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Labile organic nitrogen transformations in clay and sandy-loam soils amended with ^{15}N-labelled faba bean and wheat residues

Mervin St. Luce [a,b], Joann K. Whalen [a], Noura Ziadi [b,*], Bernie J. Zebarth [c], Martin H. Chantigny [b]

[a] Department of Natural Resource Sciences, Macdonald Campus, McGill University, 21, 111 Lakeshore Road, Ste-Anne-de-Bellevue, QC, Canada H9X 3V9
[b] Soils and Crops Research and Development Centre, Agriculture and Agri-Food Canada, 2560 Hochelaga Blvd., Quebec City, QC, Canada G1V 2J3
[c] Potato Research Centre, Agriculture and Agri-Food Canada, 850 Lincoln Road, PO Box 20280, Fredericton, NB, Canada E3B 4Z7

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ABSTRACT

Labile organic nitrogen (N) fractions are actively involved in short-term N mineralization, but the extent to which each fraction contributes to N mineralization is not fully understood. The objective of this study was to examine the flow of ^{15}N-labelled faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) residues through the soil microbial biomass N (MBN), water-extractable organic N (WEON), light fraction organic matter N (LFOMN), particulate organic matter N (POMN) and mineral N pools in sandy-loam and clay soils under controlled conditions. After 3 d, 17–30% of the residue ^{15}N was recovered in the POMN fraction, with a greater proportion of the wheat than faba bean residue recovered as POM^{15}N. This POM^{15}N probably included undecomposed residues and LFOM^{15}N. Net N mineralization was greater in faba bean- than wheat-amended soils and greater in the sandy-loam than the clay soil. The LFOM^{15}N concentrations decreased throughout the study, while POM^{15}N concentrations increased or remained constant for 28 d in the sandy-loam and until 56 d in the clay soil. This suggests possible encrustation of LFOMN with soil mineral particles causing increased densification and recovery in the POMN fraction. The subsequent decrease in POM^{15}N concentrations corresponded with mineral ^{15}N accumulation in the soils. Mineral ^{15}N concentration after 112 d was positively related to the initial POM^{15}N concentration ($r = 0.78$, $P < 0.001$) but not to the initial LFOM^{15}N concentration ($r = -0.48$, $P > 0.05$). The WEON and MBN appeared as transient, intermediary pools. The results of this study suggest that mineralization of POMN is a major pathway through which mineral N is supplied in agricultural soils, with C/N ratio of crop residues and to a lesser extent soil properties, influencing the mineralization rate.

1. Introduction

Nitrogen mineralized from soil organic N (SON) makes an important contribution to N needed by agricultural crops and may account for more than 50% of total crop N uptake (Stevens et al., 2005; Nyiraneza et al., 2010). Most of the N mineralized in agricultural soils is expected to come from the labile fractions of SON (Wander, 2004). Nonetheless, increasing the size of a given labile SON fraction may not always increase the soil N supply (Sharifi et al., 2008) if biotic and abiotic factors limit the mineralization process. While it is clear that the labile organic N fractions are actively involved in short-term N mineralization, their roles and importance are not fully understood. Investigating N flows through several labile SON fractions, namely LFOMN, POMN, MBN and water-extractable organic N (WEON), should help to elucidate potential soil N availability.

Due to the rapid generation time of soil microbial biomass, MBN is thought to be the most labile SON fraction. It represents both a source (substrate) and sink (assimilation) of mineral N (Brookes, 2001; Nicolardot et al., 2001). The WEON is widely used as a surrogate for dissolved organic N in soil solutions and includes the dissolved organic N present in macropores and some smaller pores (Chantigny et al., 2008). It is derived from microbial degradation of fresh and partially decomposed organic residues, including autochthonous SON (Murphy et al., 2000; Zsolnay, 2003). The LFOMN and POMN fractions include partially decomposed plant residues together with microbial by-products and are major sources of N for microbes (Gregorich et al., 2006), but these fractions are not structurally and chemically identical. The LFOM contains greater amounts of carbohydrates and aliphatic compounds and

* Corresponding author. Tel.: +1 418 210 5052; fax: +1 418 648 2402.
E-mail address: noura.ziadi@agr.gc.ca (N. Ziadi).

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has a larger particle size, indicative of residues in the initial stages of decomposition, compared with POM (Gregorich et al., 1996). Consequently, LFOMN is frequently linked to N immobilization and not net N mineralization (Boone, 1994; Compton and Boone, 2002), whereas POMN is often related to net N mineralization (Yakovchenko et al., 1998; Whalen et al., 2000), in part due to the lower C/N ratio of POM relative to LFOM. Cambardella and Elliott (1992) found that POM was depleted as a result of cultivation in grassland soils and concluded that POM decomposition had an important role in supplying mineral N. Assessment of N flows through POM and other labile SON fractions is expected to provide a more comprehensive understanding of N transformations and its release in available mineral N forms.

Given that crop residue is the primary substrate for SON replenishment in many agricultural soils (Campbell et al., 1991; Kumar and Goh, 1999), the response of MBN, WEON, LFOMN and POMN fractions (size and physico-chemical stability of the pool) as a function of crop residue quality needs to be studied. The quality of crop residues, e.g., C/N ratio, can influence decomposition and N processes in soils (Chivenge et al., 2011b). Crop residues with low C/N ratios tend to decompose faster (Pansu and Thuriès, 2003; Yanni et al., 2011), increase soil microbial biomass (Chotte et al., 1998; Hoyle and Murphy, 2011) and stimulate net N mineralization (Puttaso et al., 2011; Yanni et al., 2011) in comparison with crop residues with higher C/N ratios. Most models on N transformations in soils are based on or incorporate the influence of C/N ratio of added organic residues (Pansu et al., 1998, 2003; Nicolardot et al., 2001). However, the extent to which crop residue quality affects the formation and turnover of labile SON fractions depends on soil properties such as soil texture and mineral N concentration. For example, clayey soils contain greater amounts of SON and microbial biomass (Six et al., 2006), but tend to have lower N transformation rates than sandy soils due to greater physical protection of crop residues and organo-mineral complexation (Kölbl et al., 2006; Chivenge et al., 2011a). Furthermore, studies show that high initial soil mineral N availability and N additions can stimulate decomposition, particularly of N-poor crop residues (Recous et al., 1995; Liu et al., 2006; Chivenge et al., 2011b).

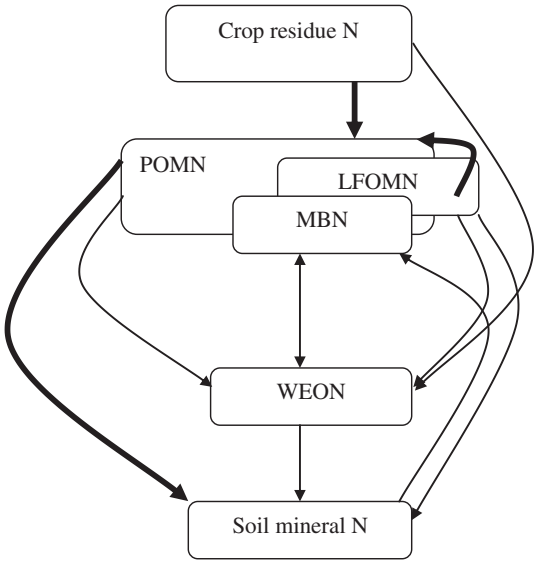

Fig. 1. Conceptual model illustrating the transfer of crop residue N through labile SON fractions. Darker lines indicate a greater level of transfer. The magnitude of N transfer is due to residue C/N ratio and soil controls but the actual fate of residue N shows similar patterns irrespective of residue quality or soil condition.

A conceptual model describing the pattern and magnitude of N flow from crop residues through the labile SON fractions and subsequent release as soil mineral N is illustrated in Fig. 1. Crop residues are generally incorporated into agricultural soils as coarse fragments. The breakdown of the crop residues is mediated by soil microbes, which derive their energy and other nutrients (including N) from the residues. The incorporation of crop residues stimulates microbial activity and may result in an initial increase in the size of the MBN pool; however, the MBN pool is expected to remain relatively constant thereafter since soil microorganisms maintain a stable C/N ratio (Puri and Ashman, 1998; Fierer and Schimel, 2002). Decomposition of crop residues results in the formation of LFOM and POM, which are subsequently further degraded by microorganisms and generate WEON as a by-product (Murphy et al., 2000; Chantigny, 2003). However, the LFOM and POM are not entirely distinct pools in that POM may contain LFOM (Carter, 2002; Six et al., 2002). Under N limiting conditions, WEON may be used as an N source by soil microorganisms (Chantigny et al., 1999b; Marschner and Kalbitz, 2003), part of which may be mineralized to soil mineral N. The rate of crop residue decomposition, the formation of LFOMN and POMN, followed by microbially-mediated mineralization to produce mineral N, is controlled by crop residue quality (e.g., the initial C/N ratio of the residue, Nicolardot et al., 2001; Chivenge et al., 2011b) and the soil texture (Chivenge et al., 2011a), but the actual fate of N is similar between the residues.

To test the interactive effects of residue quality and soil texture on N transfer from crop residues to various N pools, ^{15}N-labelled faba bean (C/N ratio = 29) and wheat (C/N ratio = 91) residues were incubated in clay and sandy-loam soils for 112 d. Our hypotheses were that (i) recovery of crop residue ^{15}N in LFOMN, POMN, WEON, MBN and mineral N will be greater for faba bean than wheat residues because of the lower C/N ratio of the former; and (ii) N mineralization from recently added ^{15}N-labelled crop residues will be slower in the clay soil than the sandy-loam soil due to more physical protection in the clay soil. Another objective of this study was to determine if the LFOMN, POMN and MBN pool sizes were related to soil N mineral concentration. This was done by relating initial ^{15}N concentration in the labile SON fractions to the final ^{15}N concentration in the soil mineral N pool at the end of the incubation.

2. Materials and methods

2.1. Soil sampling

Soils were collected in spring 2011 from the 0–15 cm depth of a field that was previously under corn (*Zea mays* L.) at Agriculture and Agri-Food Canada's Harlaka Research Farm (46° 47′N, 71°08′W) and from a timothy (*Phleum pratense* L.) field in St-Nicolas near Quebec City, Canada (46° 41′N, 71°28′W). The soil from Harlaka was a Kamouraska clay (fine, mixed, frigid, Typic Humaquept; Soil Survey Staff, 2006) and contained 295, 295 and 410 g kg^{-1} of sand, silt and clay, respectively. The soil from St-Nicolas was a St-Antoine sandy-loam (loamy, mixed, frigid, Typic Dystrochrept; Soil Survey Staff, 2006) with 678, 165 and 157 g kg^{-1} of sand, silt and clay, respectively. Large plant materials were removed manually from the field-moist soils before passing through a 6-mm sieve. The soils were air-dried and ground to pass through a 2-mm sieve. Soil chemical characteristics are provided in Table 1.

2.2. ^{15}N labelling of plants

Wheat and faba bean were grown in 2 L pots under controlled environment conditions (16 h day length; maximum day

Table 1
Selected chemical characteristics of soils and crop residues used in the incubation study. Values are mean ± SE ($n = 3$).

Soils and residues	pH[a]	Total N[b]	Organic C[b]	C/N	Mineral N[c]	^{15}N abundance[d]
		g kg^{-1}			mg N kg^{-1}	atom %
Clay	5.53 ± 0.01	2.9 ± 0.02	32 ± 0.1	11.0	12 ± 0.6	ND[e]
Sandy-Loam	6.11 ± 0.01	1.7 ± 0.03	19 ± 0.2	10.9	21 ± 1.2	ND
Faba bean	ND	14.7 ± 0.01	420 ± 0.1	29	ND	2.53
Wheat	ND	4.8 ± 0.01	438 ± 0.5	91	ND	2.84

[a] 1:2 (soil:water).
[b] Dry combustion (Elementar Analysensysteme GmbH, Hanua, Germany).
[c] 2 M KCl extracts.
[d] Mass spectrometer.
[e] ND, not determined.

temperature 21 °C; minimum night temperature 17 °C) and fertilized with ^{15}N (10 atom% $^{15}NH_4^{15}NO_3$) at a rate of 130 mg N pot^{-1} (equivalent to 100 kg N ha^{-1}). Phosphorus and potassium were applied two weeks after germination at rates equivalent to 90 kg P_2O_5 ha^{-1} (120 mg P_2O_5 pot^{-1}) and 110 kg K_2O ha^{-1} (150 mg K_2O pot^{-1}). The plants were irrigated when necessary for optimal growth. Due to differences in growth rate and maturation date, faba bean was harvested after 6 wk, while wheat was harvested after 10 wk. Above-ground tissues were dried at 60 °C for 48 h and ground in a Wiley mill to pass through a 2 mm screen. A sub-sample was further ground to pass through a 1 mm screen for total C and N analyses by dry combustion using an Elementar CN Analyzer (Elementar Analysensysteme GmbH, Hanua, Germany) and ^{15}N enrichment using an isotope ratio mass spectrometer (Delta V Plus, Thermo Fisher Scientific) at Agriculture and Agri-Food Canada's Lethbridge Research Centre in Alberta, Canada. The ^{15}N enrichment and chemical characteristics of the residues are given in Table 1.

2.3. Incubation

Approximately 1.5 g of crop residue (0.1–2 mm) was thoroughly mixed with 200 g of air-dried soil, packed to a bulk density of 1.1 Mg m^{-3} in a 500-mL glass jar and moistened to 60% water-filled pore space with deionized water. Total N added by the faba bean and wheat residue was 110 and 36 mg kg^{-1}, respectively. Four replicates of each treatment were prepared for each sampling date (3, 7, 14, 28, 56 and 112 d after incorporation) to allow for destructive sampling. The jars were covered with parafilm punched with pin holes to maintain aerobic conditions, arranged in a randomized complete block design by sampling date and incubated in the dark at 25 °C. Water content was adjusted every 2–3 d by weighing and adding deionized water as needed. On designated sampling dates, jars were removed from the experiment and thoroughly mixed before they were destructively sampled.

2.4. Analysis of labile SON fractions and mineral N

A 5 g sub-sample was taken for determination of gravimetric water content by oven drying at 105 °C for 24 h. Mineral N (NH_4–N + NO_3–N) was immediately extracted with 2 mol KCl L^{-1} from 12 g moist soil (1:10 soil: solution ratio) followed by filtration through Whatman #42 filter paper (Maynard et al., 2008). Mineral N forms were determined on an automated colorimeter (QuickChem 8000 Lachat autoanalyzer, Lachat Instruments, Loveland, CO); NO_3–N was determined by cadmium reduction and NH_4–N was determined using salicylate–nitroprusside reaction.

Chloroform fumigation-direct extraction (Brookes et al., 1985; Voroney et al., 2008) followed by potassium persulfate digestion (Cabrera and Beare, 1993) was used to determine the MBN concentrations of moist soil. Briefly, 25 g (oven-dry weight) of soil was either directly extracted or fumigated for 24 h followed by extraction with 0.5 mol K_2SO_4 L^{-1} (1:4, soil: extractant ratio). The potassium persulfate reagent (50 g $K_2S_2O_8$ + 30 g H_3BO_4 + 15 g NaOH in 1 L deionized water) was added to the 0.5 mol K_2SO_4 L^{-1} extracts (ratio 1:1) in a Kimax glass tube, capped and autoclaved at 120 °C for 1 h. The MBN concentration was subsequently calculated as [(total N in digests of fumigated soil extracts − total N in non-fumigated soil extracts)/k_{EN}] where k_{EN} is the extraction coefficient 0.54 (Brookes et al., 1985).

Water-extractable organic N of moist soil was determined by gently stirring 60 g (oven-dry basis) of soil with 120 mL of 5 mmol $CaCl_2$ L^{-1} for 1 min (Chantigny et al., 2008). The slurry was then centrifuged at 9000 rpm (11 720 g) for 10 min, followed by filtration through a 0.45 μm nylon membrane filter (Pall Corp., Ann Arbor, MI). A portion of the filtrate was subjected to potassium persulfate digestion (Cabrera and Beare, 1993) as previously described and WEON was calculated as the difference between total dissolved N concentration in potassium persulfate digest and mineral N (NH_4–N + NO_3–N) in fresh filtrate. Total N and mineral N forms were determined on an automated colorimeter as previously described.

The LFOM was separated from 25 g air-dried soil by shaking in sodium iodide solution (NaI; specific gravity of 1.8 g cm^{-3}) for 1 h and allowing the soil mineral particles to settle for 48 h before recovering the floating LFOM (Gregorich and Beare, 2008). The recovered LFOM was washed with 0.01 mol $CaCl_2$ L^{-1} and then rinsed with deionized water to remove the residual NaI. A modification of the procedure described by Gregorich and Beare (2008) was used to isolate soil POM. Briefly, 25 g of air-dried soil was dispersed by shaking in 100 mL of deionized water, instead of sodium hexametaphosphate, and 10 glass beads (6 mm) for 16 h. The dispersed soil was passed through a 53-μm sieve. The retained sand and macroorganic matter were dried at 50 °C, weighed and ground using a mortar and pestle to pass a 250-μm sieve. Whole soil was also sampled at 112 days after residue incorporation and ground to pass through a 250-μm sieve. Total N in LFOM, POM and whole soil were determined by dry combustion using an Elementar CN Analyzer (Elementar Analysensysteme GmbH, Hanua, Germany) while their ^{15}N enrichment was determined using an isotope ratio mass spectrometer (Delta V Plus, Thermo Fisher Scientific) at Agriculture and Agri-Food Canada's Lethbridge Research Centre in Alberta, Canada. All measurements were expressed as concentration per kg of soil (oven-dry basis).

2.5. Isotope analysis

The ^{15}N abundance in the soil extracts (mineral N, MBN and WEON) were measured following a modified micro-diffusion procedure outlined by Stark and Hart (1996). Filter disks (6 mm diameter) were cut from glass microfiber filter circles (Whatman #934-AH), acidified with 10 μL of 2.5 mol $KHSO_4$ L^{-1} and sealed in

Teflon tape (50 mm length, 20 mm width). Since NH_4–N extracted by 2 mol KCl L^{-1} was low throughout the experiment (<3 mg NH_4–N kg^{-1}), NO_3–N and NH_4–N were diffused together to determine mineral ^{15}N. Briefly, 15–20 mL of the 2 mol KCl L^{-1} extracts (containing 45–160 μg N) were pipetted into 250 mL acid- and KOH-washed glass jars, followed by additional 2 mol KCl L^{-1} to obtain a final volume of 30 mL. Samples with <2 μg N mL^{-1} were spiked with 10 mL of a 2 mol KCl L^{-1} solution containing 4 μg N mL^{-1} at 1.0 atom % ^{15}N. One drop of 30% (w/v) Brij-35 was added to reduce spattering and entrapment of NH_4 in H_2 bubbles during NO_3 diffusion (Herman et al., 1995). For comparable headspace in all jars, the volume was brought to 30 mL by adding 2 mol KCl L^{-1}. Then, 0.4 g of MgO, 0.2 g Devarda's alloy and one sealed filter disk were added to each jar. The jar was sealed quickly, incubated at room temperature and swirled once per day for 7 d. Thereafter, the sealed filter disk was removed, rinsed with deionized water, and dried in a desiccator over concentrated H_2SO_4. Dried filter disks were packed in tin capsules for ^{15}N analysis by mass spectrometry.

For $MB^{15}N$, 5–10 mL of K_2SO_4 extracts digested with potassium persulfate (MBN fumigated and non-fumigated extracts) containing 80–160 μg N were pipetted into the jars, followed by 2 mol KCl L^{-1} to obtain a volume of 30 mL. The 2 mol KCl L^{-1} served to increase the molarity of the solution at a level similar to that of the acidified filter disk, thereby reducing the potential for swelling and breaking of the Teflon tape. The pH was raised to >12 by adding 2 mL of 6 mol NaOH L^{-1}. All other procedures were the same as described above for mineral ^{15}N.

Two to 15 mL of 5 mmol $CaCl_2$ L^{-1} extracts digested with potassium persulfate (containing 1–160 μg N) were diffused to determine $WEO^{15}N$. Samples with <3 μg N mL^{-1} were spiked with 10 or 15 mL of a 2 mol KCl L^{-1} solution containing 4 μg N mL^{-1} at 1.0 atom % ^{15}N. All other procedures were the same as described above for mineral ^{15}N.

2.6. Calculations

The atom% ^{15}N in the spiked soil extracts ($At.^{15}N_{extract}$) was determined using the equation of Whalen et al. (2001):

$$At.^{15}N_{extract} = \frac{At.^{15}N_{diffused} \times \left(AN_{extract} + AN_{spike}\right) - \left(AN_{spike} \times At.^{15}N_{spike}\right)}{AN_{extract}} \quad (1)$$

where $At.^{15}N_{diffused}$ is the atom% ^{15}N measured from the disk in the spiked soil extract; $AN_{extract}$ is the μg of N in the soil extract, AN_{spike} is the μg of N added with the spike; $At.^{15}N_{spike}$ is the atom% ^{15}N measured from the disk in the spike solution.

The atom% ^{15}N excess (E_N) was subsequently determined as the difference between $At.^{15}N_{extract}$ and the natural abundance value of the system (atom% ^{15}N measured from the disk in a blank solution that was treated in the same manner as the soil extracts). The ^{15}N abundance of soil mineral N could not be determined in KCl extracts for the first 3 sampling dates because mineral N concentrations were too low relative to the amount of N added with the spike. Due to incomplete diffusion of the $CaCl_2$ extracts digested with potassium persulfate, determination of the ^{15}N abundance in WEON was not possible.

The E_N in the microbial biomass was calculated as the difference in ^{15}N enrichment between the fumigated and non-fumigated extracts:

$$E_N \text{ in MBN} = \frac{\left(E_N \text{ in } N_f \times N_f\right) - \left(E_N \text{ in } N_{nf} \times N_{nf}\right)}{N_f - N_{nf}} \quad (2)$$

where N_f and N_{nf} are the total N concentration (mg kg^{-1}) of the fumigated and non-fumigated extracts, respectively. Recovery of residue ^{15}N in the different N forms was calculated using the equation of Clark et al. (2009):

$$^{15}N \text{ recovered} = \frac{Q_N \times E_N}{Q^{15}N} \quad (3)$$

where Q_N is the amount of N (mg kg^{-1}) measured either in the whole soil, NH_4–N + NO_3–N, MBN, LFOMN or POMN fractions; E_N is the ^{15}N excess of the analysed sample; $Q^{15}N$ is the amount of ^{15}N added with the faba bean (2.35 mg ^{15}N kg^{-1}) or wheat (0.88 mg ^{15}N kg^{-1}) residue.

2.7. Statistical analyses

Data was checked for normality with the Shapiro–Wilk test and log transformed when necessary. The data presented in the tables and figures are untransformed means ± standard error (SE). The effect of crop residue quality and soil texture (between subject effects) on soil mineral N, MBN, WEON, LFOMN, POMN concentrations and ^{15}N recovered in these pools were assessed using repeated measures analysis of variance (ANOVA). The total N and ^{15}N recovered in the labile SON fractions and soil mineral N over time were regarded as repeated measurements (within subject effects). We used the PROC MIXED procedure Repeated Measures ANOVA of SAS statistical software, version 9.2. The choice of covariance structure, as outlined in PROC MIXED of SAS, was based on goodness-of-fit (Littell et al., 1998). Differences were considered statistically significant at $P < 0.05$. When significant effects were observed, means were compared with a post-hoc least square means test at 95% confidence level. Pearson correlation coefficients were used to examine the relationships between mineral ^{15}N recovered at the end of the incubation (112 d) and initial ^{15}N recovered in MBN, LFOMN and POMN (3 d).

3. Results

3.1. Soil and crop residue characteristics

Prior to crop residue addition, total N and organic C concentrations were more than 60% greater in the clay than the sandy-loam soil, while initial mineral N concentration was nearly 2-fold higher in the sandy-loam soil than the clay soil (Table 1). The clay soil was slightly (0.6 pH units) more acidic than the sandy-loam soil. Finally, the C/N ratio of the wheat residue was 3 times greater than the faba bean residue.

3.2. Soil mineral N dynamics

Soil mineral N concentrations increased over time ($P < 0.001$) and were influenced by soil type, residue type and their interaction ($P < 0.001$). Net N mineralization (i.e., the increase in soil mineral N concentration above the concentration before incubation) was

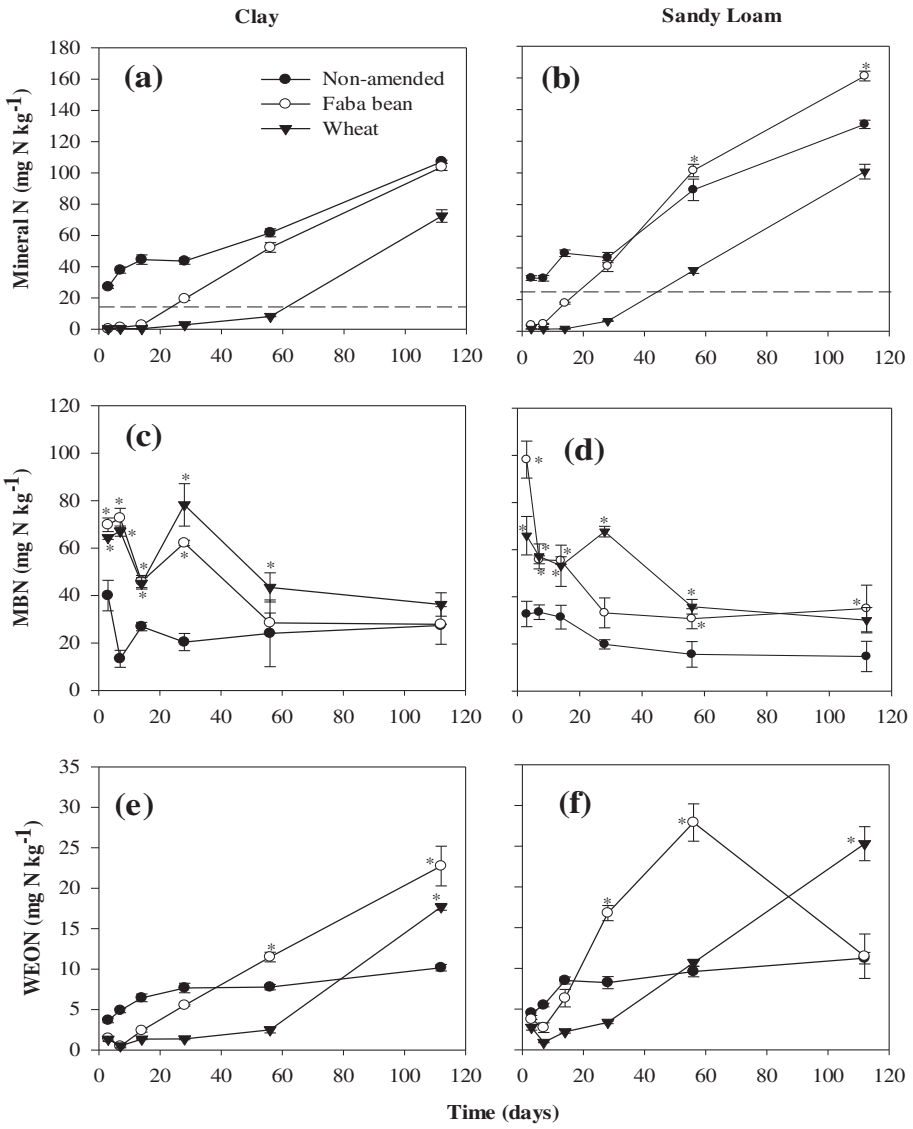

Fig. 2. Mineral N, MBN and WEON dynamics in clay and sandy-loam soils amended with faba bean and wheat residues and a non-amended control. Dotted line represents the initial soil mineral N concentration before the start of the incubation. Bars represent SE ($n = 4$). (*) Indicates significantly ($P < 0.05$) greater than the non-amended for each date.

greater in the sandy-loam than the clay soil, highest in faba bean-amended sandy-loam and lowest in wheat-amended clay soil (Fig. 2a and b). At 56 and 112 d, mineral N concentrations in sandy-loam soil were greater ($P < 0.001$) for the faba bean-amended than the non-amended soil. In the clay soil, mineral N concentrations were always lower with than without crop residues. Net N mineralization took place after 14 d in the faba bean-amended soils. However, the initial mineral N concentration was not exceeded until day 28 and 56 in the wheat-amended sandy-loam and clay soils, respectively (Fig. 2a and b).

3.3. Labile SON fractions

The MBN concentrations were significantly ($P < 0.001$) influenced by residue type but not by soil type or their interaction ($P > 0.05$). The MBN concentrations in the non-amended clay soil significantly ($P < 0.001$) decreased from 3 to 7 d but remained relatively constant after 14 d (Fig. 2c). In the non-amended sandy-loam soil, MBN concentrations were relatively stable during the first 14 d, then significantly ($P < 0.001$) decreased and remained stable thereafter (Fig. 2d). The MBN accounted for 0.9–2% of total soil N in the clay soils and from 1.5 to 3% in the sandy-loam soils. Residue incorporation increased MBN in both soils compared with the non-amended soils during the first 60 d ($P < 0.001$), but thereafter, MBN in the residue-amended clay soil and the wheat-amended sandy-loam soil (Fig. 2c and d) were similar to the corresponding non-amended soils. After 112 d, MBN was still greater in the faba bean-amended sandy-loam soil than in the non-amended sandy-loam soil. The initial (3–7 d) increase in MBN in residue-amended soils tended to be greater with faba bean than wheat, particularly in the sandy-loam soil (Fig. 2d). There were no significant ($P > 0.05$) interactions between soil and residue types on soil MBN concentrations.

Total WEON concentrations were significantly ($P < 0.001$) influenced by soil type, residue type and their interaction (Fig. 2e and f). The WEON was greater in the sandy-loam than the clay soil and accounted for 0.1–0.3% of total N in the clay soil, and 0.4–0.7% in the sandy-loam soil. The WEON concentrations followed the order of faba bean-amended > non-amended > wheat-amended soils ($P < 0.001$). The WEON concentrations were greater with faba bean

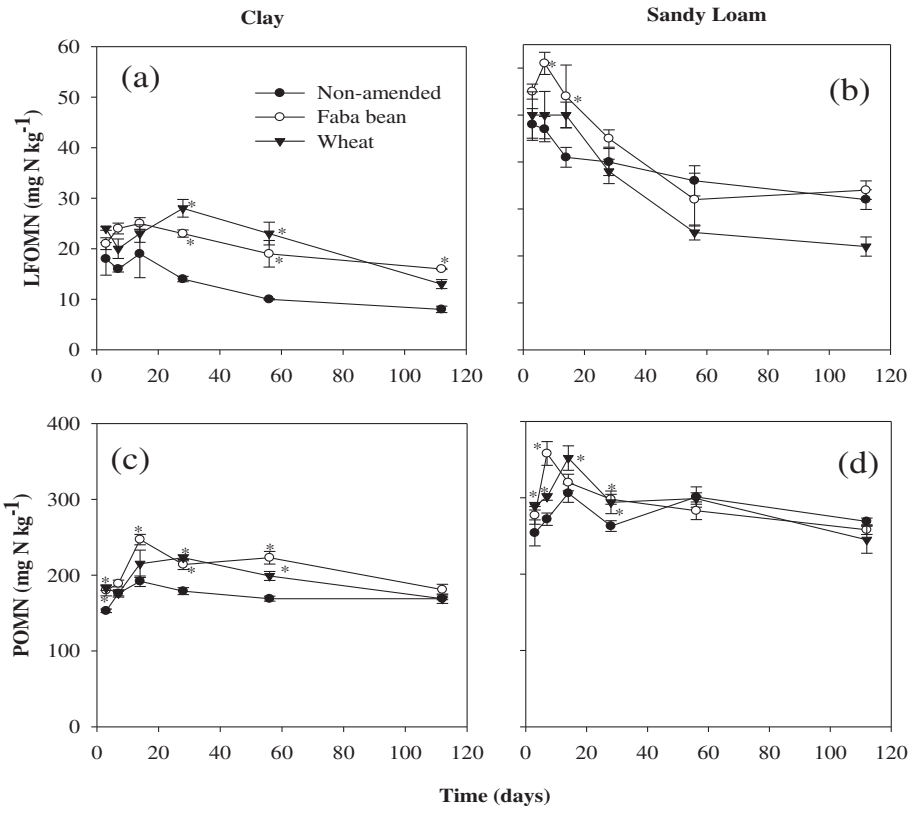

Fig. 3. LFOMN and POMN dynamics in clay and sandy-loam soil amended with faba bean and wheat residues and a non-amended control. Bars represent SE ($n = 4$). (*) Indicates significantly ($P < 0.05$) greater than the non-amended for each date.

than non-amended after 56 ($P < 0.01$) and 112 ($P < 0.001$) d in the clay soil, and after 28 ($P < 0.001$) and 56 d ($P < 0.001$) in the sandy-loam soil. However, WEON was greater with wheat than in non-amended soils only at 112 d ($P < 0.001$) after residue incorporation.

The LFOMN was influenced by soil type, residue type and their interaction ($P < 0.001$), and was greater in the sandy-loam than in the clay soil (Fig. 3a and b). The proportion of total soil N recovered as LFOMN at the end of the incubation was 0.3–0.6% in the clay soil and 1.3–2% in the sandy-loam soil. Compared to the non-amended clay soil, adding faba bean increased LFOMN ($P < 0.05$) from 28 d after incorporation until the end of the incubation, whereas, adding wheat significantly increased it at 28 and 56 d only ($P < 0.01$). In the sandy-loam soil, adding faba bean increased LFOMN ($P < 0.01$) at 7 and 14 d after residue incorporation, while incorporation of wheat residues did not increase LFOMN ($P > 0.05$).

The POMN was significantly ($P < 0.001$) influenced by soil type and residue type (Fig. 3c and d). The sandy-loam soil had greater total POMN than the clay soil. The proportion of total soil N recovered as POMN at the end of the incubation ranged from 6 to 7% in the clay soil and from 14 to 16% in the sandy-loam soil. The POMN was greater with than without crop residues during the first 60 d in the clay soil, compared with the first 28 d in the sandy-loam soil. Over the 112 d, POMN remained fairly constant in the non-amended soils, whereas LFOMN declined ($P < 0.05$) (Fig. 3). After 112 d, LFOMN was higher ($P < 0.05$) in the faba bean-amended clay than in the non-amended clay. In the sandy-loam soil, LFOMN followed the pattern faba bean = non-amended > wheat ($P < 0.05$).

3.4. ^{15}N in mineral N and labile SON fractions

Based on ^{15}N added from the crop residues (2.35 and 0.88 mg ^{15}N kg^{-1} for faba bean and wheat, respectively) and subsequently released as mineral N after 112 d, 22% and 36% of the faba bean residue N was mineralized in the clay and sandy-loam soil, respectively, compared with 13% and 20% of the wheat residue N, respectively. Soil type, residue type and their interaction affected ($P < 0.001$) residue ^{15}N in the mineral N pool (Fig. 4). The mineral ^{15}N accumulation followed the order of faba bean-amended sandy-loam > faba bean-amended clay = wheat-amended sandy-loam > wheat-amended clay ($P < 0.05$). The mineralization of residue ^{15}N progressed over time in all treatments, but at a faster rate in the residue-amended sandy-loam than the residue-amended clay soils.

The MB^{15}N concentrations were influenced ($P < 0.001$) by soil and residue type but not by their interaction (Fig. 4). On the third day of the incubation, 5% of faba bean and 4% of wheat residue N were recovered as MBN in the clay soil, whereas 11% and 8% were recovered in the sandy-loam soil. The MB^{15}N remained relatively stable throughout the experiment in the wheat-amended clay soils, whereas it deceased ($P < 0.001$) from day 3 to day 7 and remained stable thereafter in the sandy-loam soils. Although not statistically significant ($P > 0.05$), there was a slight temporal variation in the MB^{15}N in the faba bean-amended clay soil. After 56 days, about 4% of the wheat and 6% of the faba bean residue ^{15}N remained in the MBN in the clay soil, while about 6% remained in the sandy-loam soil.

The LFOM^{15}N concentrations were not influenced by soil type, residue type or their interaction ($P > 0.05$), but decreased ($P < 0.001$) as the incubation progressed (Fig. 4). The POM^{15}N concentrations were significantly influenced by residue type ($P < 0.001$) and the soil type × residue type interaction ($P < 0.01$), but the soil type effect was not significant. The POM^{15}N slightly increased and remained relatively stable during the first 60 d in the wheat-amended clay soil, whereas it gradually decreased after 14 d

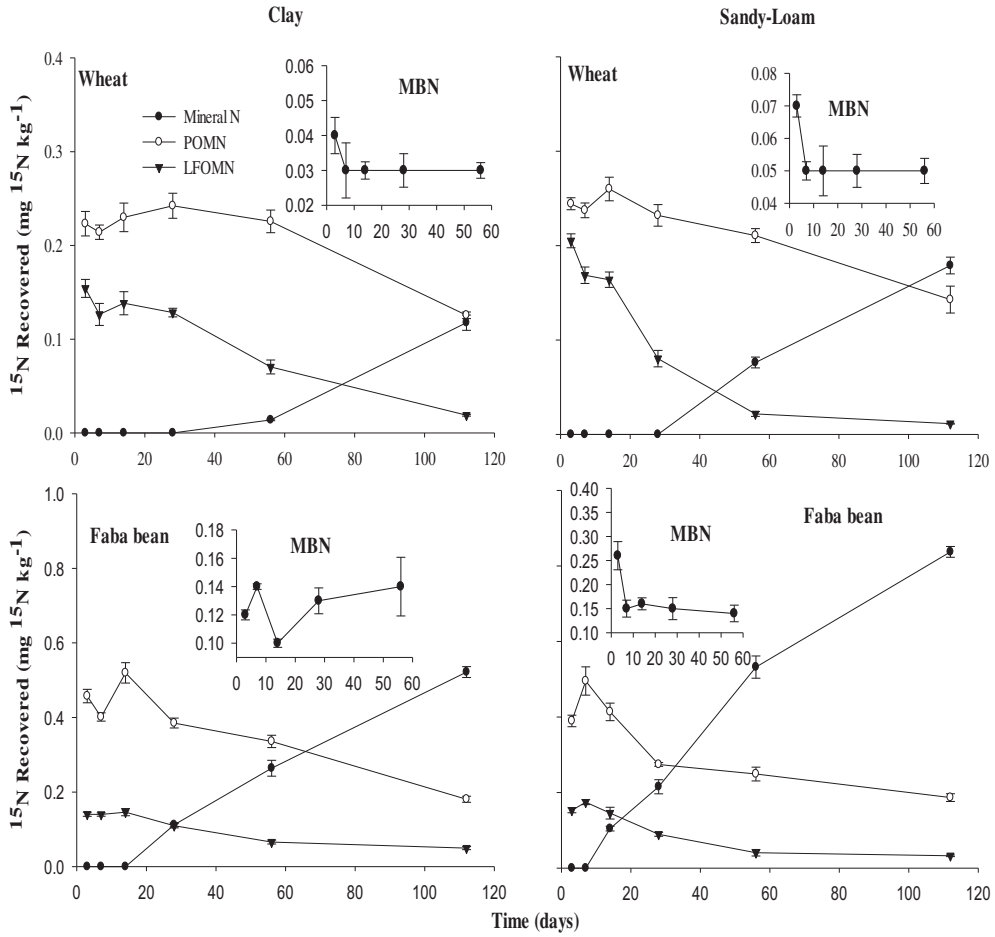

Fig. 4. ^{15}N recovered in mineral N, MBN, POMN and LFOMN in clay and sandy-loam soils amended with faba bean and wheat residues. Bars represent SE ($n = 4$).

in the faba bean-amended clay soil. In the wheat-amended sandy-loam soil, POM^{15}N increased slightly during the first 14 d and decreased slowly thereafter, with a sharper decrease after 60 d. However, after an initial increase, POM^{15}N sharply decreased from 7 to 28 d in the faba bean-amended sandy-loam soil, and then slowly decreased thereafter. The POM^{15}N concentrations were greater in faba bean- than wheat-amended soils, however, a greater proportion of the wheat (average of 24–25%) than faba bean (average of 14–16%) residues were recovered as POMN in both soils (Fig. 4). Generally, POM^{15}N concentrations decreased during the incubation but at a faster rate in faba bean- than wheat-amended soils.

A greater proportion of the ^{15}N from both residues was initially recovered as POMN (17–30%) than LFOMN, MBN or mineral N in both soils (Fig. 4). By the end of the incubation, 11–12% of the residue ^{15}N remained as POMN in both soils, compared with only 1–2% as LFOMN (Table 2). Average ^{15}N recovered in whole soils at the end of the incubation was high and accounted for 85% and 92% of initial wheat and faba bean residue ^{15}N applied, respectively (Table 2). The LFOM^{15}N concentrations decreased throughout the study, while POM^{15}N concentrations increased or remained constant for 14–28 d in the sandy-loam and 28–56 d in the clay soil. As illustrated in Fig. 4, the decline in POM^{15}N concentrations corresponded to an accumulation in mineral ^{15}N. This was particularly obvious in the wheat-amended soils. The mineral ^{15}N concentration at the end of the incubation was positively correlated to initial (d 3) POM^{15}N ($r = 0.78$, $P < 0.001$, $n = 15$) and MB^{15}N concentration ($r = 0.93$, $P < 0.001$, $n = 13$), but was not related to LFOM^{15}N concentration ($r = -0.48$, $P > 0.05$, $n = 14$). In addition, MB^{15}N concentration at d 3 was positively related to POM^{15}N concentration at d 3 ($r = 0.72$, $P < 0.01$, $n = 14$) but was not related to LFOM^{15}N concentration at d 3 ($r = -0.39$, $P > 0.05$, $n = 13$). Finally, POM^{15}N and LFOM^{15}N concentrations at d 3 were negatively correlated ($r = -0.60$, $P < 0.05$, $n = 15$).

4. Discussion

4.1. Effect of residue quality

More ^{15}N accumulated in the mineral N pool from the faba bean residue in both soils compared with the wheat residue, which presumably resulted from the narrower C/N ratio and higher N content of the faba bean residue (Trinsoutrot et al., 2000; Nicolardot et al., 2001). Conversely, more ^{15}N from the wheat residue than from the faba bean residue was recovered in the POMN fraction at the beginning and at the end of the incubation. As the POMN fraction is composed of partially decomposed plant residues together with microbial by-products (Gregorich et al., 2006), our finding suggests that a greater portion of the wheat residue N accumulated and was retained in the pool of undecomposed and partially decomposed residues, with less N incorporated into the microbial biomass and transferred to the mineral N pool than from faba bean residue because of the wider C/N ratio of wheat residues. Alternatively, less of the faba bean residue N initially accumulated and remained as POMN since it was mineralized at a much faster rate than the wheat residue. These results are in agreement with

Table 2
^{15}N recovered in whole soil, mineral N and labile SON fractions after 112 days in clay and sandy-loam soil amended with faba bean and wheat residues. Values are mean ± SE ($n = 8$).

Soils and residues	Whole soil	Mineral N	MBN[a]	LFOMN	POMN
	% of applied ^{15}N				
Clay	89.7 ± 2.0	17.2 ± 1.9	4.5 ± 0.5	2.1 ± 0.1	11.0 ± 1.3
Sandy-Loam	87.3 ± 2.4	28.0 ± 2.9	5.7 ± 0.4	1.3 ± 0.1	12.1 ± 1.8
Faba bean	91.7 ± 1.6	29.9 ± 2.7	6.1 ± 0.5	1.7 ± 0.2	7.8 ± 0.3
Wheat	85.3 ± 2.2	16.9 ± 1.5	4.6 ± 0.4	1.6 ± 0.2	15.3 ± 0.9

[a] Values are after 56 d.

previous findings that C/N ratio and N content of crop residues influences N mineralization in soils (Trinsoutrot et al., 2000; Nicolardot et al., 2001; Pansu et al., 2003; Schimel and Hättenschwiler, 2007; Yanni et al., 2011; Gul et al., 2012). Nicolardot et al. (2001) showed that most of the N assimilated by the microbial biomass in wheat straw-amended soil (high C/N ratio residue) was derived from soil mineral N, with only a minor fraction coming from the wheat straw residue. Conversely, N from low C/N ratio residues was rapidly assimilated by the soil microbial biomass.

The greater WEON concentrations in the faba bean- than wheat-amended soils were probably also related to the narrower C/N ratio and greater N concentration of the faba bean residues compared with the wheat residues. In the residue-amended soils, WEON concentrations increased gradually over 112 d but the concentrations remained relatively low. The increase in WEON concentrations can be attributed to the release of soluble organic N following microbial degradation of the crop residues (Murphy et al., 2000; Chantigny, 2003). The relatively constant WEON concentrations in the non-amended soils suggest that WEON was probably being utilized by the microbial biomass (Whalen et al., 1999; Cookson et al., 2005) at a rate similar to its production rate.

4.2. Effect of soil texture and initial soil mineral N concentration

Our second hypothesis was that N transformation from recently added ^{15}N-labelled crop residues would be faster in the sandy-loam soil than the clay soil. The release of residue ^{15}N to the mineral N pool was consistent with this hypothesis in that mineral ^{15}N was greater in the sandy-loam than the clay soil. These results were in agreement with other studies (Hassink, 1994; Griffin et al., 2002; Thomsen et al., 2003; Cookson et al., 2006) and may be due to greater microbial access to the residues in the sandy-loam than the clay soil, which would be consistent with the greater WEON and initial MB^{15}N concentration in the sandy-loam soil. Microbial access is usually lower in clay soils, particularly at low residue application rates as in our study, due to binding by clay particles. This can significantly reduce microbial respiration (Colman and Schimel, 2013; Roychand and Marschner, 2013), and thereby result in lower decomposition and N mineralization rates (Colman and Schimel, 2013). Ammonium fixation by clay particles in a non-extractable form may have also reduced the concentration of mineral N extracted in the clay soil (Chantigny et al., 2004). The greater initial mineral N concentration in the sandy-loam than in the clay soil may have also led to faster decomposition of the residues in the sandy-loam soil. In the non-amended soils, mineral N accumulation was also greater in the sandy-loam than the clay soil due to less physical protection and organo-mineral complexation (Kölbl et al., 2006; Chivenge et al., 2011a). In comparison with this study, previous studies have reported greater net N mineralization in clay than sandy soils (Chivenge et al., 2011a; Gul et al., 2012). In a mesocosm experiment, Chivenge et al. (2011a) reported faster decomposition of organic residues in a clay in comparison to a sandy soil due to higher soil water content and total soil N concentration in the clay soil. In our study, soil total N concentration was also higher in the clay than the sandy-loam soil, but soil available N was greater in the sandy-loam soil. Other studies (Scott et al., 1996; Thomsen et al., 2001) reported no or minor soil texture effects on N mineralization in short-term incubations where sieved and homogenized soils were used and optimal conditions such as moisture and finely ground residues for microorganism growth and activity were provided. The residue size in our study ranged from 0.01 to 2 mm but was ≤1 mm in the other studies.

The tendency for a longer immobilization period in the wheat- than faba bean-amended soils, particularly in the clay soil which had a lower initial soil mineral N concentration, provides support to an already established fact that initial soil N availability has a greater impact on net N mineralization from N-poor than N-rich residues (Recous et al., 1995; Liu et al., 2006).

4.3. N transformation through labile SON fractions

To our knowledge, this was the first study in which ^{15}N-labelled residues of differing quality (C/N ratio) were incubated in different soil types to simultaneously assess N flows through key labile SON fractions (LFOMN, POMN, MBN and WEON) before entering the soil mineral N pool. More than 85% of the added ^{15}N was recovered in each whole soil after 112 d. As soils and residues were incubated in closed vessels, the unrecovered ^{15}N was probably lost through gaseous N emissions. The fact that POM^{15}N concentrations were influenced by residue type and not by soil type suggests that the formation and subsequent mineralization of POMN was governed more by the biochemical composition of the crop residues and less by soil properties. This also suggests that residue quality may exhibit a greater control than soil properties in the initial stages of residue decomposition, which supports previous findings (Nicolardot et al., 2001; Zeller and Dambrine, 2011; Gul et al., 2012). Most models, e.g., the Transformation of Added Organics (TAO) model (Pansu and Thuriès, 2003; Pansu et al., 2003) and the MOMOS N model (Pansu et al., 1998), conclude that residue decomposition and N mineralization depend on organic residue quality.

Consistent with the conceptual model, a greater proportion of the ^{15}N from both crop residues was recovered in the POMN fraction than the other labile SON fractions. The relatively low recovery of ^{15}N in POMN and LFOMN after 3 d could be explained by (i) the presence of readily soluble N and (ii) incomplete recovery of LFOM and POM. It is possible that encrustation of the residues with mineral particles was already initiated by day 3 (Golchin et al., 1994; Chantigny et al., 1999a), causing an increase in residue density and thereby negatively affecting LFOM recovery. Additionally, mechanical breakdown of macroorganic materials with the action of glass beads may have occurred during POM extraction, whereby some of the residues were probably broken down into fragments smaller than 53 μm. It is also possible that a proportion of the crop residue was probably tightly bound to clay minerals (Kölbl et al., 2006; Roychand and Marschner, 2013). The greater recovery of residue ^{15}N in the POM^{15}N fraction compared to other fractions measured may be due to several reasons. Firstly, the POMN fraction recovered likely included undecomposed residues in the initial stages, particularly in the wheat-amended soils. Secondly, at the beginning of the incubation, the fresh crop residues were most likely recovered in the LFOM fraction. Since POMN and LFOMN were not sequentially extracted, the LFOMN fraction was therefore likely recovered in the POMN fraction (Carter, 2002; Six et al., 2002). In addition, encrustation of the crop residues with microbial by-products (e.g., polysaccharides) and soil mineral particles may have caused the LFOMN to be recovered in the POMN fraction

(Golchin et al., 1994; Chantigny et al., 1999a). We used NaI at a specific gravity of 1.8 g cm^{-3}. It is likely that the density of some of the LFOM was >1.8 g cm^{-3} due to adherence of microbial by-products and soil mineral particles, thereby causing them to sink during LFOM extraction (Golchin et al., 1994). We observed that the decrease in POM^{15}N concentrations started at a later stage compared with LFOM^{15}N concentrations and, in some cases, POM^{15}N concentrations actually increased while LFOM^{15}N concentrations decreased. Hence, microbial colonization, encrustation and degradation of LFOM^{15}N probably enhanced the recovery of ^{15}N in the POMN fraction, but the decline in LFOM^{15}N was not directly associated with an increase in mineral ^{15}N. It is widely known that LFOMN contains greater amounts of carbohydrates and aliphatic compounds and more recently decomposed residues than POMN (Gregorich et al., 1996). Vanlauwe et al. (1998) and Zeller and Dambrine (2011) also reported that most of the ^{15}N in ^{15}N-labelled residues were recovered in the POMN fraction.

In this study, the progressive decrease of POM^{15}N and LFOM^{15}N were associated with an increase in mineral ^{15}N. More specifically, soil mineral ^{15}N accumulation commenced at the stage where POM^{15}N and not LFOM^{15}N started to decrease, while at the same time, MB^{15}N remained relatively constant. The ^{15}N-mineral N at the end of the incubation was correlated to the initial POM^{15}N but not to the LFOM^{15}N. When the crop residues were added to the soils, the soil microorganisms colonized the surfaces of the residues, thus initiating the start of the decomposition process (Golchin et al., 1994; Ladd et al., 1996; Chotte et al., 1998). The large and active microbial community associated with this process released polysaccharides and most likely resulted in encrustation of the residues with mineral particles (Golchin et al., 1994, 1998; Chantigny et al., 1999a). This may have caused rapid densification of the residues (Chantigny et al., 1999a), whereby less of the crop residues were recovered as LFOM and more were recovered as POM. The encrusted POM likely contained SON at various stages of decomposition and the newly encrusted crop residues. As decomposition continued, the encrusted POM was gradually broken down into smaller pieces by the colonizing microbes and was transferred to the fine SOM fraction (Golchin et al., 1994; Chantigny et al., 1999a), explaining the later decline in POMN recovery. As this process unfolded, more of the organic N became accessible to decomposers and N mineralization rate increased, thereby explaining the correspondence in temporal dynamics of POMN and soil mineral N.

The positive relationship between POM^{15}N and MB^{15}N concentrations suggests that POMN was the site of localized biological activity (Chotte et al., 1998; Magid and Kjærgaard, 2001) in these soils. In forest soils, Zeller and Dambrine (2011) reported that ^{15}N abundance of soil mineral N produced was closer to that of the POM fraction than that of mineral-bound fractions (<50 μm). Cambardella and Elliott (1992) suggested that POM accounts for the majority of SOM that is initially lost due to cultivation of grassland soils. The MB^{15}N remained relatively stable because the total pool size did not vary significantly during the experiment (Puri and Ashman, 1998; Fierer and Schimel, 2002, 2003). Our results are in agreement with previous findings which indicate that while soil MBN accounts for a small portion of total soil N, it plays a major role in the N cycle by acting upon most N transformation processes in soils (Pansu et al., 1998; Manzoni and Porporato, 2009). After an initial increase in the size of the microbial biomass due to crop residue incorporation, MBN concentrations were similar in residue-amended and non-amended soils after about 60 d, suggesting that N was being cycled very quickly through the microbial biomass, most likely in a matter of days (Whalen et al., 1999; Gregorich et al., 2000). In addition, the initial increase in MBN was probably derived more from N released from the soil and dead microbial cells than the added residues, due in part to soil disturbance and rewetting (Jackson, 2000; Wu and Brookes, 2005), since only 4–11% of the added residues were initially recovered as MBN. Overall, our results suggest that mineralization of POMN may increase N availability in agricultural soils and that MBN is a transient, intermediary pool responsible for N mineralization. The importance of soil microbial biomass in controlling the transfers between organic and inorganic compartments was previously shown in the MOMOS N model (Pansu et al., 1998). It should be noted that our results concerning POMN may be partly due to the method of POM extraction. Nevertheless, studies reported that POMN was the major source of mineral N while LFOMN may be a sink (Boone, 1994; Whalen et al., 2000; Compton and Boone, 2002). Vanlauwe et al. (1998) also reported that ^{15}N present in POMN was highly related ($P < 0.001$) to corn (*Zea mays* L.) N uptake. Results from a field experiment on a clay loam soil in Quebec, Canada indicated that POM is not a strong sink for mineral fertilizer N and that N storage in POM was only transient (Nyiraneza et al., 2010). Conversely, Janzen (1987) reported that LFOMN was significantly related to N mineralization ($r = 0.87$) but this fraction may have contained LFOMN and POMN because it was collected by a combination of density and size separation. Although the determination of the ^{15}N abundance in WEON was not possible, our results provide support to the conceptual model (Fig. 1), which illustrates the possible flow of N from crop residues in agricultural soils.

5. Conclusion

Tracing the fate of ^{15}N-labelled faba bean and wheat residues showed that after 3 d, most of the ^{15}N was recovered in the POMN fraction. It is likely that the POMN fraction included LFOMN as well as undecomposed residues. The quantity of crop residue N recovered in different pools and the turnover rate were influenced more by the C/N ratio of the crop residue than by soil properties. The ^{15}N recovered in the POMN fraction after 3 d was related to ^{15}N recovered in the soil mineral N pool after 112 d. The WEON and MBN appeared to be transient, intermediary pools. Our results are consistent with the hypothesis that POMN is a key labile SON fraction and its decomposition by soil microorganisms is a major pathway through which soil mineral N is supplied in agricultural soils. Practices that increase POMN concentrations, such as inclusion of legumes in crop rotations, should be encouraged to enhance N availability and soil fertility.

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