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Incorporation of nitrogen from crop residues into light fraction organic matter in soils with contrasting management histories

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Abstract The proportion of N from crop residues entering the light fraction organic matter (LFOM) pool was investigated in soils with contrasting soil organic matter and microbial characteristics arising from different management histories. A laboratory experiment was conducted in which ¹⁵N-labelled sugarbeet, Brussels sprout or ryegrass shoots, which possessed a range of C/N contents, and hence different biochemical qualities, were incorporated into a sandy-loam soil collected from within a field (FC), or from the field margin (FM). Amounts of C and N incorporated into LFOM were determined after 112 d. The FC and FM soils had organic C contents of 0.9 and 2.5 % respectively. Addition of crop residues increased total LFOM N content and reduced its C/N in FC soil, but had no effect on total LFOM N or its C/N in FM soil. Ryegrass incorporation into FC was the only treatment in which there was a net increase in LFOM C. Isotopic analysis indicated that more crop residue derived N became incorporated into the LFOM N pool in FM relative to FC soil, with % crop residue N incorporated ranging from 25.9 to 35.3 % in FC, and between 38.9 and 68.5 in FM. Incorporation of crop residues had a positive priming effect on pre-existing LFOM N in FM but not FC soil. We conclude that the characteristics of plant

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1 material, together with differences in soil organic matter and microbiology resulting
2 from contrasting management, determined the amount of crop residue C and N
3 incorporated into both HFOM and LFOM.

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5 *Keywords:* soil organic matter; Light fraction organic matter; crop residue quality;
6 decomposition; priming

8 **Introduction**

9 Understanding the factors controlling the interplay between mineralisation and
10 stabilisation of soil organic matter (SOM) is a prerequisite for managing both nutrient
11 dynamics and C sequestration, and thereby optimising the ecosystem service provided
12 by a given soil (Janzen, 2006). SOM is a heterogeneous substrate comprising materials
13 with a range of origins and characteristics, and SOM pools have been separated and
14 characterised using both physical and chemical methods (von Lutzow et al., 2006).
15 Physical fractionation of SOM attempts to separate SOM according to the degree to
16 which it is protected against microbial degradation, and pools are quantified by
17 determining the organic material protected through chemical interactions, association
18 with clay and silt particles, physically protected within aggregates, or that remaining
19 unprotected (Six et al., 2002).

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20 One of the key pools defined within physical fractionation schemes is light fraction
21 organic matter (LFOM). The LFOM pool largely represents partially degraded plant
22 materials together with microbial tissues and products which are not associated with
23 mineral soil particles (Six et al., 2002). LFOM represents an unprotected pool of SOM
24 and is readily degradable relative to protected pools. It is therefore considered to be

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1 one of the most labile pools of SOM. As a result, the size of the LFOM pool responds
2 much more quickly to agricultural management than the total SOM pool, and LFOM
3 is considered to represent an early indicator to determine long term impacts of
4 management on soil quality and C sequestration (Janzen et al, 1992; Bending et al,
5 2004; Leifeld and Kogel-Knaber, 2005). Soil C, N and P mineralisation have all been
6 correlated with LFOM, confirming that it represents an active pool of SOM with
7 importance to plant nutrient supply (Hassink, 1995; Sierra, 1996; O'Hara et al, 2006).
8 Much is known about the influence of soil, environmental and management variables
9 on the dynamics of LFOM, with the size of the LFOM pool influenced by crop
10 rotation (Bending et al, 2000, 2004; Marriott and Wander, 2006), N fertilisation (Malhi
11 et al, 2003), and tillage (Beare et al, 1994). However, mechanisms controlling the
12 formation and turnover of LFOM are poorly understood. The biochemical quality of
13 inputs clearly has a role in directing amounts of C and N incorporated into LFOM.
14 Bending et al. (1998) indicated that net amounts of crop residue C and N incorporated
15 into the LFOM pool depended on crop residue quality, with N content and cellulose in
16 particular being important predictors of the amount of C and N immobilised.
17 However, the amount of LFOM can vary widely in different soil types (Hassink,
18 1995), and the role of specific major soil factors such as texture, clay and existing
19 SOM content in controlling retention of crop residue inputs as LFOM remains to be
20 determined.

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21 In the current study we used paired soils with identical mineralogical
22 composition but differing organic matter contents to investigate how soil and crop
23 residue characteristics affect amounts of crop residue C and N stabilised into LFOM.

24

1 **Methods**

2 Soil

3 Soil was collected from 2 sites with identical mineralogical composition, but different
4 amounts of organic matter, from Bradley's field at Warwick HRI, Wellesbourne,
5 Warwickshire, UK. The soil is an undifferentiated sandy-loam of the Wick series with
6 74 % sand and 14 % clay (Whitfield, 1974). The first site (FM) was located in the field
7 margin near to a hawthorn (*Crataegus monogyna* Jacq.) hedge. The second site (FC)
8 was located within the farmed part of the field, approximately 8 m from the FM site
9 (Bending et al., 2002). The field had been ploughed following a crop of winter wheat.
10 At both sites, soil was collected from 0-30 cm depth. Surface litter was removed from
11 the FM site prior to sampling.

12 Soil from both sites was sieved (<3 mm), air dried overnight, and stored at 4°C
13 for 4 weeks. Total organic C and N were determined using an automated C/N analyser
14 (CB-2000, Leco Corporation, Michigan, USA). The FC and FM soils were shown to
15 possess, respectively, organic C contents of 0.86 and 2.5 %, organic N contents of
16 0.08 and 0.21 %, and pH values of 5.3 and 5.4. While respiration in the soils prior to
17 the start of the experiment was equivalent, microbial biomass-N was 3.2 and 38.3 µg
18 g⁻¹ dw soil in FC and FM respectively (Bending et al., 2002). Prior to use, the soils
19 were moistened to a water holding capacity of 60 %, and incubated at 15°C for 7 days.

20

21 Plant materials

22 Sugarbeet (*Beta vulgaris* L.), Brussels sprout (*Brassica oleracea* L. var *gemmifera*)
23 and rye grass (*Lolium perenne* L.) were grown in sand culture under controlled
24 environment glasshouse conditions (16 h day length, maximum day temperature 25°C,

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1 minimum night temperature 15°C), and fed weekly with Hewitt's solution (Hewitt and
2 Smith, 1975), in which the N source was 10 at % ¹⁵N-labelled ¹⁵NH₄¹⁵NO₃. After 8
3 weeks growth, mature leaves were removed, and the lamina and petiole cut into
4 approximately 1 cm square pieces, and was incorporated into soil fresh without
5 drying. The biochemical quality of oven dried plant materials was determined by
6 sequential fractionation, to give soluble carbohydrate, phenolic, cellulose, and lignin
7 contents (Rahn et al. 1999). Total C and N were determined by C/N auto-analysis.

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9 Incubation study

10 Five g fresh weight (fw) of plant material was mixed into 100 g fw soil, and poured
11 into polystyrene containers. The ryegrass, sugarbeet, and Brussels sprout leaves had
12 moisture contents of 77.5, 86.1 and 80.4 % respectively. The additions provided
13 carbon inputs of 5.2, 3.2 and 4.6 mg C g⁻¹ dw soil for the ryegrass, sugarbeet and
14 Brussels sprout respectively (Table 1). The base of the pot was tapped firmly to allow
15 the contents to settle, providing a water filled pore space of approximately 22 %.
16 Control treatments containing unamended soil were also included. Five replicates of
17 each treatment were set up for each harvest. Containers were incubated using a
18 randomised block design in the dark at 15°C, inside 15 L plastic tubs through which
19 moist air was continuously circulated to maintain an aerobic atmosphere (Bending and
20 Turner 1999).

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22 Analysis of soil C and N pools

23 After 28, 56 and 112 days, pots were destructively harvested and the soil mineral-N
24 pools determined as described in Bending et al., (1998). Soil mineral-N was extracted

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1 in 0.5 M K₂SO₄, and NH₄⁺-N and NO₃⁻-N quantified using an EnviroFlow 5012 flow
2 injection system (Tecator AB, Sweden).

3 After 112 days, light fraction organic matter (LFOM) was extracted from 30 g
4 fw soil (equivalent to 26 g dw soil) using a 1.75 g cm⁻³ solution of NaI, and was washed
5 in 0.1 M CaCl₂ and distilled H₂O (Strickland and Sollins, 1987). After drying in an
6 80°C oven, sub-samples of the plant materials and the LFOM were weighed before
7 being milled to a fine powder (<500 µm). Approximately 5 mg samples were analysed
8 for total C and N content at the Scottish Crops Research Institute (SCRI), Dundee,
9 UK using a Roboprep automatic C/N analyser (Europa Scientific, Crewe, UK).

10 The ¹⁵N atom % content of the plant materials, 0.5 M K₂SO₄ extracts and
11 LFOM were determined at SCRI-Dundee, using a Micromass 622 mass spectrometer
12 (VG Isogas, Northwich, Cheshire, UK). The N in each pool that was derived from the
13 plant inputs, and the % crop residue-N recovered in each pool, were calculated
14 according to Ehaliotis et al. (1998).

15

16 Statistical analysis

17 The data was not normally distributed, and was subject to log transformation prior to
18 statistical analysis. The statistical significance of differences in the effect of crop
19 residue and soil type on net N mineralization, the amount of LFOM C and N between
20 treatments and the proportion of crop residue N incorporated into LFOM were
21 determined by Analysis of Variance. All statistical analysis was conducted using
22 GenStat (7th edition, VSN International Ltd.) software.

23

24 Results

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1 Composition of crop residue materials

2 There was variation between the crop residue types with respect to most quality
3 attributes (Table 1). Ryegrass had a low C/N (15), and was rich in cellulose. Brussels
4 sprout shoot had a high C/N (28) and a large soluble carbohydrate content. Sugarbeet
5 had an intermediate C/N (20) and comparable cellulose and soluble carbohydrate
6 contents to Brussels sprout and ryegrass respectively. All three crop residues types
7 had over 9 % ¹⁵N atom content.

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9 Mineralisation of N

10 Most net mineralisation of N from ryegrass and sugarbeet occurred within the first 28
11 d following incorporation (Fig 1 a, b). For Brussels sprout there was little net
12 mineralisation of N within the first 28 d, with most net mineralisation occurring
13 between 56 and 112 d in FC soil, but between 28 and 56 d in FM soil. Nitrogen
14 mineralisation was significantly affected (P<0.001) by the type of crop residue
15 incorporated and soil type, and there were significant interactions between all of the
16 variables, including crop residue type and soil, and soil and harvest time (P<0.001).

17
18 Light fraction organic matter

19 Light Fraction Organic Matter N and C in unamended FM soil were 14.1 and 5.8
20 times higher respectively than in FC soil, with LFOM C/N ratios of 38.9 and 14.6 in
21 FC and FM soils respectively (Table 2). Light Fraction Organic Matter C and N
22 content, and C/N were significantly (P<0.01) affected by both crop residue and soil.
23 In the case of N and C/N there were significant interactions between crop residue and
24 soil.

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1 In FC soil, incorporation of crop residues significantly ($P<0.05$) increased net
 2 amounts of LFOM N , with increases in the ryegrass treatment twice that in the
 3 Brussels sprout and sugarbeet treatments. Incorporation of ryegrass, but not the other
 4 materials, increased LFOM C content. Differences in the net enrichment of LFOM C
 5 and N resulted in a significant decrease in the LFOM C/N of all treatments. In the FM
 6 soil, crop residue incorporation had no effect on net amounts of LFOM C or N , or
 7 C/N .

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8 Results of ^{15}N analysis showed that both the amount and the % crop residue N
 9 incorporated into LFOM were significantly affected ($P<0.001$) by crop residue and
 10 soil type (Table 3). A significantly larger ($P<0.001$) proportion of crop residue- N was
 11 incorporated into the LFOM N pool in FM soil relative to FC soil, with amounts of
 12 crop residue derived N in the LFOM pool ranging between 25.9 to 35.3 % and 38.9
 13 and 68.5 % in FC and FM soils respectively. In FM soil, significantly more ($P<0.05$)
 14 ryegrass N was incorporated into LFOM relative to N from sugarbeet and Brussels
 15 sprout, but this represented a lower proportion of the crop residue- N added. In FC
 16 soil, the amount of ^{15}N derived from crop residues (Table 3) very closely matched the
 17 increase in total LFOM N (Table 2) resulting from crop residue incorporation,
 18 accounting for between 92.4 and 96.6 % of the increase in total LFOM N .

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19 Analysis of ^{15}N enrichment allowed us to determine how much of the LFOM
 20 N present in the soil prior to crop residue incorporation remained as LFOM after 112
 21 days. Crop residue type had no significant effect on the % of this original LFOM N
 22 which remained at the end of the incubation period. However, significantly less of the
 23 original LFOM N remained in FM relative to FC soil ($P<0.05$). The greatest loss of
 24 original LFOM N was seen in the sugarbeet treatment, in which only 65.8 % of the
 25 original LFOM N remained in the FM soil.

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1
2 Incorporation of crop-residue N into soluble and heavy fraction organic matter
3 pools

4 N in the soluble pool represents both mineral N and dissolved organic N. Both
5 crop residue type and soil affected the proportion of crop residue N contained in the
6 soluble pool after 112 days (Table 4). Significantly (P<0.001) less crop residue N
7 from ryegrass was incorporated into the soluble N pool than was the case for
8 sugarbeet and Brussels sprout leaves. Significantly less crop residue N became
9 incorporated into soluble N in FM relative to FC soil (P<0.001).

10 Since N losses via denitrification are known to be minimal following
11 incorporation of green manures in the soil type and experimental conditions used
12 (Rahn et al., 2003), incorporation of N into heavy fraction organic matter (HFOM)
13 was determined by calculating the N remaining once amounts incorporated into the
14 LFOM and the soluble N pools had been summed. It was found that significantly
15 (P<0.001) more crop residue N was incorporated into HFOM in FC relative to FM
16 soil. Crop residue type also affected amounts of N incorporated into HFOM. Amounts
17 of sugarbeet and Brussels sprout leaf N incorporated into HFOM were similar, at 15.6
18 and 17.9 % and 1.5 and 2.5 % in FC and FM soil respectively. However, much larger
19 amounts of N from ryegrass became incorporated into HFOM, representing 30.8 and
20 38.1 % of the crop residue N incorporated in the FM and FC soils respectively.

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22 **Discussion**

23 Light Fraction Organic Matter represents partially degraded, unprotected plant
24 materials and microbial tissues and products (Golchin et al., 1994; Marriott and

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1 Wander, 2006), and quantification of the amount of crop residue C and N which was
2 incorporated into HFOM provides information on the extent to which the added
3 materials had been mineralised or converted to other more recalcitrant SOM pools.

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4 Furthermore, changes in the amounts of C and N in pre-existing LFOM provides
5 information on the susceptibility of LFOM to microbial activity resulting from
6 decomposition of the plant materials, and hence its lability.

7 The FM and FC soils showed qualitative and quantitative differences in
8 organic matter, reflecting contrasting mechanisms of incorporation of organic material
9 into soil. In the case of FC soil, fresh litter is introduced by tillage, while in FM soil,
10 litter is incorporated into soil through mixing of partially degraded litter. Our data
11 shows that the characteristics of plant material incorporated into soil, together with
12 differences in soil organic matter and microbiology resulting from differences in
13 management, determined the amount of crop residue C and N remaining as LFOM
14 following the end of net mineralisation of N from the crop residues. Although greater
15 amounts of crop residue N became incorporated into LFOM in the FM soil, there were
16 net increases to LFOM N only in the low SOM FC soil. In the case of sugarbeet and
17 Brussels sprout there was only net enrichment in LFOM N in FC soil, while for
18 ryegrass, both C and N were increased. Furthermore, both crop residue type and soil
19 influenced amounts of N entering the HFOM pool, which is has greater physical
20 protection than LFOM, and is considered more stable (Six et al., 200)

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21 Differences between crop residues in the relative amount of C and N
22 immobilised into the LFOM and HFOM pools may relate to differences in the
23 biochemical quality of the materials incorporated. Bending et al. (1998) showed that
24 cellulose content was a good predictor for net increases in LFOM C and N content
25 following decomposition of a range of crop residue materials in soil. In the current

1 study, the cellulose content of ryegrass was over a third higher than sugarbeet and
2 Brussels sprout, and ryegrass was associated with greater immobilization of both crop
3 residue C and N into LFOM, and N into HFOM, than the other materials.

4 The amount of LFOM in the unamended FC soil was extremely low and had a
5 high C/N relative to FM soil. Isotopic analysis showed that the net increase in LFOM
6 N in FC soil following crop residue decomposition matched the amount of crop
7 residue N incorporated into LFOM. However, in the FM soil, more N derived from
8 the crop residues was incorporated into LFOM relative to FC soil, but there was no
9 net increase in N content, suggesting that N present in pre-existing LFOM was
10 replaced by N from the crop residues. This indicates that turnover of LFOM N in FC
11 soil was not affected by the increased microbial activity resulting from decomposition
12 of the added crop residues, but that the reverse was true in FM soil. This suggests that
13 LFOM N in FM soil, but not FC soil, had labile components in which turnover of N
14 was 'primed' during decomposition of the added plant materials. The high C/N of
15 LFOM in FM soil could have reduced N availability and limited the possibility of
16 priming effects.

17 Light Fraction Organic Matter represents a heterogenous pool and the flotation
18 method used to extract LFOM ~~extracts materials with a range of origins and~~
19 recalcitrance, including residual plant debris, living and senescent microbial and
20 faunal tissues, and charcoal (Marriott and Wander, 2006). Clearly the relative
21 proportion of reactive and non-reactive components varied in the LFOM of FC and
22 FM soils. This conclusion is also supported by the LFOM C/N, which was higher in
23 FC (38.9) relative to FM soil (14.6). Differences in the characteristics of LFOM in the
24 soils will reflect contrasting plant inputs from which LFOM is derived, and the

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1 impacts of cultivation and management techniques on turnover of LFOM in the
2 cropped soil.

3 Priming effects, in which incorporation of a substrate to soil changes the
4 mineralisation rate of native SOM, have been the subject of much debate in the
5 literature (Kuzyakov et al., 2000), and can reflect ‘real’ priming in which actual
6 mineralisation rates are altered positively or negatively, or ‘apparent’ priming in
7 which exchange of labelled mineral-N with unlabelled soil pools causes the apparent
8 priming. In this case apparent priming would involve exchange of labelled mineral-N
9 derived from the crop residues with unlabelled N in the LFOM, as the result of
10 microbial growth and turnover. It is not clear whether the priming effect observed in
11 the current experiment reflects real priming or apparent priming caused by pool
12 substitution. Whichever the mechanism, the presence of a priming effect in the FM
13 but not FC soil indicates differences in the biological activity of LFOM in each soil.

14 Differences in the fate of crop residue N and C in the two soils could be due to a
15 variety of factors. For example, SOM has strong effects on soil structure and
16 aggregate stability, which may directly influence soil texture and porosity with
17 implications for the survival and longevity of bacterial and fungal biomass, including
18 its protection against predators (Six et al., 2006). Furthermore SOM content may
19 directly affect microbial community structure (Bending et al., 2002), including fungal
20 to bacterial ratio, which increases with SOM content (Frey et al., 1999). Differences in
21 the structure of microbial communities degrading incorporated materials could affect
22 the characteristics of the microbial metabolites and tissues produced following
23 growth, and therefore the nature of organic materials stabilised as SOM. For example,
24 fungal tissues are generally considered to produce chitin rich biomass with higher C/N

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1 than bacteria, which could result in increased recalcitrance and slower breakdown
2 than bacterial biomass (Guggenberger et al., 1999; Six et al., 2006).

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3 We conclude that the characteristics of plant material incorporated into soil, together
4 with differences in soil organic matter and microbiology resulting from differences in
5 management, can have a major influence on the amount of crop residue C and N
6 incorporated into LFOM and HFOM.

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Table 1 Characteristics of crop residue materials and amounts of C and N added to soil

Crop residue	C/N	%_N	% Cellulose	% Lignin	% Soluble carbohydrate	At % ¹⁵ N abundance	Amount of C applied to soil (mg g ⁻¹ dw soil)	Amount of N applied to soil (mg g ⁻¹ dw soil)
Ryegrass	15	3	22	3	17	9.8	5.2	0.34
Sugarbeet	20	2.3	12	3	19	9.1	3.2	0.16
Brussels sprout	28	1.7	14	4	26	9.4	4.6	0.16

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Table 2 Light fraction organic matter C and N after 112 days

FC, soil from the tilled centre of the field; FM, soil from the untilled field margin

Figures in brackets represent log transformed data to which Least Significant Difference (LSD) for soil and crop residue type relates

Treatment	Total-N ($\mu\text{g g}^{-1}$ dw soil)		Total-C ($\mu\text{g g}^{-1}$ dw soil)		C/N	
	FC	FM	FC	FM	FC	FM
Sugarbeet	94.4 (4.53)	439.0 (6.07)	1535.0 (7.32)	5973.2 (8.68)	16.3 (2.79)	13.6 (2.61)
Brussels sprout	80.1 (4.37)	449.6 (6.08)	1366.6 (7.20)	6102.9 (8.69)	17.0 (2.83)	13.6 (2.61)
Ryegrass	128.1 (4.84)	578.2 (6.34)	2998.9 (7.99)	8904.6 (9.08)	23.4 (3.15)	15.6 (2.74)
Unamended	34.0 (3.42)	482.0 (6.12)	1214.3 (7.05)	7069.0 (8.81)	38.9 (3.63)	14.6 (2.69)
LSD (P<0.05)	0.37		0.34		0.14	

Significance of main treatment effects

Main effects and interaction	Significance		
	C	N	C/N
Crop residue	***	***	***
Soil	***	***	***
Crop residue x soil	NS	***	***

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Table 3 Fate of crop residue-N in the Light Fraction Organic Matter (LFOM) pools

FC, soil from the tilled centre of the field; FM, soil from the untilled field margin

Figures in brackets represent log transformed data to which Least Significant Difference (LSD) for soil and crop residue type relates

Treatment	N from <u>crop</u> residue ($\mu\text{g g}^{-1}$ dw soil)		% <u>crop</u> residue-N in LFOM		% original LFOM N remaining	
	FC	FM	FC	FM	FC	FM
Sugarbeet	62.5 (4.10)	121.7 (4.77)	35.3 (3.53)	68.5 (4.20)	93.7 (4.53)	65.8 (4.18)
Brussels sprout	48.7 (3.86)	92.6 (4.52)	28.2 (3.32)	53.5 (3.97)	92.4 (4.50)	74.1 (4.27)
Ryegrass	101.8 (4.61)	153.4 (5.03)	25.9 (3.24)	38.9 (3.66)	77.3 (4.30)	88.1 (4.44)
LSD (P<0.05)	0.31		0.31		0.35	

Significance of main treatment effects

Main effects and interaction	Significance		
	N from <u>crop</u> residue	% <u>crop</u> residue-N in LFOM	% original LFOM N remaining
Crop residue	***	***	NS
Soil	***	***	*
Interaction	NS	NS	NS

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Table 4 Incorporation of of crop residue-N into the soluble and Heavy Fraction Organic Matter (HFOM) pools

FC, soil from the tilled centre of the field; FM, soil from the untilled field margin

Figures in brackets represent log transformed data to which Least Significant Difference (LSD) for soil and crop residue type relates

Treatment	% crop residue in soluble pool		% crop residue N in HFOM	
	FC	FM	FC	FM
Sugarbeet	49.1 (3.89)	46.5 (3.83)	15.6 (2.09)	1.5 (1.26)
Brussels sprout	53.9 (3.98)	47.3 (3.85)	17.9 (2.81)	2.5 (1.41)
Ryegrass	35.9 (3.58)	30.2 (3.41)	38.1 (3.63)	30.8 (3.41)
LSD (P<0.05)	0.10		0.31	
<u>Significance of main treatment effects</u>				
<u>Main effects and interaction</u>				
		% crop residue in soluble pool		% crop residue N in HFOM
Crop residue		***		***
Soil		***		***
Interaction		NS		NS

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Figure legends

Fig 1 Mineralisation of N following incorporation of crop residues

(●, Sugarbeet; ▼, Brussels sprout; ■, Ryegrass; ◆, unamended)

Bars represent +/- standard error of the mean

a) Mineral-N pool in FC soil

b) Mineral-N pool in FM soil