nicolardot et al. (1994b) for holocellulose, by Witter and Dahlin (1995) for barley residues or Wu et al. (1993) for ryegrass. A slightly higher C assimilation yield was found for the N-rich residues. This was probably related to their higher soluble C content. In fact, the more easily accessible the added C, the greater seems to be the assimilation of the carbon by the biomass (Bremer and Kessel, 1992). A rapid decrease of biomass C was observed between 7 and 28 day, particularly for high-N content residues, confirming the rapid turnover of this pool associated with rapid exhaustion of the easily-degradable compounds, as shown by Bremer and Kessel (1992) and Aita (op. cit.). Consequently the residues did not differ to much extent either in the proportion of their C incorporated into the microbial biomass or in the dynamics of the labeled biomass over time.

The N nutrition of oilseed rape resulted in a significant difference in biochemical composition between the high- and low-N that translated into different initial rates of decomposition. Later on, differences were erased as decomposition proceeded, the larger soluble C pool being more rapidly exhausted for high-N residues, and this resulted in a similar cumulative mineralisation for high- and low-N treatments at day 168. In reality, this lack of difference in the medium-term between residues with very different N contents is mainly due to the fact that decomposition of the residues was not limited by the availability of mineral N, and only determined by the biochemical composition of the tissues themselves. We, therefore, conclude that the N nutrition of a crop influences the biochemical composition of the tissues and its N content, but the N content does not have a significant effect on decomposition in the medium term. This was also observed for the oilseed rape leaves decomposing in the soil (Dejoux et al., 2000). Conversely, Recous et al. (1995) showed that the rate of decomposition of maize residues of similar biochemical composition significantly varied whether or not the initial amount of N available in soil was above the threshold that limits decomposition (i.e. at 40 mg N g\(^{-1}\) of added C in the conditions explored). In that situation, the observed kinetics of decomposition were no longer determined by the residue chemical quality (which was similar whatever the treatment) but depended on the overall availability of N. Furthermore the ratio of immobilized N: decomposed C did vary according to the availability of N, suggesting that the C–N relationships were modified by N availability. It is thus important to understand the effect of the biochemical quality of different residues on their decomposition, by differentiating the specific role of the N contained in the residues from that of general availability of N for decomposition, which depends on experimental conditions or soil type. Consequently, it is essential that the experimental conditions are chosen in such a way that the amount of mineral N in the soil is above the threshold beyond which the amount of N immobilized per unit of C decomposed no longer varies. On the other hand, models which aim at describing the bio-transformations of C and N in soil need to include the simulation of control of the decomposition of residues by the availability of N (Hadas et al., 1998).

4.2. N fluxes involved

Despite rather similar decomposition kinetics, the different tissues with low- and high-N contents caused very different patterns in the course of mineral N in soil. The use of net changes in \(^{15}\)N both with labeled residues and by labeling the soil N pool, only partially revealed the soil N processes involved. Only a small proportion (14–27%) of the N coming from the residues was mineralized after 168 day, which is comparable to figures published for similar crop residues (e.g. Wagger et al., 1985; Jensen, 1997). This demonstrates that most of the residue-N released during decomposition was assimilated by the decomposing organisms or remained in more recalcitrant soil organic fractions. Still this net mineralization revealed by \(^{15}\)N is higher than net mineralization calculated from the N balance between amended soil and control soil, showing that soil N was immobilized in the amended treatment in place of the residue-N, i.e. that substitution effects occurred between labeled and unlabeled sources as immobilization was enhanced by C addition (Hood et al., 1999). Consequently net mineralization determined by \(^{15}\)N mineral accumulation in soil both underestimates the actual decomposition of the residue-N and overestimates the actual availability of that mineralized N.

The maximum immobilization of soil N revealed by labeling the soil mineral N pool, represented 14–21 mg N g\(^{-1}\) of added C for the low-N residues and 10–16 mg N g\(^{-1}\) of added C for the high-N residues, the difference (4–5 mg N g\(^{-1}\) residue) being assumed to be the extra contribution of high-N residue to microbial immobilization. Jensen (1997) observed maximum immobilization of 12 and 18 mg N g\(^{-1}\) of added C for peas (N% = 2.51) and barley (N% = 0.98), respectively.

An alternative approach consists of estimating the gross N fluxes involved in decomposition of the residues. Watkins and Barraclough (1996) have proposed a method of calculation based on paired labeled treatments such as those used here, enabling the direct
assimilation of the residue-N by the microflora to be estimated. This method failed here to give estimates of the fluxes because the amounts of N in the low-N residues were too small to provide the necessary sensitivity for this calculation. Moreover, the calculation of gross fluxes by isotopic dilution and enrichment requires samples to be taken at very short time of intervals, or use of successive “pulses” of labeled mineral N. Finally, the integration of instantaneous fluxes, measured with this approach, during prolonged decomposition remains rather dubious (Recous et al., 1999).

This is why the approach finally chosen consisted of estimating the gross N fluxes with a model that simulated C–N biotransformations. The advantage of such an approach is to take into account all the fluxes coming from the residue N, soil mineral N, and N recycled by the microbial biomass, that is usually not considered, and to cumulate them over time. The simulations showed that the amounts of N involved in the decomposition process were very large. The requirements of the microflora for decomposition of the stems, pod walls and roots of oilseed rape were on average over the 168 day decomposition period 91 (±16) mg N g⁻¹ of added C or 287 (±41) mg N g⁻¹ of C mineralized. These values are higher than those calculated previously from short-term estimates of gross N fluxes with wheat straw decomposing either in controlled incubation or in field conditions (Mary et al., 1998; Recous et al., 1999), which were at 174 and 126 mg N g⁻¹ of C mineralized, respectively. The calculation for net mineralization obtained with the NCSOIL model was in good agreement with the measured net N mineralization. This led us to assume that the model had correctly simulated the gross N fluxes and therefore gave a reliable picture of the contribution of the various processes to the net dynamics of soil N. By way of example, the difference in net mineralization between stems of low- and high-N content therefore appears to be simply due to a difference in the intensity of the gross mineralization flux which itself is explained by a difference in the N mineralization of the residues (5.2 and 25.8 mg N g⁻¹ of added C for low- and high-N stems, respectively). These conclusions contradict those of Barraclough et al. (1998) who suggested that the differences in net mineralization fluxes between residues were mainly due to differences between gross immobilization fluxes. This apparent contradiction may arise from the fact that the N availability was probably limiting of the microbial needs in the work of Barraclough et al. (1998), therefore, modifying the relationship between N immobilized and C decomposed.

By considering the contribution of each tissue on the dynamics of soil mineral N, it is possible to calculate the effect that the incorporation of all the residues obtained from two rape crops of low- or high-N content might produce on soil N dynamics. The proportions of stems, pod walls and roots at harvest in the related field study (Trinsoutrot et al., 2000a) were 49, 31 and 20%, respectively for a rape crop without N fertilization and 61, 31 and 8% for the crop which had previously received 270 kg N ha⁻¹. Using the reported values obtained for the various tissues and the NCSOIL model, the simulated net mineralization values for the whole residue would be −15.7 mg N g⁻¹ of added C for the low-N crop residue and +4.1 mg N g⁻¹ of added C for the high-N crop residue after 111 days at 15°C. These figures can be compared with those of Justes et al. (1999) who estimated the net mineralization rate for whole rape residues (with N contents of 0.4 and 0.9%) to be −1.1 and +1.7 mg N g⁻¹ of added C, respectively, in a field experiment of the same time length (i.e. equivalent to 111 day at 15°C). The differences are particularly large for the low-N residue, with the laboratory incubation overestimating the net N immobilization compared to the field situation. This was also observed for rape leaves decomposing at the soil surface in the field or incubated under controlled conditions by Dejoux et al. (2000). Despite the differences in composition of the residues (they were not exactly the same and the field experiment included the leaves which had fallen during crop growth), the differences observed between laboratory and field experiments may arise from the N availability in soil, which is likely to have limited N immobilization in the field (Justes et al., 1999; Trinsoutrot et al., 2000a). The differences in size of the residue fragments and their localization in the soil, which determine the closeness of contact between soil and residues, may have also exerted a substantial effect on decomposition and N cycling as shown by Jensen (1994) and Ambus and Jensen (1997). This demonstrates the importance of understanding and modeling the role of initial location and physical characteristics of plant residues in soil on the subsequent dynamics of C and N.

4.3. Conclusion

The incorporation of rape residues in soil leads to rapid decomposition of the C-substrate added and large N immobilization of N in soil. Over a 168-day period during which most of the C was decomposed, the decomposition of residues induced net immobilization of soil mineral N (compared to a control) in all cases except stems with high N content which only showed slight net mineralization. As the decomposition of residues of various composition was not limited by the overall availability of N, the kinetics of decomposition of the added C depended closely on the soluble compounds, cellulose and lignin content, regardless of
the N content of the residue itself. Conversely the initial residue-N content had a major effect both on the intensity of net immobilization and on the duration of the immobilization phase. In order to identify parameters to describe the relationship between residue quality and C decomposition, and C–N relationships during OM cycling, it appears, therefore, essential to avoid any confounding effect of the control of C dynamics and changes in C–N relationships resulting from the N availability of the soil + residue system. It also requires being able to model the effect of N shortage on the C and N biotransformations, because in most situations the N availability will control the decomposition, due to low soil mineral N content or the residue location.

The use of 15N tracing to label either the residue or the soil mineral N pool gave incomplete and biased pictures of the fate of residue-N, owing to substitution effects and assimilation of residue-N by decomposing microorganisms. But combined with the use of a C–N biotransformation model such as NCSOIL, it allowed access to the total N fluxes involved over time, and understanding of the processes by which residue decomposition influence the net dynamics of N in soil.

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