Predictors of gross N mineralization and immobilization during decomposition of stabilized organic matter in agricultural soil

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Summary

Long-term additions of different types of organic amendments affect the amount of soil organic matter. Less is known about how this in turn affects carbon (C) and nitrogen (N) mineralization from the pool of stabilized soil organic matter, or the extent to which gross N immobilization influences the net amount of N mineralized. Soils, differing in the quantity and quality of organic matter inputs they had received since 1956, were sampled approximately 6 or 18 months after the most recent applications of organic amendments. Two laboratory experiments were carried out to: (i) evaluate if, and how, the organic amendments had affected C mineralization, gross and net N mineralization; (ii) examine the relation between gross N immobilized and free-light fraction of organic matter; and (iii) assess predictors for gross N mineralization and immobilization rates in soils. The amount of soil organic C and N were major determinants of C and gross N mineralization, but not of net N mineralization. Carbon mineralization was related to gross N mineralization, but the ratio between the two was not constant. Gross N immobilization was related to the amount of free-light fraction material in the soil with 90% variation explained. For most common organic amendments applied in autumn, our results support the use of total soil organic N and C mineralization as predictors of gross N mineralization from stabilized soil organic matter. In addition, we propose that the amount of free-light fraction material present in the soil in spring is adequate as a predictor of the immobilization potential of the soil, without a need to consider the C-to-N ratio of this material.

Introduction

Over an entire year or growing season, virtually all agricultural soils show positive net nitrogen (N) mineralization (Jarvis *et al.*, 1996). This net N mineralization may, however, be accompanied by substantial N immobilization in grassland (Ledgard *et al.*, 1998), as well as forest (Davidson *et al.*, 1992) and arable soils (Recous *et al.*, 1999). Predictions of net N mineralization may therefore need to take account of gross N mineralization and immobilization. Failure to do so is probably an important contributing factor as to why the search for a rapid method to predict the N mineralization capacity of soils has been so elusive (Wang *et al.*, 2001; Curtin & McCallum, 2004).

Many attempts have been made to find fractions of soil N through either physical or chemical fractionation that relate to N mineralization (e.g. Wang *et al.*, 2001; Accoe *et al.*, 2004;

Mishra *et al.*, 2005). But, it appears that bulk soil N may in many cases be an adequate predictor of gross, although often not of net, N mineralization. Booth *et al.* (2005) compiled results from more than 100 studies on agricultural, grassland and woodland ecosystems and found that 42% of the variation in gross N mineralization could be explained by the amounts of soil N and soil carbon (C), which were the main determinants. Gross N mineralization has also been found to relate to C mineralization (Hart *et al.*, 1994; Bengtsson *et al.*, 2003), though the C-to-N ratio of the decomposing material may have to be taken into account in this relation, at least when comparing decomposition of diverse plant materials (Luxhøi *et al.*, 2006). Carbon mineralization and bulk soil N, rather than specific soil N fractions, may therefore in many cases be adequate predictors of gross N mineralization.

Density fractionation techniques have been used to separate the heterogeneous pool of soil organic matter classes differing in age, quality or origin (e.g. Golchin *et al.*, 1994; Gregorich *et al.*, 2006). Free-light fraction organic matter and occluded light fractions (Golchin *et al.*, 1994) are intermediates between

undecomposed plant residues and stabilized soil organic matter (Gregorich *et al.*, 2006). Their characteristics are closer to that of undecomposed crop residues and organic amendments (Baisden *et al.*, 2002) and therefore more influenced by the characteristics of such materials than stabilized organic matter recovered in the mineral-associated heavy-fraction. The free-light fraction usually has a wide C-to-N ratio (Gregorich *et al.*, 2006) and its decomposition makes it therefore a likely candidate to induce N immobilization. Free-light fraction organic matter has, however, variable chemical and biological properties, depending amongst other things, on its source and degree of decomposition (Golchin *et al.*, 1994), but also on the method of isolation, with especially density and size fractionation resulting in material with different properties (Gregorich *et al.*, 2006). Such differences may explain why free-light fraction organic matter sometimes is (e.g. Whalen *et al.*, 2000) and sometimes is not (e.g. Cookson *et al.*, 2005) found to significantly contribute to N immobilization.

Using soils from the Ultuna Long-Term Soil Organic Matter Experiment (Persson & Kirchmann, 1994), the present work builds on the following hypotheses.

1 Bulk soil organic N and C mineralization are major determinants of gross N mineralization and they can therefore be used as predictors of gross N mineralization.

2 The free-light fraction organic matter causes substantial gross N immobilization and thus affects the relation between total soil organic N and net N mineralization.

3 The amount and quality of free-light fraction soil organic matter can be used as a predictor of gross N immobilization rates.

To test these hypotheses we evaluated if, and how, the quantity and quality of organic matter inputs affects C mineralization, gross and net N mineralization in some treatments of the Ultuna Long-Term Soil Organic Matter Experiment, and we examined the relation between gross N immobilization and free-light fraction organic matter.

Materials and methods

Soils

Soil samples were taken from the Ultuna Long-Term Soil Organic Matter Experiment (Uppsala, Sweden; 60°N, 17°E) (Persson & Kirchmann, 1994). The experiment was started in 1956 on a post-glacial clay loam classified as a Typic Eutochrept (Soil Survey Staff, 1987) or a Eutric Cambisol (FAO, 1988). The mineral fractions consist of 36.5% clay, 41% silt and 22.5% sand and soil pH is between 6.2 and 6.8. In this experiment, soils (2 × 2 m plots) have been treated with different N fertilizers or organic amendments. On all soils, except the fallow, arable crops (mainly cereals) have been grown. Crops are sown in spring and all above-ground material is removed at harvest in autumn. All treatments are replicated in four blocks in a semi-randomized design. Seven treatments were selected: (i) bare fallow; (ii) cropped without N-fertilizer (unfertilized); (iii) $Ca(NO_3)_2$ (N-fertilized); (iv) Hay; (v) straw + $Ca(NO_3)_2$ (Straw + N); (vi) farmyard manure (FYM); and (vii) sawdust + $Ca(NO_3)_2$ (SD + N). Treatments (i) – (iv) were considered to approximate a series of increasing levels of C input of similar quality, whereas series (iv) – (vii) have similar levels of C input, but of different quality. In the selected N-fertilized treatments, $Ca(NO_3)_2$ has been applied every year at the time of sowing at a rate of 80 kg N ha^{-1} $year^{-1}$, and the organic amendments every other year in the autumn at a rate of 8 Mg ha^{-1} ash-free organic matter. Soil data are given in Table 1. Eight soil sub-samples to a depth of 0–7 cm were taken from each plot in May 2002 and 2003, sieved through a 4-mm aperture sieve, bulked together to give one representative sample for each replicate block, thoroughly mixed and stored at −18°C until used.

Experimental design

The study consisted of two experiments and a schematic representation of the experimental design is given in Figure 1. In the first experiment, the effects of long-term organic matter inputs on C mineralization, gross and net N mineralization were studied in soils sampled 18 months after the most recent application of organic amendments (May 2003). A preliminary experiment showed that the free-light fraction had the highest C-to-N ratio among all density fractions (free-light fraction, occluded light fractions and heavy fraction; data not shown). The aim of the second experiment was therefore to study the role of free-light fraction material in gross N immobilization. In order to obtain a wider range of free-light fraction contents in the latter experiment, soils were sampled both 6 and 18 months (in May 2002 and 2003, respectively) after the most recent addition of amendments.

Table 1 Soil characteristics. Mean values of four replicates. Means suffixed by a different letter are significantly different at $P < 0.05$ (Duncan's multiple range test)

Soil[a]	Soil C /%	Soil N /%	C-to-N ratio	Organic amendment N[b] /%	pH H_2O
Fallow	1.1[A]	0.09[A]	12[A]	–	6.2[A]
Unfertilized	1.3[B]	0.11[B]	12[A]	–	6.5[B]
N-fertilized	1.5[C]	0.14[C]	11[B]	–	6.8[C]
Hay	1.9[D]	0.18[D]	11[B]	1.78[A]	6.3[AB]
Straw + N	2.1[E]	0.19[E]	11[BC]	0.39[B]	6.7[C]
FYM	2.3[F]	0.22[F]	10[C]	2.48[C]	6.6[BC]
SD + N	2.4[F]	0.18[D]	13[D]	0.02[D]	6.7[C]

[a]Soil chemical data are for spring 2003. Initial in 1956: soil C 1.5%, pH 6.5.
[b]Mean values for organic amendments applied in autumn 1997 and 2001. Single determination of total N contents of organic amendments; all amendments had a total C content of *c.* 45%.
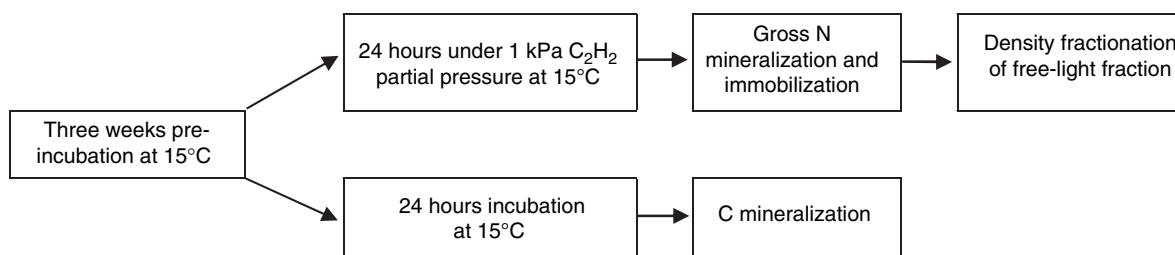
Experiment1: Effects of long-term organic matter inputs on C mineralization,
gross and net N mineralization

Soils taken in May 2003 (18 months after most recent application of organic amendments)
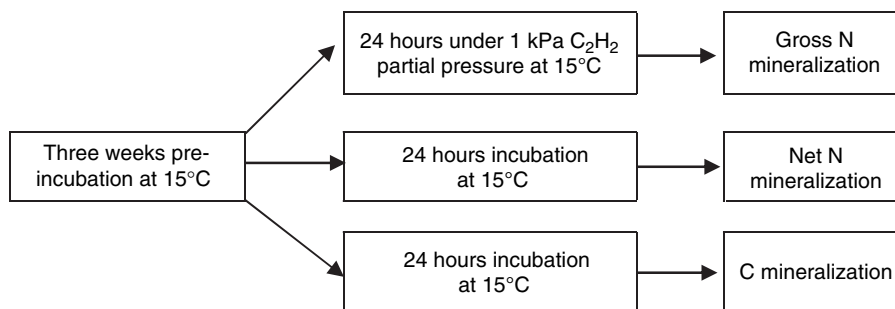
Experiment 2: Role of the free-light fraction in gross N immobilization

Soils taken in May 2002 (six months after most recent application of organic amendments)

Soils taken in May 2003 (18 months after most recent application of organic amendments)
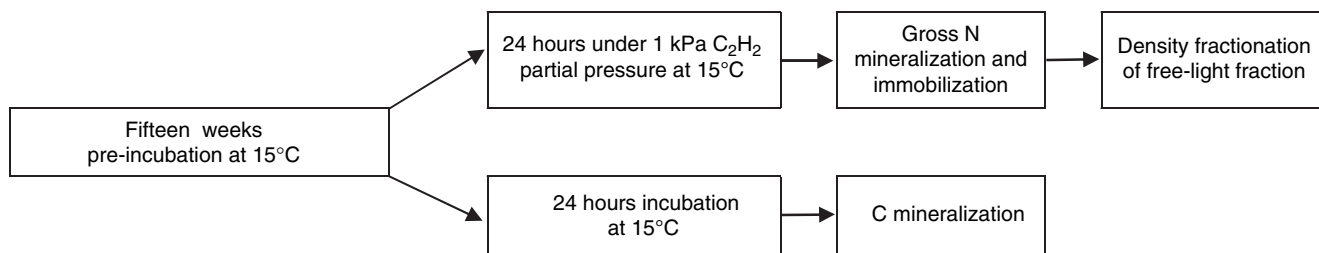

Figure 1 Schematic representation of the experimental design.

Experiment 1: effects of long-term organic matter inputs on C mineralization, and gross and net N mineralization. After 15 months storage at −18°C, soils taken in May 2003 from all selected seven treatments were thawed and wetted to 45% of their WHC and pre-incubated at 15°C for 3 weeks. A lengthy 3-week pre-incubation period was chosen to minimize the risk of measuring microbial cycling and recycling of small pools of highly labile, N-rich compounds, rather than of soil organic matter processes (Fierer *et al.*, 2001). The soils were divided into three sets (Figure 1). To estimate gross N mineralization rates one set of soil samples (equivalent to 100 g dw soil) was weighed into 0.3-litre Schott flasks. The other two sets (equivalent to 100 g dw soil) were weighed into 0.5-litre glass honey jars for determination of net N and C mineralization, respectively.

The set of samples in the 0.3-litre Schott flasks were sealed with lids modified with a gas septum. Acetylene (C_2H_2) generated from calcium carbide was added with a syringe to a partial pressure of 1 kPa C_2H_2 and samples were incubated at 15°C for 24 hours to allow time for the added $^{15}NH_4^+$-N to equilibrate, thus avoiding biases in the estimation of gross N mineralization rates (Herrmann *et al.*, 2007). This pre-treatment was required as these soils have been shown to have rapid nitrification of added $^{15}NH_4^+$-N (Herrmann *et al.*, 2004). The flasks were then opened and amended with 3 ml per 100 g dw soil of a solution containing ^{15}N-labelled $(NH_4)_2SO_4$ (10 atom% ^{15}N) giving 5 μg N g^{-1} dry soil, and sealed without further addition of C_2H_2. The remaining two sets in the 0.5-litre glass honey jars were amended with 3 ml deionized water per 100 g dw soil for determination of net N and C mineralization, respectively, and

incubated at 15°C for 24 hours without C_2H_2. A previous study (Herrmann *et al.*, 2007) showed that there are no differences in C mineralization, gross N mineralization and immobilization rates between soils pre-treated with or without C_2H_2. All solutions were added drop-wise onto the soil surface, bringing the soil moisture content up to 50% WHC, thoroughly mixed and incubated at 15°C. The amounts of mineral N (25 g dw soil) were determined after 24 and 72 hours at 15°C for estimation of gross N mineralization. To determine net N mineralization, the amounts of N mineralized were determined after 24 hours and after a subsequent 8-day incubation period at 15°C. The amounts of C mineralized were determined over an incubation period of 24–72 hours at 15°C.

Experiment 2: role of the free-light fraction in gross N immobilization. In this experiment soil samples were used from only the Hay, Straw + N, FYM and SD + N treatments. After storage at −18°C for 30 months, soils sampled in May 2002 (6 months after the most recent application of organic amendments) were adjusted to 45% WHC and pre-incubated at 15°C for 3 weeks. The soils sampled in May 2003 (18 months after the most recent application of organic amendments) were stored at −18°C for 15 months and then incubated for 15 weeks at 45% WHC to further reduce the amounts of labile, free-light fraction organic matter.

After pre-incubation, two sets of soil samples (equivalent to 75 g dw) were weighed into 0.3-litre Schott flasks (Figure 1). One set of soil samples was used to estimate gross N mineralization and immobilization and samples were incubated under a partial pressure of 1 kPa C_2H_2 at 15°C for 24 hours and then amended with ^{15}N-labelled $(NH_4)_2SO_4$ as described above. The amounts of N mineralized and residual ^{15}N of 25 g dw soil were determined after incubation for 24 and 72 hours at 15°C. The remaining 25 g dw of soil was used to determine the amount of free-light fraction organic matter at the end of the 72-hours incubation period. The other set of soil samples was incubated without C_2H_2 at 15°C for 24 hours, amended with deionized water and amounts of C mineralized were determined over an incubation period of 24 to 72 hours at 15°C. The C-to-N ratio of the material undergoing decomposition was estimated using Equation (1) as described below.

Analytical methods

Evolved CO_2 was trapped in a NaOH solution and determined by titration with 0.1–0.5 M HCl after addition of excess $BaCl_2$, with airtight honey jars without soil as a control (Zibilske, 1994).

Mineral N in soil samples was extracted by shaking with 2 M KCl (soil : extractant mass-to-volume ratio of 1:4) for 2 hours followed by centrifugation (5 minutes at 2000 g) and filtration using Munktell M00 filter paper. All extracts were stored at −18°C prior to colorimetric analysis for NH_4^+-N and NO_3^--N on a TRAACS 800 auto-analyser (Bran and Luebbe, Norderstedt,

Germany). The atom% [15]N of mineral N in KCl soil extracts was determined using a modified diffusion technique (Herrmann *et al.*, 2007) and the atom% [15]N of dried paper discs was determined as described below.

To determine recovery of residual atom% [15]N in soils after extraction, KCl extracted soil samples (see above) were once more extracted with 100 ml 2 M KCl for 2 hours followed by centrifugation (5 minutes at 2000 g) and filtration with Munktell M00 filter paper to remove remaining mineral N in soil solution. The supernatant was discarded, and samples were then extracted twice with 100-ml deionized water for 30 minutes followed by centrifugation (5 minutes at 2000 g) and filtration. The adequacy of this procedure to remove all extractable mineral N from the soil was tested in preliminary experiments (data not shown). Organic matter particles recovered on the filter paper and soil samples were pooled together, dried at 60°C (approximately 2 days), milled and the atom% [15]N determined as described below.

Free-light fraction organic matter was separated from the heterogeneous pool of soil organic matter using a density fractionation procedure (Golchin *et al.*, 1994). Air-dried soil (20 g) was placed in a 250-ml centrifuge tube and the free-light fraction was separated with 200-ml sodium polytungstate solution (density 1.6 Mg m^{-3}) (SOMETU, Berlin, Germany). The tube, with a plastic stopper, was inverted gently by hand five times to avoid disruption of aggregates and the suspension was allowed to stand for 30 minutes before centrifuging at 2000 g for 30 minutes. The supernatant with floating particles was poured into a funnel fitted with a 0.45 μm pore-size membrane filter (Schleicher & Schuell, Dassel, Germany) and filtered under vacuum. Free-light fraction organic matter was then washed with deionized water, dried at 60°C for 2 days and homogenized by manually grinding with a mortar. The atom% [15]N and total amounts of C and N were determined as described below.

The atom% [15]N of the dried paper discs obtained after micro-diffusion, atom% [15]N recovery of residual and free-light fraction as well as total amounts of C and N in the free-light fraction were determined after converting organic C and N to CO_2 and molecular N_2, respectively, using an automatic elemental analyser (EA 1110) coupled to an isotope ratio mass spectrometer (Finnigan MAT Deltaplus, Thermo Finnigan, Bremen, Germany).

Calculation of net N mineralization, gross N processes and C-to-N ratio of organic material undergoing decomposition

Net N mineralization rates were determined as the changes in 2 M KCl-extractable mineral N (NH_4^+-N and NO_3^--N) over an 8-day incubation period.

Gross N mineralization and immobilization rates were estimated over the 24 to 72 hour periods after ^{15}N addition. Gross N mineralization rates were estimated by the analytical equations proposed by Kirkham & Bartholomew (1954), while the

numerical FLUAZ-model (Mary *et al.*, 1998) was used to estimate gross N immobilization rates. The latter were based on residual ^{15}N remaining in soil after extraction of mineral N.

The C-to-N ratio of organic matter undergoing decomposition was calculated from Murphy *et al.* (2003):

$$C/N = \frac{C_{\min}}{(1 - C_{\text{soil}})} \times \frac{1}{m}, \qquad (1)$$

where *C/N* is the C-to-N ratio of the decomposing material, $C_{\min}$ is the amount of C mineralized (µg C g^{-1} soil day^{-1}), *m* is the gross N mineralization rate (µg N g^{-1} soil day^{-1}) and C_{soil} is a constant that combines the microbial C use efficiency and the fraction of soil organic matter stabilized and was assumed to be 0.42 (Bradbury *et al.*, 1993).

Statistical analysis

All experiments were carried out on soil samples from each of the four replicate blocks of the field experiment, and the mean values are reported for the four blocks. Characteristics of free-light fraction organic matter, C mineralization and gross N processes were normally distributed; consequently, differences between treatments were assessed using analysis of variance for a randomized block design (Statsoft, 2000). To calculate differences in gross N processes and characteristics of free-light fraction organic matter between the years 2002 and 2003, year was included as an independent factor in a repeated measures design. Multiple least squares regression analysis was used on data from all replicates to assess the relationship between characteristics of free-light fraction material as predictor variables and immobilized N or recovered added ^{15}N as dependent variables. Stability of the predictors in the regression model was verified by ensuring that forward and backward hierarchical regression, as well as single-block regression analysis, yielded similar results. Weighted 95% confidence intervals calculated within the FLUAZ-model were transformed into standard errors of the mean; for further details of the model see Mary *et al.* (1998). Unless indicated otherwise, statistically significant refers to $P < 0.05$.

Results

Effects of long-term organic matter inputs on C mineralization, and gross and net N mineralization

In the first experiment, C and gross N mineralization rates were linearly related to total soil organic C ($r = 0.91$; Figure 2a) and N ($r = 0.95$; Figure 2b), respectively, but net N mineralized was unrelated to total soil organic N (Figure 2b). Gross N mineralization was also linearly related to C mineralization ($r = 0.95$; Figure 2c), but with the SD + N treatment a clear outlier showing smaller gross N mineralization rates per amount of C mineralized. Gross N mineralization rates in the

Hay, Straw + N, FYM and SD + N treatments were also measured separately in the second experiment (also sampled in May 2003, but with 15 instead of three pre-incubation weeks), and except for the SD + N treatment these rates were similar on both occasions (cf. Figure 2b, Table 2).

A comparison of gross N mineralization rates in soils sampled 6 months (in May 2002) and 18 months (May 2003) after the most recent addition of organic amendments showed that gross N mineralization rates had decreased by between 32 and 55% in the Hay, Straw + N and SD + N treatments, but was little changed in the FYM treatment (Table 2). Using Equation (1), the C-to-N ratio of the material undergoing decomposition was estimated to be between eight and 20 among all treatments and years, with generally no significant differences between treatments, except for the SD + N treatment in 2002, which had a statistically significant larger ratio (Table 2).

Role of the free-light fraction in gross N immobilization

In soils sampled in May 2002, on average 8% of added ^{15}N-labelled NH_4^+-N was recovered in the free-light fraction in the SD + N treatment 72 hours after addition, whereas in the other treatments only 1–2% of added N was recovered in that fraction (Table 3). In the FYM and SD + N treatments, the amount of added N recovered in the free-light fraction in the soils sampled in May 2003 was statistically significantly less than that recovered in that fraction in the soils sampled in 2002, but in the Hay and Straw + N treatments there were no differences between years (Table 3). Recovery of added N was 1.30 ± 0.06 (SE) ng N mg^{-1} free-light fraction across all treatments and years, except for the FYM and SD + N treatments in year 2002, which showed a recovery of 2.4 ± 0.2 (SE) ng N mg^{-1} free-light fraction (Table 3).

Across all treatments and both years recovery of added N in the free-light fraction could be largely explained by the amount of this material in the soil (with close to 90% of the variation explained or 55% when the two SD + N treatments were excluded; Figure 3a) and the C-to-N ratio affording no additional explanation (multiple regression analysis; data not shown). The SD + N treatment, however, had a wide range of C-to-N ratios ranging from 36 to 62 between replicate blocks, compared with a narrow range and a much smaller average C-to-N ratio of 22 for all other treatments (Table 3, Figure 3b). As a result, the variation in recovery of added N in the free-light fraction of the SD + N treatment (Figure 3b) was best explained by a regression model that included both the amount and the C-to-N ratio of the free-light fraction. Both these predictors carried approximately equal weight in the model (standardized regression coefficients 0.47 ± 0.006 (SE) and 0.55 ± 0.003 (SE), respectively; $R^2 = 0.94$, $P < 0.001$), reflecting that both were correlated to each other.

Gross N immobilization rates were largest in the SD + N treatment when estimated using the numerical FLUAZ-model (Table 2). Except in the Straw + N treatment, gross N
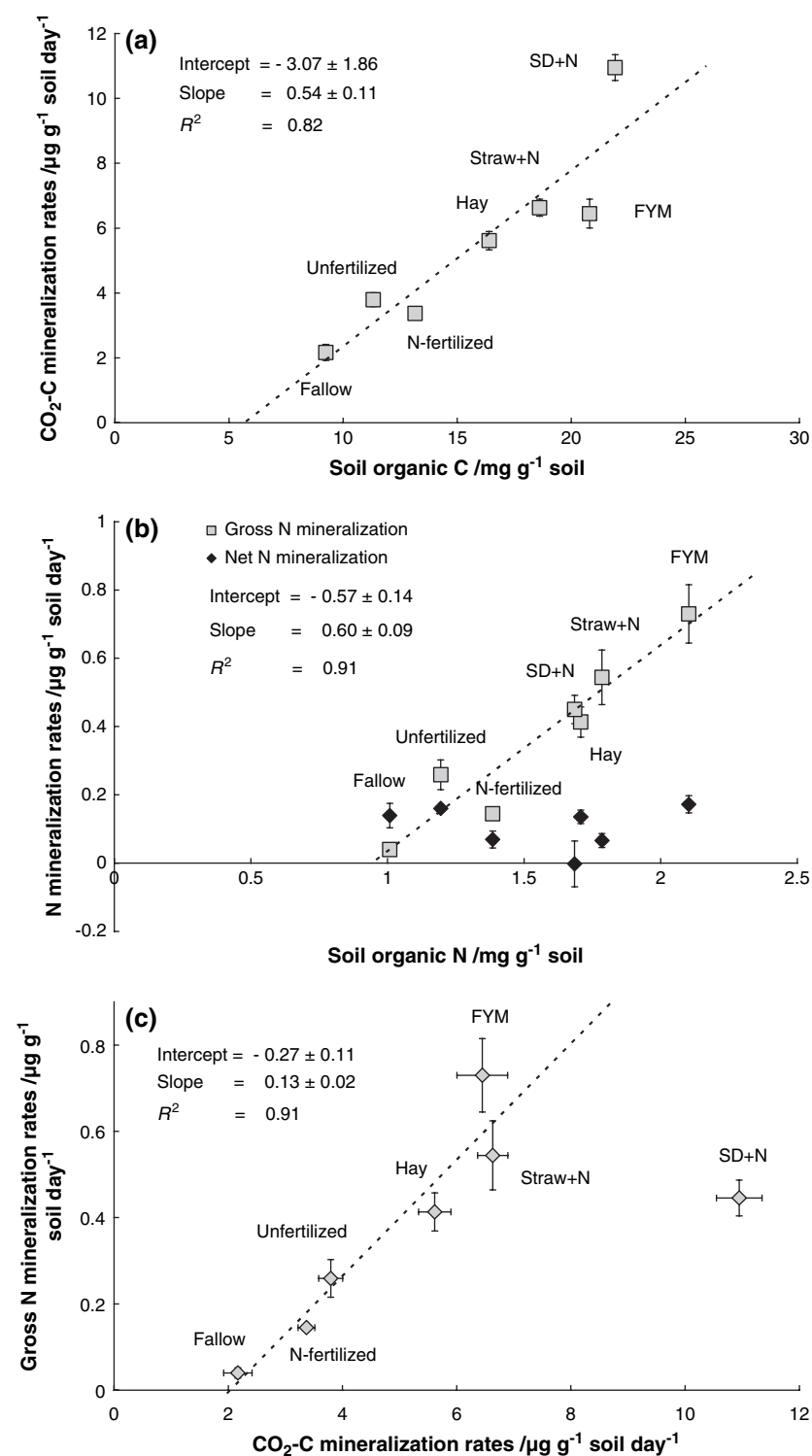
Figure 2 (a) Rates of C mineralization (μg CO_2-C g^{-1} soil day^{-1}) and (b) rates of gross and net N mineralization (μg N g^{-1} soil day^{-1}) in relation to total soil organic C and N, respectively, for soils taken in May 2003 (18 months after the most recent addition of organic amendments), and (c) gross N mineralization rates in relation to C mineralization among all soils (the SD + N treatment was treated as an outlier in the regression). Mean value and standard error of the mean of four replicates.

immobilization rates estimated by the FLUAZ-model were statistically significantly larger 6 months (May 2002) compared with 18 months (May 2003) after the most recent addition of organic amendments, indicating the importance of relatively undecomposed organic matter for immobilization (Table 2).

These immobilization rates were linearly related to both C mineralization (Figure 4a) and to the calculated C-to-N ratio of the decomposing material (Figure 4b), suggesting that both the amount and qualitative differences in the material undergoing decomposition influenced the rate of immobilization. This was

Table 2 C mineralization and gross N processes (μg N g^{-1} soil day^{-1}) as well as C-to-N ratio of organic matter undergoing decomposition for soils taken in May 2002 and 2003. Mean values and standard error of the mean are given for four replicates

	C mineralized	Gross N mineralized	Gross N immobilized[a]	Gross N immobilized[b]	Material undergoing decomposition[c]
			/μg N g^{-1} soil day^{-1}		C-to-N ratio
Soils taken in May 2002 (6 months after the most recent application of organic amendments and pre-incubated for 3 weeks at 15°C)					
Hay	4.1 ± 0.2	0.8 ± 0.2	1.00 ± 0.04	0.6 ± 0.2	9.4 ± 1.5
Straw + N	6.0 ± 0.2	0.9 ± 0.2	1.13 ± 0.11	0.5 ± 0.4	12.0 ± 1.9
FYM	5.1 ± 0.1	0.7 ± 0.1	0.89 ± 0.07	0.01 ± 0.3	13.2 ± 2.4
SD + N	11.1 ± 1.0	1.0 ± 0.1	1.84 ± 0.43	1.4 ± 0.1	20.3 ± 2.7
Soils taken in May 2003 (18 months after the most recent application of organic amendments and pre-incubated for 15 weeks at 15°C)					
Hay	2.9 ± 0.2	0.6 ± 0.1	0.61 ± 0.02	0.7 ± 0.3	9.0 ± 0.5
Straw + N	3.4 ± 0.2	0.4 ± 0.2	1.03 ± 0.18	1.7 ± 0.4	18.0 ± 4.3
FYM	3.0 ± 0.1	0.7 ± 0.1	0.58 ± 0.03	1.1 ± 0.3	7.6 ± 1.1
SD + N	5.0 ± 0.3	0.7 ± 0.1	1.42 ± 0.20	0.5 ± 0.3	14.6 ± 3.0

[a]Estimated using the numerical FLUAZ-model (Mary *et al.*, 1998).
[b]Calculated as the differences between gross and net N mineralization rates.
[c]C-to-N ratio of material undergoing decomposition was estimated using Equation (1), assuming 0.42 for a constant that combines the microbial C use efficiency and the fraction of soil organic matter stabilized (C_{soil}) (Bradbury *et al.*, 1993).

despite the fact that there were few statistically significant differences between treatments in the calculated C-to-N ratios of the decomposing material (Table 2). Some of the qualitative differences in the material undergoing decomposition that are relevant for N immobilization may relate to the amount of free-light fraction material as this is the fraction with the largest C-to-N ratio (Table 3). It was, however, only in the FYM and SD + N treatments that statistically significantly more free-light fraction material was found in 2002 than in 2003 (Table 3). Nevertheless, gross N immobilization rates, as was the case for recovery of ^{15}N (Figure 3a), were closely related to the amount of free-light fraction material in soils across all treatments and both years (Figure 4c). The amount of free-light fraction explained 90% of the variation (70% when the two SD + N treatments were excluded) in gross N immobilization rates (Figure 4c). This

relation was not further improved by including the C-to-N ratio of the free-light fraction in the regression model (data not shown). That immobilization rates were also related to the C-to-N ratio of the free-light fraction (Figure 4d) was mainly due to the larger amounts of this material in the SD + N treatment, which covaried with its C-to-N ratio.

Discussion

Validation of gross N rate estimates

We followed the procedure of Herrmann *et al.* (2007) to estimate gross N transformation rates. The procedure is based on measurement of rates over the 24–72 hour period after ^{15}N addition to avoid effects of disturbance caused by ^{15}N

Table 3 Characterization of the free-light fraction (FLF) from soils taken in May 2002 and 2003, respectively. Mean values and standard error of the mean are given for four replicates

	Amount FLF /mg g^{-1} soil	Total C FLF /mg C g^{-1} soil	Total N FLF /mg N g^{-1} soil	C as % soil C	N as % soil N	C-to-N ratio of FLF	^{15}N-labelled recovered in FLF /μg added N g^{-1} soil[a]	/ng added N mg^{-1} FLF
Soils taken in May 2002 (6 months after the most recent application of organic amendments and pre-incubated for 3 weeks at 15°C)								
Hay	4.4 ± 0.4	0.8 ± 0.1	0.031 ± 0.003	4.6 ± 0.4	1.8 ± 0.2	24.7 ± 1.2	0.06 ± 0.01	1.4 ± 0.1
Straw + N	6.3 ± 0.8	0.8 ± 0.1	0.037 ± 0.003	4.4 ± 0.7	2.1 ± 0.2	21.7 ± 1.7	0.10 ± 0.03	1.5 ± 0.2
FYM	4.9 ± 0.3	0.9 ± 0.1	0.042 ± 0.005	4.5 ± 0.3	2.0 ± 0.2	22.6 ± 1.4	0.12 ± 0.04	2.4 ± 0.3
SD + N	12.5 ± 1.9	3.0 ± 0.5	0.059 ± 0.005	13.5 ± 2.2	3.5 ± 0.3	49.7 ± 4.7	0.39 ± 0.17	2.5 ± 0.3
Soils taken in May 2003 (18 months after the most recent application of organic amendments and pre-incubated for 15 weeks at 15°C)								
Hay	4.5 ± 0.6	0.7 ± 0.1	0.033 ± 0.005	4.4 ± 0.5	1.9 ± 0.3	21.9 ± 1.1	0.05 ± 0.01	1.1 ± 0.1
Straw + N	5.6 ± 1.1	0.8 ± 0.1	0.039 ± 0.007	4.5 ± 0.7	2.2 ± 0.4	21.6 ± 0.3	0.08 ± 0.02	1.5 ± 0.1
FYM	3.1 ± 0.5	0.5 ± 0.1	0.025 ± 0.004	2.5 ± 0.3	1.2 ± 0.2	21.3 ± 1.7	0.03 ± 0.01	1.1 ± 0.1
SD + N	9.0 ± 0.8	1.6 ± 0.1	0.042 ± 0.003	7.2 ± 0.4	2.5 ± 0.2	37.7 ± 1.1	0.12 ± 0.05	1.2 ± 0.2

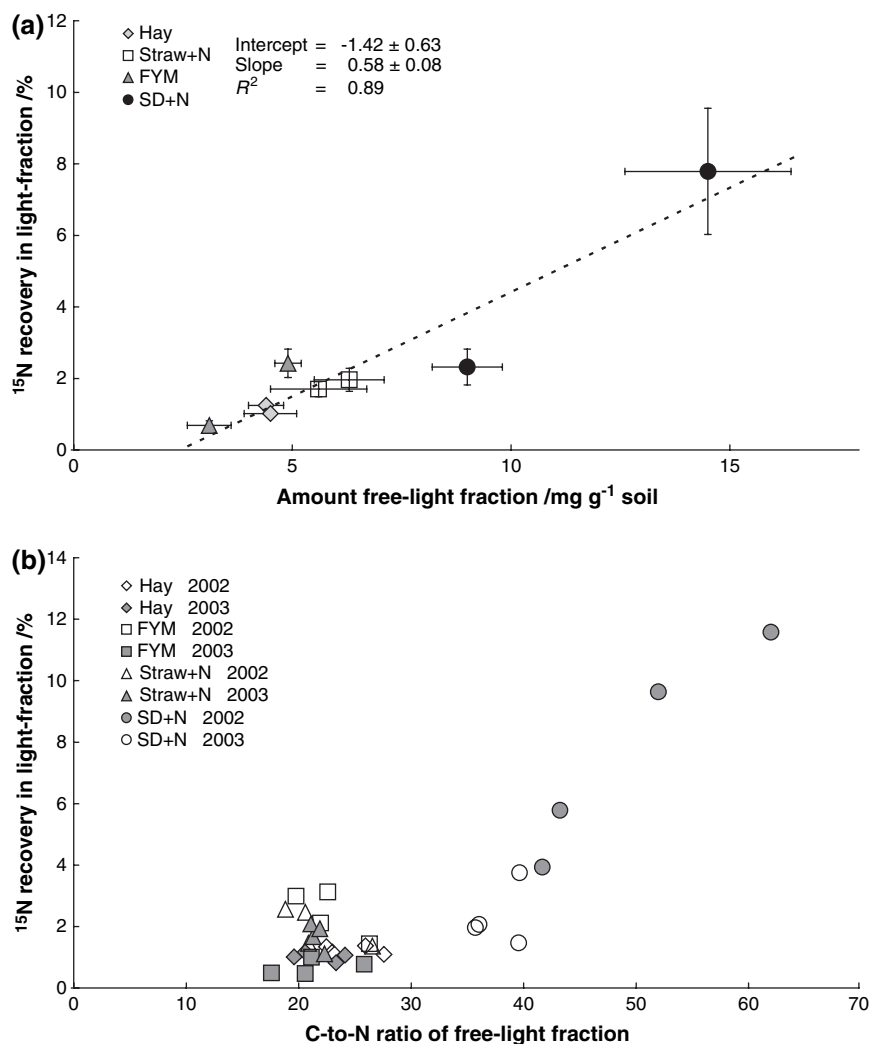[a]Soils were amended with ^{15}N-labelled $(NH_4)_2SO_4$ (10 atom% ^{15}N) giving 5 μg N g^{-1} dry soil.

Figure 3 Recovery of added $^{15}NH_4^+$-N in free-light fraction (% of N added) in relation to (a) amount of free-light fraction (mg g^{-1} soil) and (b) C-to-N ratio of free-light fraction. Mean value and standard error of the mean of four replicates are given in (a), whereas all replicates are presented in (b).

(Herrmann *et al.*, 2007), water addition (Davidson *et al.*, 1991; Murphy *et al.*, 1997) and to allow equilibration between added and native N (Cliff *et al.*, 2002). Differences in the microbial use of added and native N was minimized through the addition of relatively small amounts of NH_4^+-N and through inhibition of nitrification, which may be the process that is particularly subject to differential use of native and added N (Herrmann *et al.*, 2007). Inhibition of nitrification also ensured that sufficient N remained in the NH_4^+-N pool 72 hours after addition in these strongly nitrifying arable soils. Even though this procedure by no means guarantees correct estimation of gross N transformation rates, it does minimize some of the more common sources of bias. Recovery of added $^{15}NH_4^+$-N was less than 100% (on average 89%; $P < 0.05$) but remained constant over the 24–72 hour period that was used to estimate gross N transformation rates, so that no losses were assumed in the calculations. There were discrepancies between gross N immobilization calculated as the difference between gross and net N mineralization and N immobilization estimated on basis

of residual ^{15}N after extraction of mineral N and using the FLUAZ-model (Table 2). As the former estimates had much larger standard errors and were based on indirect estimation of rates, they were not deemed to be reliable.

Effects of quantity and quality of organic matter inputs on soil organic matter decomposition

We considered that the Fallow, Unfertilized, N-fertilized and Hay treatments represent a gradient in the quantity of C input on the assumption that the hay (consisting of *Phleum pratense*) has a comparable C quality to the crop residues (stubble and roots) in the Unfertilized and N-fertilized treatments. Among the treatments receiving organic amendments (i.e. Hay, Straw + N, FYM and SD + N) the main differences are in the quality of organic matter input as yield levels and therefore C input through crop residues are similar (Witter, 1996).

Because free-light fraction organic matter mainly consists of more recently added and less degraded organic matter than bulk
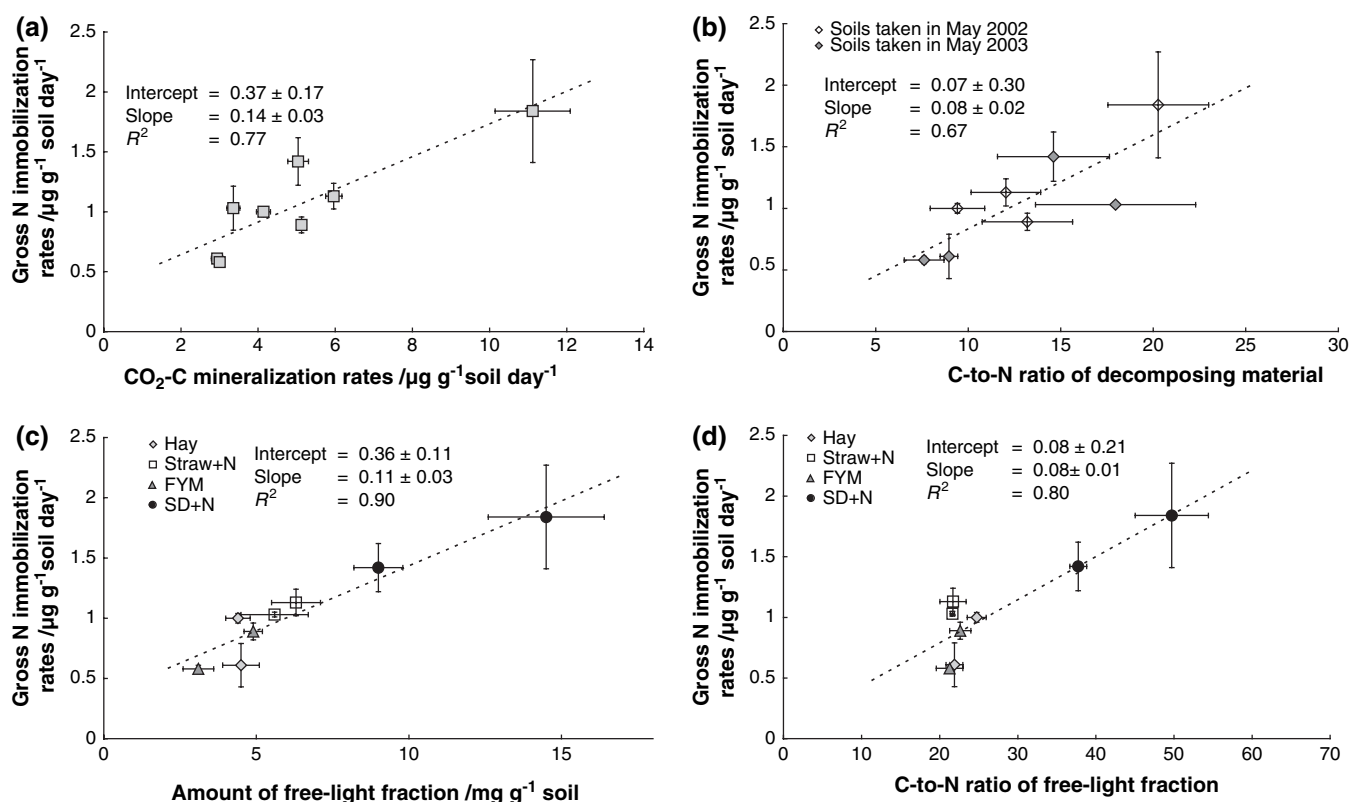
Figure 4 Gross N immobilization rates (μg N g^{-1} soil day^{-1}) estimated using the FLUAZ-model (Mary *et al.*, 1998) in relation to (a) C mineralization rates (μg CO_2-C g^{-1} soil day^{-1}), (b) C-to-N ratio of decomposing material, (c) amount of free-light fraction (mg g^{-1} soil) and (d) C-to-N ratio in free-light fraction. Mean value and standard error of the mean of four replicates.

soil organic matter, the former has been found to be the most sensitive indicator of soil organic matter changes (Gregorich *et al.*, 2006). In our study, the free-light fraction density fractions did not, however, reflect long-term changes in the amount of C in this experiment (Table 3 and Witter, 1996). It may be that differences in the amounts of free-light fraction material between the soils had become less pronounced due to decomposition of this material, as the soils had been sampled 18 months after the last addition of organic amendments. Among the treatments with different qualities of organic matter input, only the soil that had received sawdust deviated consistently by showing a greater C-to-N ratio of the organic matter in the free-light fraction, and about 50% more soil C was recovered in the free-light fraction (Table 3), reflecting the recalcitrant nature and large C-to-N ratio of the sawdust amendment. The amount of soil C recovered in the free-light fraction in all other soils (on average 5%) and the C-to-N ratio of this fraction (on average 22) was very similar to that found in most agricultural soils (Gregorich *et al.*, 2006).

Predictors of gross N mineralization rates

Gross N mineralization rates were linearly related to the soil organic N content, with 91% of the variation in gross N miner-

alization rates between the treatments explained by differences in soil N (Figure 2b). This is similar to the 90% explained variation found by Accoe *et al.* (2004) across grassland soils and the 70% variation explained found by Wang *et al.* (2001) for 19 agricultural soils in Australia. However, gross N mineralization was not a constant fraction of soil N either in our study or in studies by Wang *et al.* (2001) and Accoe *et al.* (2004), as it is influenced by a range of factors, including edaphic factors such as soil texture (Accoe *et al.*, 2005). The use of bulk soil N as sole predictor of gross N mineralization is therefore not sufficient and additional predictors are required.

Our study corroborates that gross N mineralization can be predicted from C mineralization (Figure 2c). But if the C-to-N ratio of the material undergoing decomposition varies, then the relation between C and gross N mineralization will be obscured by differences in concurrent gross N immobilization as evident in the SD + N treatment (cf. Figure 2c, Table 2). Taking the C-to-N ratio of such materials into account may be then needed to improve the estimate of N mineralization from C mineralization data (Luxhøi *et al.*, 2006). In an earlier study (Herrmann *et al.*, 2004), with six different soil treatments from the Ultuna Long-Term Soil Organic Matter Experiment, gross N mineralization was proportional to C mineralization over 17 weeks of incubation, irrespective of incubation time and treatment (with the

exception of the sawdust treatment after 2 and 7 weeks of incubation). However, the amount of gross N mineralized per unit C mineralized in the present study differed from that obtained by Herrmann *et al.* (2004) with about half the amount of gross N mineralized per unit C mineralized (Figure 2c vs. Herrmann *et al.*, 2004). Differences in the ratio of C-to-gross N mineralization have also been observed among forest soils and suggest qualitative differences in the substrate undergoing decomposition, or differences in characteristics of the substrate-utilizing microbial communities (Bengtson *et al.*, 2005). Such differences may also have existed between the soil treatments taken at different times from the Ultuna Long-Term Soil Organic Matter Experiment and incubated after different lengths of pre-incubation and storage, explaining differences in the ratio of C-to-gross N mineralization between the present study and that reported in Herrmann *et al.* (2004).

Lack of proportionality and the presence of possible outliers in the relation between C and gross N mineralization (Figure 2c) meant that the ratio between these two was not constant, which is reflected in the calculated C-to-N ratio of the material undergoing decomposition (Table 2). There may, however, have been differences in the parameter C_{soil} in Equation (1) (i.e. the microbial C use efficiency and the fraction of soil organic matter stabilized) among the treatments. However, sensitivity analysis showed that the calculated C-to-N ratio of organic matter undergoing decomposition remained similar (83 to 116% of that calculated using a factor of 0.42), when assuming a factor of 0.3 or 0.5 (Saetre & Stark, 2005; Luxhøi *et al.*, 2006). Differences in the ratio of C-to-gross N mineralization were therefore more likely to be due to differences in the C-to-N ratio of the material undergoing decomposition (Table 2), rather than differences in the parameter C_{soil}. As the C-to-N ratio of the material undergoing decomposition was related to gross N immobilization (Figure 4b), differences in the ratio of C-to-gross N mineralization between the soils amended with organic manures (i.e. Hay, Straw + N, Farmyard manure and SD + N) will have been at least partially due to gross N immobilization.

Predictors of gross N immobilization rates

Only the free-light fraction in the SD + N treatment had a C-to-N ratio that would make it a likely candidate as a pool of nitrogen-deficit organic matter to drive N immobilization during its decomposition (Table 3). Nevertheless, the strong correlation between the amount of free-light fraction with both recovery of added $^{15}NH_4^+$-N in the free-light fraction and gross N immobilization rates (Figures 3a, 4c), suggests that the amount of this fraction in all treatments was important for determining gross N immobilization and that free-light fraction material with a C-to-N ratio of around 22 (Table 3) was sufficient to induce N immobilization during its decomposition.

In addition to the amount, the quality of the free-light fraction, as indicated by its C-to-N ratio, was only a predictor of ^{15}N

recovery in the free-light fraction in the SD + N treatment (Figure 3b). Variations in ^{15}N recovered per gram free-light fraction (Table 3) suggest that the quality of free-light fraction and the potential for N immobilization in the FYM and SD + N treatments was statistically significantly different from free-light fraction in the Hay and Straw + N treatments in year 2002. These qualitative differences were, however, not apparent in the following year (Table 3), suggesting that the quality of the free-light fraction had become more similar between the treatments. As these differences were not related to differences in the C-to-N ratio of the free-light fraction, some additional characteristics of the material, such as, for example, its lability, must explain the differences in recovery of ^{15}N in the free-light fraction. These differences were, however, relatively small and do not distract from the main conclusion that for most common organic amendments applied in autumn, the amount of free-light fraction material present in the soil in spring is a sufficient predictor of the immobilization potential of the soil. The C-to-N ratio of the free-light fraction needs only to be taken into account when it exceeds about 40 (Figure 3b).

Conclusions

Our study confirms the appropriateness of bulk soil N and C mineralization as predictors of gross N mineralization from stabilized soil organic matter. Comparison between studies suggests, however, that neither predictor is universally valid; that is, across studies gross N mineralization is not a constant fraction of either bulk soil N or C mineralization. Even though it has been suggested by others that edaphic factors may affect the relation between gross N mineralization and bulk soil N as well as C mineralization, these factors are yet to be quantified. The presence of free-light fraction material was largely responsible for obscuring the relation between soil organic N and net N mineralization, as gross N immobilization rates were related to the amount of free-light fraction soil organic matter. The C-to-N ratio of this fraction needs only to be taken into account as a predictor of gross N immobilization when above 40. We conclude that bulk soil N, C mineralization and the amount of free-light fraction organic matter in soils are promising predictors of gross N mineralization and immobilization, respectively. This will inform the development of models predicting net N mineralization from stabilized soil organic matter.

Acknowledgements

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