

**Predicting Nitrogen Mineralization  
from Soil Organic Matter  
- a Chimera?**

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## Abstract

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Predicting nitrogen (N) mineralization from soil organic matter is difficult because N mineralization is affected by several environmental factors, while being the net outcome of concurrent N processes that produce and consume mineral N. One aim of the present thesis was to study the effects of freezing and thawing on carbon (C) and N mineralization. A second aim was to elucidate if, and how, the quantity and quality of organic matter inputs affect N mineralization from the pool of soil organic matter.

C and net N mineralization were determined in soils from the Ultuna Long-Term Soil Organic Matter Experiment exposed to repeated freezing and thawing (temperatures ranging from  $-5^{\circ}\text{C}$  to  $+5^{\circ}\text{C}$ ). C, gross and net N mineralization in relation to quantity and quality of organic matter inputs were determined during long-term laboratory incubations at  $20^{\circ}\text{C}$ . Gross N mineralization rates were estimated using the  $^{15}\text{N}$  isotope dilution technique, which is based on several assumptions. The assumption of 'equilibrium between added and native N' was tested by using a published data set in a dynamic compartmental model.

Freezing and thawing of soils resulted in a flush in C and N mineralization, but the effect was only short-lived. It was concluded that freezing and thawing of soils during late winter and early spring is unlikely to be of importance to crop N availability in spring. Both quantity and quality of organic matter were major determinants of C and gross N mineralization, and these were proportional suggesting that C mineralization may be used as a predictor for gross N mineralization. Preferential use of added N may be a more common occurrence in  $^{15}\text{N}$  isotope dilution studies than hitherto thought and the assumption of 'equilibrium between added and native N' needs therefore critical evaluation. The data analysis presented in this thesis offers a way to estimate the potential effects of preferential use on gross N mineralization rate estimates.

This thesis indicates that studies based on the mechanisms underlying N processes may improve our understanding of the relation between soil organic matter and N mineralization. Further mechanistic studies should therefore be considered in future N research.

*Keywords:* Decomposition, agricultural soil, microbial biomass, mechanistic approach, freeze-thaw cycle, quantity and quality of soil organic matter, gross N mineralization,  $^{15}\text{N}$  isotope dilution technique, preferential use of added N

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**Man muss sich einfache Ziele setzen, dann  
kann man sich komplizierte Umwege erlauben.**

Charles deGaulle

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# Preface

## Papers I-III

The thesis is based on the following papers, which are referred to by their Roman numerals:

- I. Herrmann, A. & Witter, E. 2002. Sources of C and N contributing to the flush in mineralization upon freeze-thaw cycles in soils. *Soil Biology and Biochemistry* 34, 1495-1505.
- II. Herrmann, A. & Witter, E. Gross and net N mineralization from soil organic matter after 45 years of addition of different organic amendments. *Manuscript*.
- III. Herrmann, A., Witter, E. & Kätterer T. An attempt to quantify 'preferential use' in the  $^{15}\text{N}$  isotope dilution technique and its impact on gross N mineralization rates. *Submitted to Soil Biology and Biochemistry*.

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## Introduction

In agricultural systems, careful management of nitrogen (N) is crucial for plant production and environmental reasons. On the one hand, there is a risk of N deficiency during the growing season that reduces crop yields. On the other hand, N surpluses may cause environmental problems in temperate climates due to nitrate leaching after crop harvest. In ecological farming systems N deficiency is often seen in early spring, which is partially due to low soil temperatures limiting microbial activity and thus N mineralization, *i.e.* the transformation of organic N into mineral N. One strategy to meet crop N demand in these farming systems is to maximize the stabilization of organic N inputs in the soil organic matter and thereby over time build up the pool of soil organic matter. In such systems, N mineralization from this pool determines the amount of available crop N. But, continuing N mineralization from the large pool of soil organic matter after harvest increases the risk of nitrate leaching during the autumn and winter. In conventional farming systems, mineral N fertilizers are applied in spring to meet crop N demand, but the amount that can be added is limited by the risk of negative environmental effects. Even when mineral fertilizer N is applied, N mineralization from soil organic matter remains an important source for crop N uptake in conventional farming systems. For example, soil organic matter contributed up to 50% of N uptake by spring barley grown on soils receiving 120 kg N fertilizer per ha for maximum yield (McTaggart & Smith, 1993). However, the leaching risk is mainly due to nitrate derived from soil organic matter after harvest (Macdonald *et al.*, 1989), rather than from unused N fertilizer applied in spring. Consequently, predicting N mineralization from soil organic matter is important, in both ecological and conventional farming systems, to meet crop N demand and to reduce nitrate leaching during autumn and winter.

## Objectives

The overall aim of the present thesis was to study the mechanisms of carbon (C) and N mineralization, so that prediction of N supply can be improved through use of mechanistic models. My work was therefore underpinned by two main aims. One aim was to study the importance of freezing and thawing on C and N mineralization and how that affects N availability to crops. The working hypothesis was that freezing and thawing during late winter and early spring are important mechanisms in releasing soil organic matter for microbial decomposition. Freeze-thaw cycles combined with low temperatures may make relatively large amounts of soil organic matter available for microbial decomposition in the following spring when soil temperatures increase again. A second aim of the thesis was to elucidate if, and how, the quantity and quality of long-term organic matter input affects N mineralization from the pool of soil organic matter. The working hypothesis was that gross N mineralization is determined by the amount of organically bound N, and that the quality of past organic matter inputs affects the amount of N immobilized in association with soil organic matter undergoing decomposition. Varying amounts of N immobilization

may therefore obscure the relation between net N mineralization and the amount of soil organic matter.

The specific objectives were:

- To study the mechanism by which organic C and N is released due to freezing and thawing.
- To assess the importance of freezing and thawing on N availability for crops in early spring.
- To determine C, gross and net N mineralization in soils differing in quantity and quality of long-term organic matter input and to relate them to quantity and quality of soil organic matter.
- To evaluate the  $^{15}\text{N}$  isotope dilution technique for the estimation of gross N processes.

## **Background**

Virtually all N in soils is present in organic forms. In arable soils in Sweden, for example, on average approximately  $8 \text{ t N ha}^{-1}$  is organically bound (Eriksson *et al.*, 1997), while generally less than  $100 \text{ kg N ha}^{-1}$  (about 1% of soil organic N) is present in a directly plant-available, *i.e.* mineral N, form. Soil organic matter encompasses a continuum from very labile to very recalcitrant material, *i.e.* soil organic matter consists of various heterogeneous pools with different rates of decomposition (Schnitzer, 2000). The term ‘quality of soil organic matter’ defined in this thesis refers to the C-to-N ratio of the organic material. Mineral N is continuously released from the soil organic matter pool (N mineralization). But predicting N mineralization from soil organic matter is difficult because N mineralization is affected by several environmental factors, while being the net outcome of concurrent N processes that produce and consume mineral N. Water and temperature are thought to be the main environmental factors controlling N mineralization from soil organic matter (*e.g.* Waksman & Gerretsen, 1931; Jarvis *et al.*, 1996). Their effects on decomposition are well understood and can be quantified, but their inherent uncertainty, *i.e.* the weather over the growing season, makes it difficult to predict N mineralization. Moreover, the processes that substantially contribute to net N mineralization, *i.e.* stabilization of soil organic matter, N supply and removal from the mineral N pool, are understood qualitatively. Quantification of these processes, however, is still one of the greatest challenges in N research.

### **Predicting N mineralization from soil organic matter**

In the past decades, extensive work has been done to seek a reliable method to predict N mineralization from soil organic matter. The variety of methods is vast (biological, chemical or physical methods), but no particular one has dominated. To discuss these methods in detail would go beyond the scope of this thesis and readers seeking an overview of these methods are directed to reviews from *e.g.*

Stanford (1982), Keeney (1982) or Bundy & Meisinger (1994). It has been suggested that mechanistic approaches of N processes may improve our understanding of the relation between soil organic matter and N mineralization (Jarvis *et al.*, 1996; Powlson, 1997).

Mechanistic approaches take the internal N cycle as a starting point where gross N mineralization (N supply) and concurrent N immobilization (N removal from mineral N pool) are two fundamental processes that largely determine net N mineralization. As mentioned above, soil organic matter consists of various heterogeneous pools with different rates of decomposition. Unless the relative distribution of organic matter between these pools is the same in all soils, the total amount of soil organic matter will be a poor predictor for N mineralization. Jansson (1958) divided the soil organic matter pool into an 'active' and 'passive' pool. Mechanistic models (*e.g.* Jenkinson & Rayner, 1977; Smith *et al.* 1997; Jansson & Karlberg, 2001; Kätterer & Andrén, 2001) divide soil organic matter into several organic C pools (*e.g.* organic C from crop residues or manure, microbial biomass C and stabilized soil organic C pool) with different turnover rates and assuming a certain C-to-N ratio for each pool (*e.g.* Parton *et al.*, 1987; Hansen *et al.*, 1991; Rijkema & Kroes, 1991). Each organic C pool is treated as a homogenous substrate with an explicit turnover rate based on first-order kinetics. The turnover rates are modified by the effects of abiotic factors such as temperature, soil moisture and soil texture using empirical relations. Gross N mineralization is usually estimated from C mineralization, while the C-to-N ratio of the organic matter in the source and sink pools determines whether net N mineralization or net N immobilization occurs. It is difficult to validate these models as the different pools of soil organic matter can usually not be measured directly and are therefore conceptual, rather than real. In contrast, the model of Bosatta & Ågren (1985) and Ågren & Bosatta (1996) considers the decomposition of soil organic matter as a continuum whereby organic matter is assumed to move down a quality scale. However, the mathematics of such an approach is complex. Estimations of gross N mineralization and N immobilization may improve our understanding of the relation between soil organic matter and N mineralization in soils and these rate estimates may be put to use in mechanistic models. The challenge of a mechanistic approach is therefore to be able to quantify and predict gross N mineralization and N immobilization.

### **Mineralization and immobilization turnover in soils**

Soil organic matter is continuously decomposed by a range of soil microorganisms including bacteria, fungi and their predators resulting in release of ammonium ( $\text{NH}_4^+$ ) (mineralization). This may be oxidized to nitrate ( $\text{NO}_3^-$ ) by bacterial species belonging to the genera *Nitrosospira*<sup>1</sup>, which convert  $\text{NH}_4^+$  to nitrite, and *Nitrobacter*, which complete the oxidation to  $\text{NO}_3^-$  (nitrification) (Figure 1). Microbial N immobilization, *i.e.* the assimilation of mineral N into microbial

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<sup>1</sup> *Nitrosospira* have recently been shown to be more common in arable soils than those belonging to *Nitrosomonas* (Mendum *et al.*, 1999)

biomass, is usually concurrent to the release of mineral N.  $\text{NH}_4^+$  is preferentially immobilized compared to  $\text{NO}_3^-$  (Jansson, 1958; Recous *et al.*, 1990), but  $\text{NO}_3^-$  immobilization may dominate when  $\text{NH}_4^+$  is limited (Azam *et al.*, 1986; Rice & Tiedje, 1989; Recous *et al.*, 1990), as it is often the case in arable soils.

The net outcome of mineralization and immobilization determines the amount of available crop N hence neither should be considered separately. Together, these processes have been referred to as the ‘Mineralization-Immobilization Turnover’ (MIT) (Jansson & Persson, 1982) (Figure 1). The soil microbial biomass mediates between mineralization and immobilization and it is therefore a key factor in MIT. Even though soil microbial biomass N is only a small part, approx. 4-6% of total soil organic N (Paul, 1984), it is clear from the above why it has been referred to as ‘the eye of the needle’ (Jenkinson, 1990).

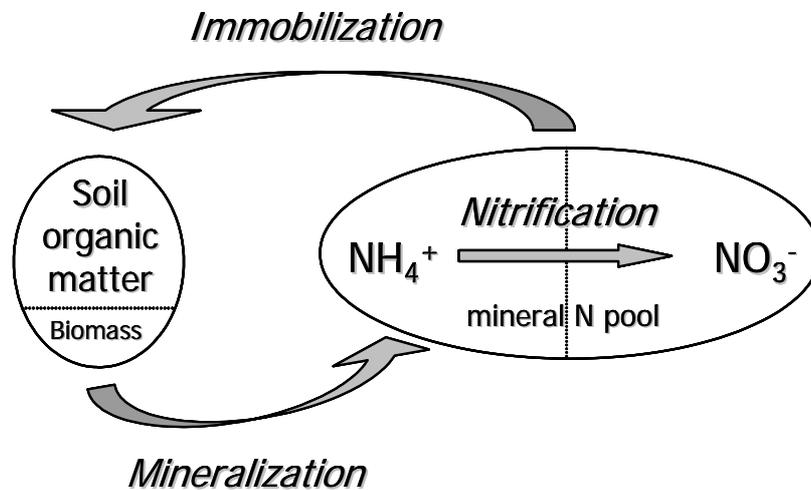


Figure 1. Processes constituting the Mineralization-Immobilization Turnover (MIT).

In addition to MIT, there may be direct microbial assimilation of soluble, low-molecular-weight, nitrogenous organic compounds such as amino acids (Hadas *et al.*, 1987; Barak *et al.*, 1990; Drury *et al.*, 1991; Barraclough, 1997). These two pathways are concurrent and direct assimilation of simple amino acids may be of similar magnitude compared to MIT (Barraclough, 1997; Gibbs & Barraclough, 1998; O'Dowd *et al.*, 1999).

Mineralization of soil organic matter provides C (energy) for microbial maintenance and growth. Net immobilization of N occurs when organic matter undergoing microbial decomposition has an N content that is insufficient to meet the N demand of the microorganisms. Thus, the net outcome in terms of available crop N, *i.e.* net N mineralization or net N immobilization is largely determined by the C-to-N ratio of the organic matter undergoing decomposition (Paul & Juma,

1981; van Veen *et al.*, 1984; Chaussod *et al.*, 1988). A well-known example is the net N immobilization of mineral N in arable soils that occurs after the addition of fresh N-poor crop residues such as straw (Ocio *et al.*, 1991). It is less well known how much N immobilization occurs during the decomposition of older, more stabilized soil organic matter because the quality, *i.e.* the C-to-N ratio of the decomposing material is rarely known. The C-to-N ratio of the entire soil organic matter pool is often too low for any net N immobilization to occur, but density fractionation of soil organic matter (*e.g.* Golchin *et al.*, 1998) reveals that soil organic matter is not a homogenous pool but contains fractions of distinctly different C-to-N ratios. Studies in a range of ecosystems highlighted that N mineralization may be accompanied by substantial N immobilization (Davidson *et al.*, 1992; Ledgard *et al.*, 1998; Murphy *et al.*, 2003).

### *Quantification of gross N processes*

Mineralization, nitrification and immobilization continuously affect the amounts of mineral N over time. The amount of mineral N at a certain time is therefore only a snapshot of the net balance between these N processes (ignoring other N losses, *e.g.* denitrification, nitrate leaching or crop N uptake from the mineral N pool). A low rate of net N mineralization may not always be the result of a low rate of gross N mineralization, but may equally well be the result of a high rate of immobilization from the mineral N pool. Because gross N processes describe the total production of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (gross mineralization and nitrification) and assimilation (gross immobilization) and not just the net balance between these processes, quantification of these processes is essential to our understanding of the turnover of mineral N in soils.

Gross N processes are studied using the  $^{15}\text{N}$  isotope dilution technique.  $^{15}\text{N}$  is a stable isotope<sup>2</sup> of nitrogen with mass number 15. Gross N mineralization, for example, is estimated by enriching the  $\text{NH}_4^+$  pool with  $^{15}\text{N}$  and measuring the changes of the  $\text{NH}_4^+$  pool size and dilution of  $^{15}\text{N}$  in the  $\text{NH}_4^+$  pool over time. The  $^{15}\text{N}$  enriched  $\text{NH}_4^+$  pool is diluted due to introduction of  $\text{NH}_4^+$  at natural abundance, *i.e.*  $\text{NH}_4^+$  with natural background concentration of  $^{15}\text{N}$  (0.3663 atom%  $^{15}\text{N}$ ), via mineralization from soil organic matter. Gross nitrification is similarly estimated; the difference being that the  $\text{NO}_3^-$  pool is enriched with  $^{15}\text{N}$  and the changes of the  $\text{NO}_3^-$  pool size and dilution of  $^{15}\text{N}$  in the  $\text{NO}_3^-$  pool are measured over time. In this case, the  $^{15}\text{N}$  enriched  $\text{NO}_3^-$  pool is diluted due to introduction of  $\text{NO}_3^-$  at natural abundance via mineralization from soil organic matter followed by nitrification from the  $\text{NH}_4^+$  pool.

Kirkham & Bartholomew (1954, 1955) first proposed differential equations to calculate gross N processes that form the basic concepts of the  $^{15}\text{N}$  isotope dilution technique. Blackburn (1979) adjusted these equations for  $^{15}\text{N}$  at natural abundance, *i.e.* taking into account the natural background concentration of  $^{15}\text{N}$ , and equivalent equations for the calculation of gross N rates were proposed by

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<sup>2</sup> Isotope is defined as the variety of an element with different mass number, but otherwise same atomic number and chemical properties

Nishio *et al.* (1985) and Barraclough (1991). In principle, all equations give similar results and can be described by the following equation (equation 1) as shown by Smith *et al.* (1994).

$$m = \frac{\left( \frac{AT_1 - AT_2}{\Delta t} \right) \times \log \left( \frac{AL_1 AT_2}{AL_2 AT_1} \right)}{\log \left( \frac{AT_1}{AT_2} \right)} \quad (1)$$

where  $m$  is the gross N mineralization rate,  $AT$  is the amount of total  $\text{NH}_4^+$ ,  $AL$  is the amount of labelled  $\text{NH}_4^+$ ,  $\Delta t$  denotes the time interval and the subscripts 1 and 2 denote the amounts at the beginning and end of the incubation period. Gross nitrification  $n$  is calculated by using the same equation, but  $AT$ ,  $AL$  and  $m$  are replaced by  $NT$  the amount of total  $\text{NO}_3^-$ ,  $NL$  the amount of labelled  $\text{NO}_3^-$  and  $n$  gross nitrification, respectively.

Gross N immobilization can then be estimated from (i) differences between gross and net N mineralization, (ii) by subtracting gross nitrification from  $\text{NH}_4^+$  consumption (Kirkham & Bartholomew, 1954; Davidson *et al.*, 1991), which gives an estimate for  $\text{NH}_4^+$  immobilization, (iii) by measuring  $^{15}\text{N}$  in the microbial biomass using the fumigation-extraction method (Ledgard *et al.*, 1998, Hatch *et al.*, 2000), (iv) by determination of residual  $^{15}\text{N}$  in soils after KCl extraction (Mary *et al.*, 1998; Recous *et al.*, 1999; Andersen & Jensen, 2001) or (v) by the use of numerical approaches.

Several numerical approaches have been established besides analytical approaches in the last decades (Myrold & Tiedje, 1986; Bjarnason, 1988; Nason & Myrold, 1991; Wessel & Tietema, 1992; Smith *et al.*, 1994; Mary *et al.*, 1998). The principle is similar in all these numerical approaches in that they combine numerical integration of differential equations and a non-linear fitting procedure resulting in the best fit between simulated and measured values. The advantages of numerical approaches are that, in contrast to the analytical approach, several N processes can be estimated simultaneously.

#### Assumptions of the $^{15}\text{N}$ isotope dilution technique

The  $^{15}\text{N}$  isotope dilution technique is based on several assumptions, which, when false, can lead to large errors in the calculation of gross rates. The following assumptions have to be met to avoid errors in the calculation of gross N rates (Powlson & Barraclough, 1992):

- 1.) No isotopic discrimination
- 2.) No re-mineralization of recently added  $^{15}\text{N}$
- 3.) All rate processes are constant during the incubation period, *i.e.* can be described by zero-order kinetics
- 4.) Equilibrium and identical behaviour between added and native N pools

Isotopic discrimination in the soil processes involved (assumption 1) is only of importance at natural abundance because discrimination is at most 20-30‰  $\delta^{15}\text{N}$  (Högberg, 1997) and therefore insignificant when the mineral N pools are  $^{15}\text{N}$  enriched to a level several times above natural abundance (Davidson *et al.*, 1991; Wessel & Tietema, 1992).

There are few studies that have tried to directly measure re-mineralization of added mineral N (assumption 2). Very low re-mineralization of mineral N immobilized through the addition of a C source (Bjarnason, 1987), as well as the absence of the appearance of labelled N in the  $\text{NH}_4^+$  pool after addition of labelled  $\text{NO}_3^-$  (Watson *et al.*, 2000; Burger & Jackson, 2003) suggest that there is no significant re-mineralization of immobilized N within one week.

The assumption of zero-order rate constants for all processes (assumption 3) holds if measurements are carried out over sufficiently short time intervals so that processes following first-order kinetics can be approximated by zero-order kinetics. Although in most studies zero-order rate constants are assumed for all processes, applied  $\text{NH}_4^+$  is rapidly nitrified within weeks (Mendum *et al.*, 1999; Burger & Jackson, 2003) and  $\text{NH}_4^+$  addition may stimulate nitrification (Myrold & Tiedje, 1986; Nira *et al.*, 1996; Willison *et al.*, 1998), indicating that nitrification is a substrate-limited process. This suggests that  $\text{NH}_4^+$  consumption may follow first-, rather than zero-order kinetics and incorrect description of process kinetics may therefore lead to serious errors in rate estimation (Nason & Myrold, 1991). Bjarnason (1988) suggested that by using a numerical model the assumption of zero-order rates is of little importance in agricultural soils over short-time intervals of less than a week. This conclusion was, however, derived from an experiment in which nitrification was inhibited, which emphasizes that the importance of the assumption of zero-order rate constants is dependent on experimental design.

Equilibrium between and identical behaviour of added and native N (assumption 4) is fundamental to the  $^{15}\text{N}$  isotope dilution technique because gross N rates cannot be calculated from the behaviour of native N alone. Instead, the behaviour of native N is inferred from that of the added  $^{15}\text{N}$ . Equilibrium is facilitated by even distribution of the added N, and uneven distribution can lead to large errors in the estimation of gross rates (Davidson *et al.*, 1991). Uniform distribution of added  $^{15}\text{N}$  is more difficult to obtain in intact soil cores in the field than in sieved soil samples that are mixed after addition of  $^{15}\text{N}$ .  $^{15}\text{N}$  additions to intact soil cores are carried out by single (*e.g.* Schimel *et al.*, 1989; Stockdale *et al.*, 1994) or multiple point injectors (*e.g.* Ledgard *et al.*, 1998; Andersen & Jensen, 2001), which give a more even (Monaghan, 1995), but still noticeably imperfect (Andersen & Jensen, 2001) distribution of the added solution. However, even distribution of the added  $^{15}\text{N}$  solution may not be sufficient to guarantee equilibrium between added and native N. The exchange of  $^{15}\text{N}$  with adsorbed  $^{14}\text{N}$  or  $^{14}\text{N}$  in soil solution located in micropores where it is held at high tension and consequently physically protected from microorganisms may affect the assumption of equilibrium. Further, irreversible clay fixation of added  $\text{NH}_4^+$  leads to overestimation of gross N rates. This process is thought to occur within 15 minutes of  $^{15}\text{N}$ -labelled  $\text{NH}_4^+$  addition and overestimation can be avoided with an

initial extraction (Davidson *et al.*, 1991; Stockdale *et al.*, 1994). The time needed to establish equilibrium between native and added N may, however, vary between soils and the recommended practice (Murphy *et al.*, 2003) is to use the amounts of  $^{15}\text{N}$  recovered in the mineral N pools after 24 h of N addition as the starting point. There is, however, no evidence when, or indeed if, equilibrium between added and native N is established and whether consequently the behaviour of added N can be assumed to be identical to that of native N.

These four assumptions are crucial for the estimation of gross N rates and must be met to minimize potential sources of error in studies using the  $^{15}\text{N}$  isotope dilution technique. The assumptions of 'no isotopic discrimination' and 'no re-mineralization of recently added  $^{15}\text{N}$ ' (assumptions 1 and 2) are relatively easy to fulfil by  $^{15}\text{N}$  addition several times above natural abundance and incubation periods of less than one week. The assumption of 'zero-order rate constants' (assumption 3) can be tested by several successive samplings, but 'equilibrium and identical behaviour between added and native N pools' (assumption 4) is difficult to test, as it is an integral in the  $^{15}\text{N}$  isotope dilution technique.

### **Stabilization of organic matter in soils**

Soils contain varying amount of organic matter depending on the balance between the rate of organic matter input and the rate of its decomposition. Some of the soil organic matter is extremely recalcitrant to decomposition. Jenkinson (1977) was still able to find some labelled C in the soil several years after addition of  $^{14}\text{C}$  labelled ryegrass to the soil, and some soil organic matter has been shown by  $^{14}\text{C}$  dating to be hundreds and even thousands of years old (Hsieh, 1992). There are three principally different mechanisms by which organic matter is stabilized against decomposition in soils: biochemical recalcitrance, chemical stabilization and physical protection (Jastrow & Miller, 1998) (Figure 2). Stabilization of organic matter is of importance for gross N mineralization because it affects the amount of organic matter susceptible to decomposition. The three mechanisms by which organic matter is stabilized in soils are therefore discussed in detail in this section.

Organic matter entering the soil becomes subject to decomposition due to microbial and faunal activity. At the same time the composition of the remaining organic matter changes: First simple compounds such as sugar and protein then cellulose *etc.* are decomposed. Microbial biomass and intermediary breakdown products are formed during this process. Some of the biochemical recalcitrance of soil organic matter is due to the chemical nature of the material entering the soil and some is due to the formation of biochemically recalcitrant molecules during the decomposition process. Organic matter that contains lignin derivatives (Stott *et al.*, 1983), for example, or melanin produced by fungi and other soil organisms (Martin & Haider, 1986) are rather difficult to decompose by microorganisms. Biochemical recalcitrance of substrate C explained the relatively higher amount of C remaining in peat- and sewage-sludge-amended soils, but there were no differences in the smaller amounts of C stabilized from straw and green manure (Witter, 1996).

Biochemical recalcitrance is only one possible explanation for the stabilization of organic matter in soils. Besides this stabilization process, organic matter may become adsorbed on minerals or included into soil aggregates. As a result of these processes both fresh and partially decomposed organic substrate is thought to be stabilized against further decomposition by two mechanisms: chemical stabilization and physical protection. Chemical stabilization is the adsorption of decomposable organic matter onto clay mineral and sesquioxide surfaces through chemical and physicochemical associations (Swift *et al.*, 1979). Physical protection, the third mechanism of soil organic matter stabilization, occurs when organic matter is situated in soils in such a way that it is physically inaccessible to microorganisms and their enzymes, and thus protected from decomposition (Swift *et al.*, 1979). Organic matter may become physically protected from decomposition by incorporation into soil aggregates (Golchin *et al.*, 1994a, b) or by deposition in micropores inaccessible to microorganisms (Foster, 1985).

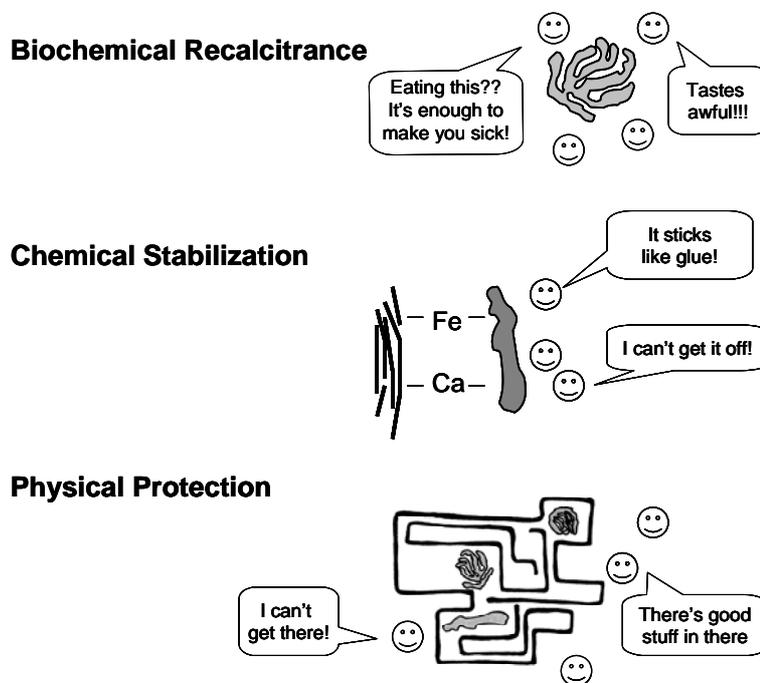


Figure 2. Mechanisms of soil organic matter stabilization (adapted from Jastrow & Miller, 1998).

Chemical stabilization varies between soils with different texture and soils with the same clay content but of different clay type (Saggar *et al.*, 1996). The main evidence for the importance of physical protection for organic matter decomposition comes from studies comparing the rate of decomposition of added substrates in soils of different texture. Such studies generally show that

decomposition rates are lower and that more of the added organic matter remains in finer textured compared to coarser textured soils (*e.g.* Hassink *et al.*, 1993; Hassink, 1995; Strong *et al.*, 1999). Fractionation of soils into their primary particles also shows that the clay- and silt-size fractions have a higher C content than the sand-size fraction (van Gestel *et al.*, 1996; Stemmer *et al.*, 1998; Kandeler *et al.*, 1999). These studies indicate that physical protection in soils is due to the presence of clay- and silt-sized particles. Clay soils have generally more micropores than sandy soils. Hassink *et al.* (1993) found that the relative increase in N mineralization after fine sieving was correlated to the percentage of soil pore space occupied by pores with diameters  $<0.2 \mu\text{m}$  in clay soils ( $R^2 = 0.81$ ), but the correlation with any other pore size class was poor. Fine sieving enlarges pores and organic matter situated in small pores may become accessible to microorganisms after sieving. The study by Hassink *et al.* (1993) suggests that physical protection plays a more important role in clay soils with relatively more micropores than in sandy soils with fewer micropores. There are, to my knowledge, no studies that compare the relative importance of physical protection versus biochemical recalcitrance or chemical stabilization. Studies dealing with stabilization of soil organic matter often refer to one of the three mechanisms mentioned above without being able to quantify the contribution of the respective mechanism.

Soil texture and the pore size distribution of soils may be important factors in physical protection of organic matter in soils. However, I want to point out that a relation between decomposition and soil texture does not provide information as to the mechanisms by which physical protection takes place, because chemical stabilization (*i.e.* adsorption onto mineral surfaces) is also related to soil texture. The term ‘physical protection’ used in the following section implies therefore both chemical stabilization and physical protection, since it is nearly impossible to separate these two stabilization mechanisms.

### *Soil aggregation and physical protection*

In the former section, the mechanism of physical protection of organic matter is described in relation to the pore size distribution of soils and therefore emphasizes the importance of soil texture. However, other physical properties, such as the arrangement of soil aggregates (soil structure) will also lead to different pore size distributions and thus contribute to physical protection of soil organic matter. Furthermore, organic matter addition and organic matter decomposition may by themselves affect the degree of soil aggregation and thus the degree of physical protection.

#### Aggregate hierarchy

Soil structure is thought to be hierarchical (Tisdall & Oades, 1982; Golchin *et al.*, 1998). According to this theory the soil matrix consists of macro- and microaggregates. Macroaggregates are larger, while microaggregates are smaller than  $250 \mu\text{m}$  (Tisdall & Oades, 1982). Primary mineral particles bind together into microaggregates ( $<250 \mu\text{m}$ ), which in turn form macroaggregates ( $>250 \mu\text{m}$ ). Macroaggregates are easily disrupted by *e.g.* drying and wetting or freezing and

thawing, whereas microaggregates are highly stable against physical disruption. Young labile organic matter may become physically protected within macroaggregates and acts as a binding agent for tying together micro- into macroaggregates (Jastrow & Miller, 1998). The binding substrate is readily decomposable organic materials, such as microbial- and plant-derived polysaccharides as well as fine roots, fungal hyphae, bacterial cells and algae (Jastrow & Miller, 1998). The organic matter in microaggregates is thought to be relatively persistent and consists of humic materials or polysaccharide polymers that are strongly adsorbed to clays by bridges of polyvalent cations (Tisdall & Oades, 1982; Golchin *et al.*, 1994a, b) or by hydrophobic interactions (Piccolo, 1996). Oades & Ladd (1977) provided chemical evidence that aggregates 1-5  $\mu\text{m}$  diameter consist of old, protected organic matter within the aggregates.

Evidence for the protection of relatively labile, inter-macroaggregate organic matter comes from laboratory studies comparing mineralization in intact and crushed macroaggregates from topsoil (Elliott, 1986; Beare *et al.*, 1994a). The latter were obtained through fine sieving, which resulted in a 30-40% increase of C and N mineralization compared to intact macroaggregates. This relatively large impact of fine sieving of macroaggregates on mineralization suggests that fine sieving of soil samples followed by incubation may be an appropriate method to evaluate the degree of physical protection of soil organic matter. Density fractionation is a further method that may be applied to study the degree of physical protection in soils. This method is often used to separate the heterogeneous pool of soil organic matter into soil organic matter classes differing in age, quality or origin. Golchin *et al.* (1994a, b) used polytungstate solution with different densities to separate soil organic matter into a free-light fraction, an occluded-light fraction, an occluded-particulate fraction and a colloidal- or clay-associated fraction. These different fractions form the basis for a conceptual model (Golchin *et al.*, 1998) that links different levels of aggregation with soil organic matter dynamics. The free-light fraction, which is thought not to be physically protected against decomposition (Gregorich *et al.*, 1994), is separated with a solution of density  $1.6 \text{ Mg m}^{-3}$ . Subsequently, ultrasonification is used to disrupt macro- and microaggregates and different density media are used to obtain the remaining fractions. The different fractions were linked to different sizes of aggregates. Fractions with a density  $<1.6$  and  $1.6-1.8 \text{ Mg m}^{-3}$  contained soil organic matter originating from microaggregates, *i.e.* organic matter with a higher degree of decomposition. Soil organic matter found in  $1.8-2.0 \text{ Mg m}^{-3}$  refers to macro- as well as microaggregates. The remaining organic matter in the soil (density  $>2 \text{ Mg m}^{-3}$ ) after fractionation is thought to be from colloidal or clay-associated origin. The age of organic matter in the different fractions and thus most likely the importance of physically protected organic matter increases from the free-light to the occluded and clay-associated fractions. Golchin *et al.* (1998) concluded this from electron microscopy, chemical characterisation by CP MAS  $^{13}\text{C}$  NMR analysis and the C-to-N ratio of the different fractions. Hence, the density fractionation proposed by Golchin *et al.* (1994 a, b) may be a valuable method to assess the degree of physical protection of organic matter and to relate it to soil organic matter dynamics.

It is known that aggregate cycling (*i.e.* their formation, stabilization and degradation) is a very dynamic process. As discussed above, soil aggregation is intimately associated with the protection of organic matter, and changes in aggregation may therefore have consequences for mineralization and stabilization of soil organic matter. Soil and crop management (Beare *et al.*, 1994 a, b; Besnard *et al.*, 1996; Balesdent *et al.*, 1998; Silgram & Shepherd, 1999), wetting and drying (Kieft *et al.*, 1987; Degens & Sparling, 1995; Appel, 1998; Strong *et al.*, 1999; Magid *et al.*, 1999) as well as freezing and thawing are thought to be important factors affecting soil aggregation and thus protection of organic matter. In the following, only freezing and thawing is discussed in detail.

#### *Effects of freeze-thaw cycles on mineralization*

Freeze-thaw cycles (FTC) generally, but not always (Wang & Bettany, 1993; Lomander *et al.*, 1998), result in a flush in either C or N mineralization (DeLuca *et al.*, 1992; Schimel & Clein, 1996; Groffman *et al.*, 1999). The effect is dependent on the rate of freezing and thawing, temperature, soil moisture content and number of FTC (Edwards, 1991; Lehrsch *et al.*, 1991; Lehrsch, 1998). Studies on tundra and taiga soils (Schimel & Clein, 1996) and other forest soils (Skogland *et al.*, 1988) showed that the effect of the mineralization flush upon FTC is short-lived and largest in the first FTC. However, little is known about the mechanism behind the release of organic material upon freeze-thawing, but the microbial biomass and the release of formerly physically protected soil organic matter are conceivable C and N sources of the mineralization flush upon FTC.

It has been suggested that lysis of microbial cells could be an important source of organic matter made available by FTC (Soulides & Allison, 1961; Skogland *et al.*, 1988). Schimel & Clein (1996), for example, found that the quality of organic matter in taiga and tundra soils was of significance for its susceptibility to FTC, but suggested that this was an indirect effect caused by the effect of organic matter quality on the amount of microbial biomass in the soils. They also concluded that the C flush after each freezing period was due to physical damage of microbial cells by FTC and that the long-term effects might therefore be largely controlled by the ability of the microbial community to recover from such stress. DeLuca *et al.* (1992) found a significant increase in the N mineralization rate and mineral N flush between moist soils frozen to  $-20\text{ }^{\circ}\text{C}$  and then thawed, compared to non-frozen soils. The freeze-thaw treatments resulted in a significant release of ninhydrin-reactive, *i.e.* amine or amide N. This release was more closely correlated with biomass than total N and their results therefore suggested that FTC in soil may disrupt microbial cells in a similar way to drying and re-wetting or chloroform fumigation.

FTC are also known to change physical properties of soils and may thus influence the degree of physical protection of soil organic matter, *i.e.* FTC may result in changes in the pore system in soils, so that microorganisms gain access to organic matter formerly situated in the micropores. Studies on the effects of FTC on soil physical properties suggest changes in soil aggregate distribution and aggregate stability, but with contradictory results. FTC has been found to both

decrease (Edwards, 1991) and increase soil aggregate stability (Lehrsch *et al.*, 1991; Lehrsch, 1998). There is no evidence in the literature that organic matter from non-microbial origin contributes to the mineralization flush upon FTC. This, however, does not imply that decomposition of non-microbial organic matter does not contribute to the flush.

## Materials and Methods

### Soils

Soil samples (Papers I and II) were taken from the Ultuna Long-Term Soil Organic Matter Experiment (Uppsala, Sweden; 60 °N, 17 °E). The experiment was started in 1956 on a post-glacial clay loam classified as a Typic Eutrochrept (Soil Survey Staff, 1987) or an Eutric Cambisol (FAO, 1988). In this experiment, soils have been treated with different inorganic N fertilizers or organic amendments and all treatments are replicated in four blocks. Inorganic N fertilizer has been applied annually in spring at the time of sowing at a rate of 80 kg N ha<sup>-1</sup> y<sup>-1</sup>, whereas organic amendments (8 t ha<sup>-1</sup> ash-free organic matter) were added, together with crop residues, every other year in the autumn. The following treatments were selected: (a) bare fallow (Fallow), (b) cropped without N-fertilizer (Unfertilized), (c) N-fertilized, (d) green manure (GM), (e) straw + N, (f) farmyard manure (FYM), (g) sawdust (SD) and (h) sawdust + N (SD + N). Soil treatments (a)-(d) represent a series of increasing levels of C input of similar quality, whereas (d)-(h) have similar levels of C input, but of different quality. The entire treatment selection was not used in every experiment (for particular soil selection, see Papers I and II). Soil samples were taken from each block in May 1999 and 2001 (approx. 18 months after the last application of organic material). For further details about this long-term field experiment, see Kirchmann *et al.* (1991).

### Importance of freezing and thawing on C and net N mineralization (Paper I)

Soil samples were exposed to repeated freeze-thaw cycles with each cycle consisting of different temperature intervals ranging from -5 °C to +5 °C and constant temperatures acted as a control. The flush in C and N mineralization (*i.e.* differences in mineralization between FTC treatment and constant temperatures) was adjusted to the mean response temperature of the FTC (+1.5 °C) by using an Arrhenius-type function (Kätterer *et al.*, 1998). This temperature adjustment was carried out in order to obtain a less biased quantification of the flush. The contribution of microbial biomass C to the C flush upon freeze-thawing was determined by labelling the native biomass with a small amount of highly <sup>14</sup>C labelled glucose (Dahlin & Witter, 1998) and comparing the specific activity of the C flush upon freeze-thawing with that upon chloroform fumigation-incubation (Jenkinson & Powlson, 1976). The release of non-microbial soil organic matter was studied based on the theory of stabilized soil organic matter, *i.e.* that loss of aggregate structure upon freeze-thawing may be related to the release of

physically protected organic matter. Aggregate size distribution (2000  $\mu\text{m}$ , 250  $\mu\text{m}$ , and 63  $\mu\text{m}$ ) was therefore determined by wet sieving (Kemper & Rosenau, 1986) on soil samples previously subjected to FTC and those incubated at constant temperature of +7 °C.

### **Long-term addition of different amendments and its impact on...**

*... the free-light fraction as well as C and net N mineralization from soil organic matter (long-term incubation)*

Carbon and net N mineralization were consecutively determined over a 27-week incubation period at 20 °C (particular soil selection see Paper I). A density fractionation procedure (Golchin *et al.*, 1994a, b) was used to separate the free-light fraction from the heterogeneous pool of soil organic matter on soil samples prior and after the long-term incubation. Size of free-light fraction and its total C and N were determined. Total C and N were also determined on bulk soils. Solid-state cross polarization magic angle spinning  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy (CP MAS  $^{13}\text{C}$  NMR) (Wilson, 1987) was used to characterize organic amendments added to the soils in the autumn 1997.

*... gross N mineralization from soil organic matter (Paper II)*

Carbon, gross and net N mineralization rates were determined over a 120-h or 72-h incubation period after 2, 7 and 17 weeks of pre-incubation at 20 °C (Period 1, 2 and 3, respectively). Gross N mineralization was determined by using the  $^{15}\text{N}$  isotope dilution technique, assuming first-order kinetics for  $\text{NH}_4^+$  consumption rates. Soil samples were amended with labelled  $(^{15}\text{NH}_4)_2\text{SO}_4$  solution (2.0 atom%) at a rate equivalent of approximately 5  $\mu\text{g N g}^{-1}$  soil after each pre-incubation period. The amounts of mineral N in soil samples were extracted by 2 M KCl, after an incubation period of 2 h and 72 h or 120 h at 20 °C. The atom %  $^{15}\text{N}$  of mineral N in KCl soil extracts was determined using a microdiffusion technique proposed by Goerges & Dittert (1998) with slightly modifications.

### **Testing the assumption of ‘equilibrium between and identical behaviour of added and native N’ by using a dynamic compartmental model (Paper III)**

The assumption of ‘equilibrium between and identical behaviour of added and native N’ (assumption 4) in the  $^{15}\text{N}$  isotope dilution technique was tested by using the raw data of Watson *et al.* (2000) in a dynamic compartmental model. The model considered added and native  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools as separate state variables (Figure 3). Gross N mineralization rates  $m$ , first-order rate constants for  $\text{NH}_4^+$  consumption  $c$ , nitrification  $n$  and  $\text{NO}_3^-$  immobilization  $i_{\text{N}}$  were obtained by combining analytical integration of differential equations with a non-linear fitting procedure to obtain the best fit between predicted and observed N values. Initially, first-order rate constants for  $\text{NH}_4^+$  consumption ( $c_{\text{add}}$ ), nitrification ( $n_{\text{add}}$ ) and  $\text{NO}_3^-$

immobilization ( $i_{N, \text{add}}$ ) were estimated for the added N pools. The first-order rate constants  $c_{\text{add}}$  and  $i_{N, \text{add}}$  were then used to estimate gross mineralization ( $m_{\text{nat}}$ ) and first-order rate constants for nitrification ( $n_{\text{nat}}$ ) of native  $\text{NH}_4^+$  on the assumption that consumption rates of native  $\text{NH}_4^+$  ( $c_{\text{nat}}$ ) and  $\text{NO}_3^-$  ( $i_{N, \text{add}}$ ) were the same as those for the added N pools, *i.e.* that  $c_{\text{add}} = c_{\text{nat}}$  and  $i_{N, \text{add}} = i_{N, \text{nat}}$ .  $\text{NH}_4^+$  immobilization of added and native  $\text{NH}_4^+$  ( $i_{A, \text{add}}$  and  $i_{A, \text{nat}}$ ) was calculated by differences between respective  $c$  and  $n$ . Finally, first-order rate constants for added and native nitrification ( $n_{\text{add}}$  and  $n_{\text{nat}}$ ) were compared and the following hypotheses were tested to see if any discrepancy between the two could be explained by native and added mineral N being subjected to different rates of either nitrification,  $\text{NH}_4^+$  or  $\text{NO}_3^-$  immobilization. This was done by either varying the first-order rate constant for nitrification  $n$ ,  $\text{NO}_3^-$  immobilization  $i_N$  or  $\text{NH}_4^+$  immobilization  $i_A$ . The following hypotheses were tested:

- (a) Only native or added  $\text{NH}_4^+$  is preferentially nitrified
- (b) Only native or added  $\text{NO}_3^-$  is preferentially immobilized or
- (c) Only native or added  $\text{NH}_4^+$  is preferentially immobilized

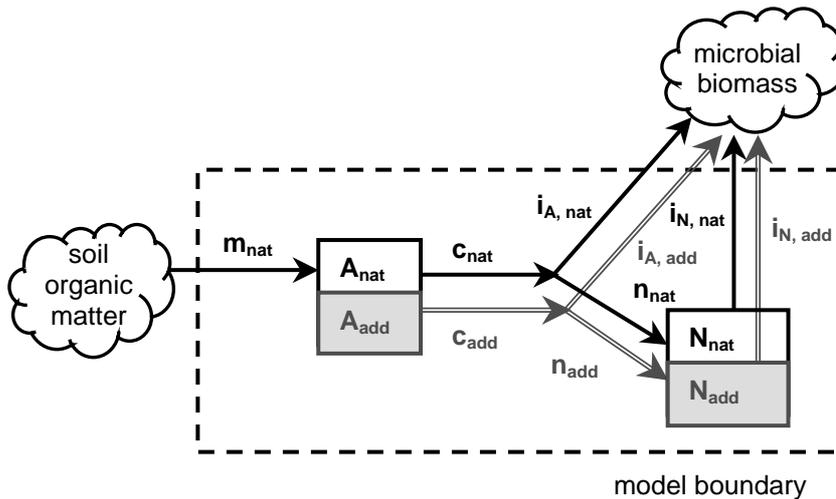


Figure 3. Internal N cycle described as a dynamic compartmental model.

## Analytical methods

Evolved  $\text{CO}_2$  was trapped in NaOH solution (Zibilske, 1994) and mineralized N was determined in 2 M KCl soil extracts by colorimetric analysis for  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N on a TRAACS 800 auto-analyzer (Bran and Luebbe, Germany). The atom%  $^{15}\text{N}$  of dried paper disks from  $^{15}\text{N}$  microdiffusion, total C and N of free-light fraction and total soil were determined after converting inorganic N to molecular  $\text{N}_2$  using an Automatic Elemental Analyser (EA 1110) coupled to an isotope ratio mass spectrometer (Finnigan MAT Delta<sup>plus</sup>, Thermo Finnigan,

USA). Solid-state CP MAS  $^{13}\text{C}$  NMR spectroscopy (Chemagnetics CMX LITE 300 MHz spectrometer) was carried out at the University of Dundee, Scotland.

## Results and discussion

### Importance of freezing and thawing for crop N availability in spring

The amount of C mineralized upon freeze-thawing was more than three times that at the mean response temperature of the FTC and on average, all soils showed a positive N flush upon FTC, but the variation between replicates in the latter was high (Figures 2 b & d in Paper I). Because of differences in experimental conditions and in calculation of the flush, it is difficult to quantitatively compare the effect of FTC on C and N mineralization among studies. The flush in C and N mineralization upon FTC is often calculated as the difference in mineralization between the FTC treatment and a rather arbitrary constant temperature, *i.e.* constant temperatures are used as a control for FTC (*e.g.* DeLuca *et al.*, 1992; Schimel & Clein, 1996). It is, however, very unlikely that microbial activity at these constant temperatures is comparable to the activity in the FTC. Adjustment of C and N mineralization to the mean response temperature of the FTC, and taking these mineralization rates as a control for FTC is more appropriate than considering constant temperatures as a control for FTC. There are few data from incubations at temperatures below 10 °C and it is therefore difficult to evaluate the true mean response temperature of the FTC. Summarizing the literature, Kätterer *et al.* (1998) suggested using functions at these low temperatures that take into account exponentially decreasing  $Q_{10}$  values with temperature, such as the Arrhenius-type function used in Paper I. The C flush was linearly related to soil and water-soluble organic C, while it was proportional to microbial biomass C and basal C respiration at constant temperature (Figure 3 in Paper I). However, correlation between the C flush and these soil characteristics does not provide any evidence for the source of organic material, because they are correlated to each other.

Calculation of the contribution of native microbial biomass C to the flush upon FTC, by comparing the specific activities between the C flush upon FTC with the flush upon fumigation, suggested that microbial biomass contributed *ca.* 65% to the C flush upon FTC. Even though the contribution of the microbial biomass was significant, the flush upon FTC was too small to cause any measurable decrease in the amount of microbial biomass. This calculated C flush derived from the biomass represented only about 5% of microbial biomass C, confirming similar observations on the effects of wet-dry cycles (Magid *et al.*, 1999). There was no evidence for the source of the remaining, non-microbial, 35% of the C flush or for the mechanism of its release. The initial hypothesis was that physically protected soil organic matter might be a source for the flush upon FTC. It appears to be difficult, however, to quantify and assess treatment effects on the degree of physical protection of soil organic matter without suitable methods. One

possibility is to relate changes of soil aggregation stability and mineralization, which in turn will provide indirect evidence. Aggregate size distribution in soils exposed to FTC differed from that in the soils that had been incubated at a constant temperature of +7 °C. The amount of soil in macroaggregates (size class 250 µm < 2000 µm) had increased by between 65 and 139%, while the amount in clay- and silt-sized particles (size class < 63 µm) had decreased by between 24 and 38% (Table 1). Even though organic matter may have become available in association with these changes in aggregation, increased aggregation is normally associated with increased protection of soil organic matter (Elliott, 1986; Beare *et al.*, 1994a). Alternatively, the degree of physical protection can be evaluated by density fractionation combined with ultrasonification (Gregorich *et al.*, 1989; Strickland *et al.*, 1992; Golchin *et al.*, 1994a, b) or by fine sieving of soil samples followed by incubation (Elliott, 1986; Beare *et al.*, 1994a). Because the absolute size of the flush upon FTC was very small, I presume that possible differences in the degree of physically protected soil organic matter between soils previously exposed to FTC and those at constant temperatures were too small to cause any measurable discrepancies using these two methods. Due to the lack of appropriate methods to quantify physical protection of organic matter, both the source and the mechanism by which the non-microbial fraction of soil organic matter becomes available for decomposition upon FTC remain unclear.

Table 1. Aggregate size distribution in soils after 40 d incubation at +7 °C and 20 FTC (corresponding to 40 d), respectively. Means suffixed by a different letter are significantly different at  $P < 0.05$  (Duncan's multiple range test). FTC treatment suffixed with \*, \*\* and \*\*\* are significantly different to constant +7 °C treatment at  $P > 0.05$ ,  $P < 0.005$  and  $P < 0.001$ , respectively.

	> 2000µm		250-2000 µm		63-250 µm		< 63 µm	
	————— % total soil —————							
	+7 °C	FTC	+7 °C	FTC	+7 °C	FTC	+7 °C	FTC
Fallow	7.2 <sup>A</sup>	10.9 <sup>A</sup>	8.4 <sup>A</sup>	20.1 <sup>A*</sup>	5.8 <sup>A</sup>	9.6 <sup>A**</sup>	78.6 <sup>A</sup>	59.4 <sup>A*</sup>
Unfertilized	18.5 <sup>B</sup>	24.4 <sup>B</sup>	14.3 <sup>AB</sup>	30.2 <sup>B*</sup>	4.5 <sup>A</sup>	4.4 <sup>A</sup>	62.7 <sup>B</sup>	41.1 <sup>B*</sup>
N-fertilized	16.7 <sup>B</sup>	14.8 <sup>AC</sup>	17.9 <sup>BC</sup>	34.6 <sup>BC*</sup>	6.2 <sup>A</sup>	9.4 <sup>A</sup>	59.2 <sup>BC</sup>	41.2 <sup>B*</sup>
GM	22.8 <sup>B</sup>	20.5 <sup>BC</sup>	19.5 <sup>BC</sup>	33.1 <sup>BE*</sup>	6.7 <sup>A</sup>	9.0 <sup>A*</sup>	51.1 <sup>BD</sup>	37.4 <sup>BC*</sup>
Straw	18.6 <sup>B</sup>	18.7 <sup>BC</sup>	27.4 <sup>BC</sup>	40.5 <sup>CD*</sup>	7.4 <sup>A</sup>	10.8 <sup>A</sup>	46.7 <sup>D</sup>	30.0 <sup>C*</sup>
FYM	21.3 <sup>B</sup>	20.2 <sup>BC</sup>	23.4 <sup>CD</sup>	38.6 <sup>CE*</sup>	6.4 <sup>A</sup>	9.8 <sup>A</sup>	48.9 <sup>CD</sup>	31.4 <sup>C*</sup>
SD + N	17.8 <sup>B</sup>	10.6 <sup>A</sup>	24.9 <sup>CD</sup>	42.0 <sup>D**</sup>	7.1 <sup>A</sup>	16.2 <sup>A*</sup>	50.2 <sup>CD</sup>	31.1 <sup>BC*</sup>

The effect of FTC on C and N mineralization was short-lived and organic matter made available by FTC was largely decomposed during the period of thawing, confirming similar results from studies on tundra and taiga soils (Schimel & Clein, 1996) and other forest soils (Skogland *et al.*, 1988). The short duration of the flush is indicated by the fact that (i) the size of the C flush decreased with each successive FTC (Table 2 in Paper I) and (ii) subsequent incubation of the soils at +20 °C revealed no differences in C mineralization between the soils previously subjected to FTC and those at constant temperatures. It has to be borne in mind

that soil samples were taken 18 months after the last application of organic manure and were deliberately pre-incubated for a further 6 months at +20 °C to reduce the amounts of relatively undecomposed residues in the soils. Consequently, organic matter made available due to FTC came from stabilized soil organic matter rather than from fresh crop residues or organic manure, which may have induced a larger effect of FTC on C and N mineralization. Although FTC increased C and N mineralization from stabilized soil organic matter, it is very unlikely that these effects are of importance for crop N availability in spring since they are only short-lived.

### **Long-term addition of different amendments and its impact on C and N mineralization from soil organic matter**

Differences between the treatments in the quantity and quality of organic matter inputs since 1956 had affected both the quantity and quality of the organic matter in the soils, which were sampled more than 40 y after the start of the experiment. In both the Fallow and Unfertilized treatments the amount of soil organic matter had decreased, it had remained approximately unchanged in the N-fertilized treatment, whereas it had increased in the treatments receiving organic amendments (soil organic matter expressed as soil C in Table 1 in Papers I and II). Treatments receiving sawdust amendments had a significantly wider C-to-N ratio compared to treatments receiving straw, green or farmyard manure in which the C-to-N ratios were similar to those in Unfertilized, N-fertilized treatments and the older organic matter in the Fallow treatment (Table 1 in Papers I and II). The organic matter in the Fallow soil is in its entirety derived from inputs before 1956. Consequently, differences between the amounts of soil organic C and N in the cropped and that in the Fallow represented 'new' soil organic matter formed from inputs since 1956, on the assumption that mineralization of the older soil organic matter in the Fallow occurred at the same rate in other treatments. The quality of organic amendment had relatively little effect on the amounts of 'new' soil organic C in soils (Table 1 in Paper II). In contrast, the amount of organic N 'newly' formed was dependent on the quality of the organic amendment, *i.e.* more soil organic N was formed per unit N input from FYM than N derived from crop residues only and N immobilizing materials such as straw and sawdust (Table 1 in Paper II). That more soil organic N was formed per unit N input from FYM compared to other amendments is probably a result of the higher degree of stabilization of farmyard manure-N prior addition to soils. In comparison to the straw and sawdust amendments, the organic matter of farmyard manure had a higher alkyl C-to-O-alkyl C ratio (Table 2), which is indicative of a greater degree of decomposition and recalcitrance, as the FYM is derived from plant materials (Golchin *et al.*, 1998). Calculation of long-term C balances (Witter, 1996) and application of soil organic matter models to C and N turnover in the soils of the Ultuna Long-Term Soil Organic Matter Experiment (Paustian *et al.*, 1992; Hyvönen, *et al.*, 1996; Andrén & Kätterer, 1997) also suggest that farmyard manure is more resistant to decomposition than crop residues, such as straw. Even though I cannot provide any direct evidence of which mechanism caused organic N stabilization in the FYM treatment, it has been observed before that application

of farmyard manure result in a larger build up of soil organic matter than applications of straw together with mineral N (Jenkinson & Rayner, 1977).

The higher degree of stabilization of farmyard manure was also demonstrated in this treatment since less C was mineralized in relation to soil organic C (Table 3 in Paper II and Figure 4). Carbon (Figure 2 in Paper II & Figure 4) and gross N (Figure 3 in Paper II) mineralization rates decreased within each treatment during the incubation, but among all treatments they were related to soil organic C and N, respectively. The relation between C and gross N mineralized to total soil organic C and N, respectively implies that the amount of organic C and N in the soil is a major determinant of ‘mineralizable’ soil organic matter, whereas the decrease in mineralization during incubation suggests that there is a limited pool of ‘mineralizable’ C and N (Figure 2 & 3 in Paper II & Figure 4). Despite the amendments of farmyard manure resulting in the highest ‘new’ soil organic C and N contents among treatments, the amounts of C and gross N mineralized over and above that in the Fallow were smallest in the FYM treatment and largest in the sawdust treatments, when expressed as a percentage of ‘new’ soil organic C and N, respectively (Table 3 in Paper II). The pool size of ‘mineralizable’ C and N was therefore determined not only by the amount of organic matter in the soil, but also by its quality as affected by differences in quality of past organic matter inputs.

Table 2. Alkyl C-to-O-alkyl C ratio of organic amendments added in autumn 1997

	Alkyl C-to-O-alkyl C ratio
Farmyard manure	0.28
Straw	0.12
Sawdust	0.09

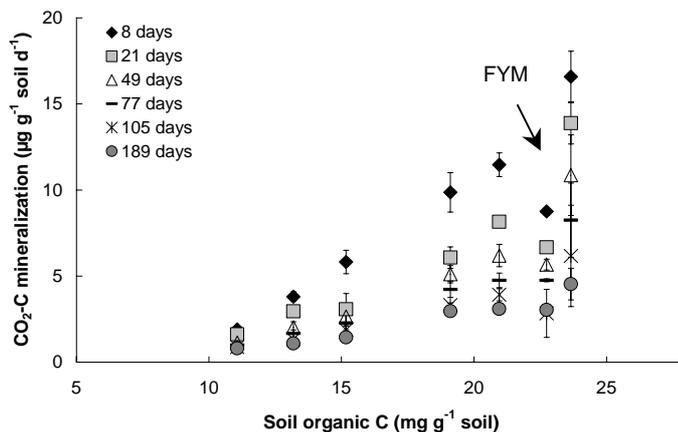


Figure 4. Rates of C mineralization ( $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil d}^{-1}$ ) from soil samples incubated at 20 °C for 27 weeks in relation to soil organic C (long-term incubation). Mean value and standard error of four replicates.

Cumulative amounts of net N mineralized from soil samples taken in May 1999 were unrelated, irrespectively of duration of incubation, to total soil organic N (Figure 5). Also, the cumulative amounts of N mineralized from soil samples taken in May 2001 were unrelated to total soil organic N after 5 d of incubation (*i.e.* in Period 1), but the correlation coefficient of this relation improved with length of incubation (Figure 5 in Paper II). The presence of organic C that caused N immobilization during its decomposition may have obscured the relation between total soil organic N and net N mineralization. The free-light fraction, with a high C-to-N ratio, undergoing decomposition would be a likely candidate as a pool providing C to drive N immobilization. For example, the SD + N treatment showed net N immobilization at the beginning of the 27-week incubation (*i.e.* in the first three weeks of the long-term incubation, see Figure 5). At the beginning

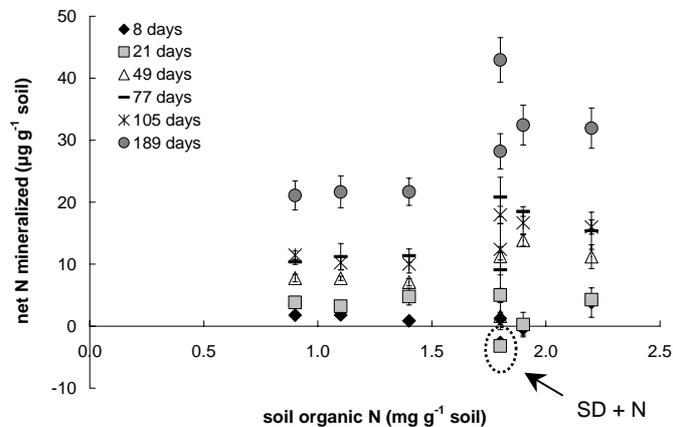


Figure 5. Cumulative net N mineralized ( $\mu\text{g N g}^{-1}$  soil) from soil samples taken in May 1999 and incubated at 20 °C for 27 weeks in relation to soil organic N (long-term incubation). Mean value and standard error of four replicates.

of this long-term incubation the SD + N treatment had larger amounts of free-light fraction material, both per weight of soil and as a fraction of total soil organic C, with a wider C-to-N ratio (Table 3) than that found in other treatments. In general, the free-light fraction is thought to be very reactive as it is not physically protected in soils (Gregorich *et al.*, 1994), has usually a wider C-to-N ratio than the total soil (Greenland & Ford, 1964) and C mineralization rates are correlated with the size of the free-light fraction (Janzen *et al.*, 1992). Furthermore, the free-light fraction has been shown to decompose quickly compared to organic matter in the soil as a whole, despite its wider C-to-N ratio (Sollins *et al.*, 1984; Bonde *et al.*, 1992, Hassink, 1995). That the free-light fraction is a very labile pool of soil organic matter that may make a substantial contribution to soil C mineralization was seen in the significant losses of free-light fraction C (up to 40% of initial amount) during the long-term incubation, with the highest losses in the SD + N treatment

(Table 3). These losses of free-light fraction C were found to be proportional to the amounts of C mineralized in these soils (Figure 6). The FYM treatment mineralized, however, more C in relation to C losses from the free-light fraction. Large amounts of the free-light fraction with a wide C-to-N ratio may therefore cause substantial N immobilization because the N content of the organic matter undergoing decomposition is likely to be insufficient to meet the N demand of the microorganisms. Consequently, varying amounts of N immobilization, depending on the C-to-N ratio of the organic material undergoing decomposition, may be accompanied with gross N mineralization, thus obscuring the relation between total soil organic N and net N mineralization.

Table 3. Characterization of the free-light fraction (FLF) prior to a long-term incubation at 20 °C for 189 days and losses of free-light fraction C during long-term incubation. Mean values are given for four replicates and standard error. Treatments followed by a different letter are significant at  $P < 0.05$  (Duncan's multiple range test).

	— FLF prior to long-term incubation —			
	mg C g <sup>-1</sup> soil	C as % soil C	C-to-N ratio	loss mg C g <sup>-1</sup> soil
Fallow	0.46 <sup>A</sup>	4.1 <sup>AB</sup>	22.7 <sup>A</sup>	-0.02 <sup>A</sup>
Unfertilized	0.69 <sup>AB</sup>	5.0 <sup>AB</sup>	23.0 <sup>A</sup>	0.25 <sup>AB</sup>
N-fertilized	0.81 <sup>AC</sup>	5.1 <sup>AB</sup>	19.0 <sup>A</sup>	0.39 <sup>ABC</sup>
Straw + N	1.18 <sup>C</sup>	5.2 <sup>AB</sup>	21.0 <sup>A</sup>	0.51 <sup>BC</sup>
GM	1.12 <sup>C</sup>	5.3 <sup>A</sup>	20.3 <sup>A</sup>	0.50 <sup>BC</sup>
FYM	0.87 <sup>BC</sup>	3.4 <sup>B</sup>	19.0 <sup>A</sup>	0.24 <sup>AB</sup>
SD + N	1.84 <sup>D</sup>	7.2 <sup>C</sup>	34.6 <sup>B</sup>	0.84 <sup>C</sup>

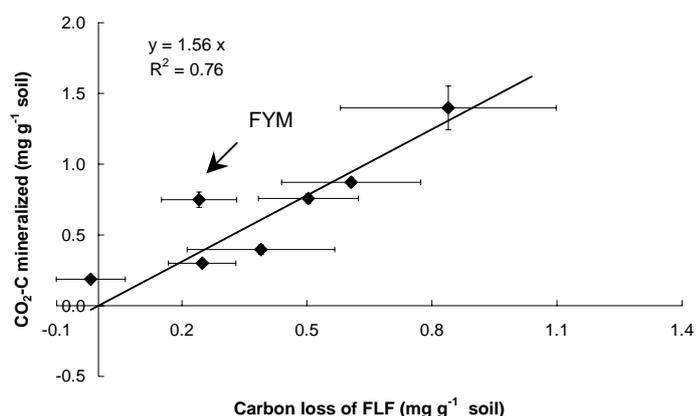


Figure 6. Amounts CO<sub>2</sub>-C mineralized (mg g<sup>-1</sup> soil) from soil samples incubated at 20 °C for 27 weeks in relation to C loss of the free-light fraction (mg g<sup>-1</sup> soil) (long-term incubation). Mean value and standard error of four replicates.

## Testing the assumptions in the $^{15}\text{N}$ isotope dilution technique

As discussed above, measurements of gross N mineralization may improve our understanding of the relation between soil organic matter and net N mineralization in soils. Violation of the assumptions on which the  $^{15}\text{N}$  isotope dilution technique is based (see section ‘Quantification of gross N processes’ on page 12) may, however, have a large impact on the estimates of gross N rates. Testing these assumptions is therefore an important part of the evaluation of such estimates.

There is very little doubt that the assumptions of ‘no isotopic discrimination’ and ‘no re-mineralization of recently added  $^{15}\text{N}$ ’ are met in Paper II. In Paper II nearly 4500‰  $\delta^{15}\text{N}$  ( $(\text{NH}_4)_2\text{SO}_4$  (2.0 atom%) were added to the soil samples and at the end of each incubation period between 2000-4000‰  $\delta^{15}\text{N}$  (1.1-1.8 atom%) was present in the Fallow, but only 100-600‰  $\delta^{15}\text{N}$  (0.4-0.6 atom%) were present in the other treatments. This is still 3- to 5-fold more than any discrimination that may have occurred during N transformation in the soil, which is thought to be at the most 20-30‰  $\delta^{15}\text{N}$  (Högberg, 1997). Consequently, ‘isotopic discrimination’ (assumption 1) should not have introduced a bias, even though there was very little extractable  $\text{NH}_4^+$  at the end of the incubation period. ‘Re-mineralization of recently added  $^{15}\text{N}$ ’ (assumption 2) is very unlikely since gross N mineralization rates were estimated over short-incubation periods of three or five days, respectively.

The assumption of ‘zero-order rate constants for all processes’ (assumption 3) was, however questionable in Paper II. The addition of  $\text{NH}_4^+$  provides a substrate for nitrification, which usually is a substrate-limited process (*i.e.* it follows first-order kinetics) since applied  $\text{NH}_4^+$  is rapidly nitrified within weeks (Mendum *et al.*, 1999; Burger & Jackson, 2003) and net nitrification rates are often enhanced by  $\text{NH}_4^+$  addition (Willison *et al.*, 1998). Consequently, first- rather than zero-order kinetics for  $\text{NH}_4^+$ -N consumption may be more appropriate. The assumption of first-order kinetics for  $\text{NH}_4^+$  consumption rates in Paper II was underpinned by the substantial amounts, up to approx. 25% of added  $\text{NH}_4^+$ -N, recovered in the  $\text{NO}_3^-$ -N pool as soon as 2 h after addition (Table 2 in Paper II), indicating that the added N was rapidly nitrified. Moreover, virtually all added N was consumed after 1-3 d with less than 0.3% of added  $^{15}\text{N}$  recovered in the  $\text{NH}_4^+$  pool (excepting the Fallow treatment) at the end of each incubation period (Figure 1 & Table 2 in Paper II). Clearly, first- rather than zero-order kinetics for  $\text{NH}_4^+$  consumption was more appropriate in this experiment because the incubation period was too long to meet the assumption of ‘zero-order rate constants’ during the incubation period. In comparison, estimated gross N mineralization rates based on all rate processes following zero-order kinetics over the incubation period (Powlson & Barraclough, 1992) differed significantly from rate estimates in Paper II (*cf.* Figure 3 in Paper II & Figure 7). Gross N mineralization rates based on ‘zero-order rate constants’ were similar in all cropped treatments and only the Fallow treatment showed lower gross N mineralization rates (Figure 7). Furthermore, gross N rate estimates did not decrease within each treatment during the incubation. First-, rather than zero-order rate kinetics was also more appropriate to describe the consumption of added  $\text{NH}_4^+$  in the study of Watson *et al.* (2000) at the low rate of  $\text{NH}_4^+$  addition

(2 mg N kg<sup>-1</sup> soil) (Figure 2a in Paper III). Analysis of their raw data using a dynamic compartmental model (Paper III) demonstrated that wrong assumption of the kinetics of NH<sub>4</sub><sup>+</sup> consumption resulted in an underestimation of both, NH<sub>4</sub><sup>+</sup> consumption and gross N mineralization (*cf.* Watson *et al.*, 2000 and Table 2 in Paper III). Clearly, whether zero- or first-order kinetics for NH<sub>4</sub><sup>+</sup> consumption is assumed has a significant impact on the outcome of gross N mineralization rates and, therefore, which is the most appropriate assumption has to be clarified.

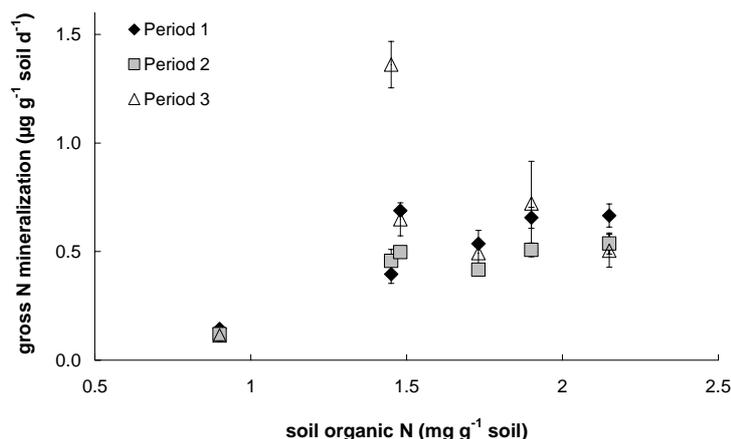


Figure 7. Gross N mineralization rates based on zero-order rate constants for all N processes in relation to soil organic N. The N-fertilized treatment showed, however, exceptionally high gross N mineralization per amount of organic N in Period 3 and was therefore treated as an outlier. Mean value and standard error of four replicates.

There are, however, some obstacles to the calculation of gross N mineralization rates based on first-order kinetics of NH<sub>4</sub><sup>+</sup> consumption in Paper II. It was assumed that added NH<sub>4</sub><sup>+</sup> was completely extractable after addition (0h) in the calculation of gross N mineralization rates based on first-order kinetics of NH<sub>4</sub><sup>+</sup> consumption (Paper II). A criticism is that it may not be valid to assume 100% recovery of added NH<sub>4</sub><sup>+</sup> immediately after <sup>15</sup>N addition, because of irreversible losses due to NH<sub>4</sub><sup>+</sup> fixation (Davidson *et al.*, 1991; Stockdale *et al.*, 1994). The importance of abiotic NH<sub>4</sub><sup>+</sup> fixation was, however, tested prior to the start of the experiment in sterilized gamma radiated soil samples (12 kGy). In this case, added <sup>15</sup>N labelled (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was completely recovered in the mineral N pool when soil samples were consecutively extracted over a time-interval of 7 days, indicating that NH<sub>4</sub><sup>+</sup> fixation was not of importance in the soils used in the present study. Ideally, several samplings, including a time zero extraction to test if added NH<sub>4</sub><sup>+</sup> is completely extractable after addition, will be needed to obtain more reliable parameter estimates than those obtained in Paper II.

Gross N mineralization rates are obtained on the assumption that the rate of consumption of native  $\text{NH}_4^+$  is the same as that for added  $\text{NH}_4^+$ , *i.e.* that there is 'equilibrium and identical behaviour between added and native N pools' (assumption 4). However, analysis of the raw data of Watson *et al.* (2000) by separate estimation of added and native N pools showed that this might not always be the case (Paper III). It is most likely that added  $\text{NH}_4^+$  is preferentially nitrified, but from the data available, it cannot be excluded that both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  immobilization are subjected to preferential use. Because the process of nitrification is part of  $\text{NH}_4^+$  consumption and parity between consumption of added and native  $\text{NH}_4^+$  is required for reliable gross N mineralization rates, preferential use in nitrification of added  $\text{NH}_4^+$  affects the estimation of gross N mineralization rates. In the study of Watson *et al.* (2000), preferential use of the added  $\text{NH}_4^+$  resulted in a 2- to 3-fold overestimation of the gross N mineralization, emphasizing the importance of testing this assumption in the  $^{15}\text{N}$  isotope dilution technique.

Luxhøi *et al.* (2003), however, suggested that the preferential use of added  $\text{NH}_4^+$  in Watson *et al.* (2000) study was specific to the experimental design and a result of non-uniform distribution of added N. Contrary to the conclusion of Watson *et al.* (2000) they suggested preferential use of the native, rather than of added N. Their assertion is, however, speculative and not supported by an analysis of Watson *et al.*'s data. To be able to test the assumption of non-preferential use an experiment should include additions of both  $^{15}\text{N}$ -labelled  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , while at least three successive samplings are required to test whether zero- or first-order kinetics should be applied. These requirements are not met in the estimation of gross N mineralization rates in Paper II though and whether non-preferential use of added N is the case in this experiment cannot be tested. The amounts of  $^{15}\text{N}$  recovered in the mineral N pools after 24 h of N addition are often used as a starting point to allow the soil to equilibrate (Ledgard *et al.*, 1998; Murphy *et al.*, 1999; Hatch *et al.*, 2000). However,  $^{15}\text{N}$  addition in these studies was carried out by multiple injectors or by uniform application of  $^{15}\text{N}$  over the soil surface, which then requires equilibration time due to low diffusion of added  $^{15}\text{N}$ . In comparison, soil samples in Paper II were mixed thoroughly after  $^{15}\text{N}$  addition to obtain even distribution between added and native N and instant equilibration was therefore assumed. Despite the uncertainty about whether non-preferential use of N can be assumed, gross N mineralization rates reported in Paper II were in the range of 0.2-2.5  $\mu\text{g N g}^{-1} \text{ soil d}^{-1}$ , as often found in other studies (Murphy *et al.*, 1999; Recous *et al.*, 1999; Andersen & Jensen, 2001; Sørensen, 2001). These similar gross N mineralization rates are corroboration that the rate estimates in Paper II represent the true gross N mineralization rates, but only if preferential use of added N was not of importance in the calculated rates published in the literature.

The outcome of Papers II and III illustrates how difficult it can be to meet the assumptions in the  $^{15}\text{N}$  isotope dilution technique. Each experiment needs thorough consideration of its experimental design because there is no scheme of how to run an experiment to avoid errors in the estimation of gross N rates due to one or more assumptions not being met. Discrimination between  $^{14}\text{N}$  and  $^{15}\text{N}$  can be avoided by  $^{15}\text{N}$  addition several times that of natural abundance, re-

mineralization of recently added  $^{15}\text{N}$  can be tested by the appearance of labelled N in the  $\text{NH}_4^+$  pool after addition of labelled  $\text{NO}_3^-$  and whether zero- or first-order kinetics should be applied can be tested by several successive samplings. Up to now, there has been no ‘method’ available for critical testing of the assumption of equilibrium and identical behaviour between added and native N pools. But, the method of data analysis presented in Paper III offers a step towards the validation of this assumption.

### **Predicting N mineralization from soil organic matter - a chimera?**

Processes that substantially contribute to net N mineralization have to be identified and quantified when a mechanistic approach is used to predict N mineralization from soil organic matter. Gross N mineralization is the process supplying mineral N from soil organic matter and its quantification is the first step in a mechanistic approach. Evidently, there were some uncertainties in the estimation of gross N mineralization rates in Paper II. Nonetheless, I want to stress the following question: Does the estimation of these rates contribute to a better understanding of the relation between soil organic matter and N mineralization? Processes that remove N from the mineral N pool also have to be considered in a mechanistic approach. N immobilization is an important process that removes N from the mineral N pool and its quantification is therefore the next step in a mechanistic approach. This raises the question: Is it possible to determine N immobilization in soils? In conclusion, I want to discuss the question: Can a mechanistic approach be used to predict N mineralization in the field?

Gross N mineralization was approximately proportional to C mineralization (Figure 4 in Paper II), which is in line with mechanistic models that estimate gross N mineralization from C mineralization (*e.g.* Jenkinson & Rayner, 1977; Smith *et al.* 1997; Jansson & Karlberg, 2001; Kätterer & Andrén, 2001). For example, the SUNDIAL model (Smith *et al.*, 1997) estimates gross N mineralization from C mineralization with knowledge of the C-to-N ratio of the decomposing material, the microbial C use efficiency ( $\alpha$ ), *i.e.* the amounts of C incorporated into microbial tissues, and the fraction of soil organic matter stabilized ( $\beta$ ) (equation 2):

$$\text{gross N mineralization} = \frac{\left( \frac{\text{C mineralization}}{1 - (\alpha + \beta)} \right)}{\text{C-to-N ratio of the decomposing material}} \quad (2)$$

This model has been found to give adequate prediction of gross N mineralization from C mineralization in the field (Murphy *et al.*, 2003). Despite the differences in the quality of total soil organic matter, the proportionality between C and gross N mineralization suggests that the quality, *i.e.* the C-to-N ratio, of the organic matter

undergoing decomposition and the sum of  $\alpha$  and  $\beta$  are approximately the same among those soils. The unlikely alternative is that the C-to-N ratio of the decomposing material and the sum of  $\alpha$  and  $\beta$  may differ among the soils, but in opposing directions, so that applying equation (2) gives proportionality between C and gross N mineralization. The C-to-N ratio of the decomposing material as part of stabilized soil organic matter is rarely known. But, using the gross N and C mineralization data in Paper II and assuming a factor of 0.42 for the sum of  $\alpha$  and  $\beta$  (derived from equation 5 in Bradbury *et al.*, 1993) resulted in an average C-to-N ratio of 9 for the decomposing material. Thus, applying equation (2) to calculate the C-to-N ratio of the decomposing material suggested that N-rich organic material was subjected to microbial decomposition. Although the proportionality between C and gross N mineralization suggests that C mineralization may be used as a predictor for gross N mineralization, further examination is needed of whether the same proportionality is valid among other soils. Moreover, the validity of the assumption of 'equilibrium and identical behaviour between added and native N pools' has to be tested since preferential use of added N may be a more common occurrence in  $^{15}\text{N}$  isotope dilution studies than hitherto thought. Testing this assumption is needed to substantiate the validity of gross N rate estimates in Paper II and its relation to C mineralization rates.

Albeit that gross N mineralization may be predicted from C mineralization, this relation is only the very first step in a mechanistic approach to enable prediction of net N mineralization from soil organic matter, which is of interest for crop N availability. The next step is to estimate N immobilization and to find a predictor for this process. Differences between gross and net N mineralization suggested substantial N immobilization in all treatments (Table 3 in Paper II), but it was not possible to obtain reliable N immobilization rates by measuring  $^{15}\text{N}$  in the microbial biomass or determination of residual  $^{15}\text{N}$  in soils after extraction due to methodological problems (data not shown). Varying amounts of N immobilization, depending on methods used, are also reported in the literature (Ledgard *et al.*, 1998; Hatch *et al.*, 2000; Andersen & Jensen, 2001). This raises the question of which method, if any, gives the true rates of N immobilization. As discussed above, the free-light fraction with its wide C-to-N ratio would be a likely candidate as a pool providing C to drive immobilization. It made a substantial contribution to the amount C mineralized in soils incubated for 27 weeks, which suggests that free-light fraction material is an important pool susceptible to microbial decomposition. Determination of amount and C-to-N ratio of the free-light fraction obtained by density fractionation (*e.g.* Golchin *et al.*, 1994 a, b) may therefore be a good predictor for N immobilization rates in soils. But, due to the lack of reliable N immobilization estimates, it was not possible to quantify the relation between free-light fraction and N immobilization in the soils. It seems that estimation of N immobilization rather than gross N mineralization is the most difficult step in predicting net N mineralization from soil organic matter when using a mechanistic approach. The development of methods for quantification of N immobilization in soils needs therefore further attention.

C mineralization and possibly the free-light fraction may be used as predictors for gross N mineralization and N immobilization, respectively. The differences

between the latter two will then give an estimate of net N mineralization from soil organic matter. Although this simplistic approach may suffice to predict N mineralization in soils under standardized conditions (*i.e.* soils incubated in the laboratory), it is not sufficient to predict N mineralization under field conditions. In the field, measuring C mineralization is complicated and a predictor for C mineralization is therefore needed. The amounts of C mineralized were linearly related to amount of soil organic C (Figure 2 in Paper II and Figure 4), while they were proportional to C losses from the free-light fraction (Figure 6). Both soil organic matter and the free-light fraction may therefore be appropriate predictors for C mineralization. Total soil organic matter alone is, however, a poor predictor for C mineralization, as decomposition rates are lower and more of the added organic matter remains in fine textured compared to coarse textured soils (Hassink *et al.*, 1993; Hassink, 1995; Strong *et al.*, 1999). Physical protection of soil organic matter may play a more important role in clay than in sandy soils. It is therefore likely that in clay soils less organic matter is susceptible to decomposition per unit soil organic C compared to that in sandy soils. Consequently, the importance of physical protection on organic matter decomposition has in some way to be taken into account when predicting C mineralization. There is little doubt about the existence of physical protection, but little is known about the factors that control this process. Obviously, soil texture affects physical protection of soil organic matter, but it is difficult to quantify the relation between these two. Fundamental studies on the relation between physical protection of soil organic matter and soil texture are needed, so that soil texture may be used as an additional predictor for C mineralization besides total soil organic C and the amount of free-light fraction C.

Apparently, predicting net N mineralization in practice requires additional variables and not only the estimation of gross N mineralization and N immobilization. For example, nitrate leaching and denitrification can contribute significantly to N losses from the mineral N pool and indices of the susceptibility of soils to these losses are needed. Further, variables have to be found that identify outliers, which do not follow the above-mentioned relations (*e.g.* the sawdust treatment was an outlier in the relation between C and gross N mineralization; and farmyard-manure-amended soils mineralized less C in relation to soil organic C, but mineralized more C in relation to C losses from the free-light fraction). Given the complexity of the N cycle, it is a chimera to believe that one single variable will succeed in predicting N mineralization from soil organic matter. The combination of several variables (*e.g.* total soil organic matter, free-light fraction, C mineralization, soil texture *etc.*) that are based on a mechanistic relation to N processes may, however, succeed in adequately estimating N mineralization from soil organic matter.

## Conclusions – Something new under the sun?

*What has been is what will be,  
and what is done is what will be done;  
and there is nothing new under the sun.  
Is there a thing of which it is said,  
'See this is new?'  
It has been already, in the ages before us.  
There is no remembrance of former things  
nor will there be any remembrance of later things yet to happen  
- Ecclesiastes 1:9-11*

The N cycle was largely formulated in the late 50s by Jansson (1958) and it has been extensively studied in the last decades. But, it is difficult to grade and summarize the findings of studies concerning prediction of N mineralization from soil organic matter because there are so many of them. However, there is not much point to science, if the presumption ‘there is no remembrance of former things’ is taken to be the case. Is there ‘something new’ in the present thesis? What conclusions can be drawn from the experiments that I have carried out in the past four years? I want to emphasize the following achievements, in the hope that ‘there will be remembrance’ in the future:

- Lysis of microbial biomass contributes up to two-third of the C and N flush upon freezing and thawing, but the mechanism and source of the remaining one-third is not known.
- Freezing and thawing of soils during late winter and early spring is of little importance to crop N availability in spring.
- The amount of soil organic matter is a major determinant of gross N mineralization, but the quality also affects gross N mineralization.
- Gross N mineralization is proportional to C mineralization in soils, so that C mineralization may be used as a predictor for gross N mineralization.
- Gross N mineralization may be accompanied by substantial N immobilization. But, it is difficult to evaluate how the quality of past organic matter affects N immobilization and thus net N mineralization from the pool of soil organic matter, since reliable N immobilization estimates are missing.
- The <sup>15</sup>N isotope dilution technique is a method that is and will be based on several assumptions. The potentially large impact of preferential use of added N on the estimated rate parameters warrants further examination of the validity of <sup>15</sup>N isotope dilution technique to estimate gross N processes.

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